Advancing Sexual Health for Cisgender Women: A Series of Studies Addressing Doxycycline Post-Exposure Prophylaxis, Vaginal Health, and Antimicrobial Resistance in Kenyan Women

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A dissertation

submitted in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2025

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Abstract

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Globally, high rates of sexually transmitted infections (STIs) persist even in the face of effective antibiotic treatment, with young women living in Africa facing a disproportionate burden. Sexually transmitted infections can lead to dire reproductive health consequences for women, including severe complications and significant economic burdens related to lost productivity and quality of life. Among these infections, *Trichomonas vaginalis* stands as the most prevalent curable STI globally and can cause vaginitis, urethritis, pregnancy complications, and increased HIV susceptibility. Treatment relies solely on 5-nitroimidazole medications (metronidazole and tinidazole), but emerging resistance has necessitated extended treatment protocols, highlighting the urgent need for novel prevention strategies. Doxycycline post-exposure prophylaxis (doxy-PEP) has emerged as an innovative method for STI prevention. While laboratory evidence suggests doxycycline may effectively treat *T. vaginalis* by disrupting cellular metabolism, no studies have investigated doxyPEP's potential in preventing *T. vaginalis* infections—an important gap in developing comprehensive STI prevention approaches for high-burden settings.

The first study investigated the potential utility of doxyPEP in preventing the protozoal infection of *T. vaginalis*, a pathogen not traditionally targeted by doxycycline therapy. In this secondary analysis of a randomized clinical trial involving 449 cisgender Kenyan women followed for 12 months, the study assessed incident infections. There was no significant difference in *T. vaginalis* incidence when comparing the doxyPEP group to the standard of care group (RR: 0.96, 95% CI: 0.54-1.73, p=0.9). Low doxycycline use was reported in a subset of participants in the parent trial. Further studies are needed to investigate whether alternative dosing strategies, higher concentrations, or different administration schedules might enhance doxycycline's efficacy against protozoal pathogens, potentially bridging the gap between promising in vitro mechanisms and clinical outcomes.

Building on this doxyPEP research, the second study examined antimicrobial resistance patterns in *Neisseria gonorrhoeae*, which is ranked as the second most frequent bacterial STI globally, with Africa accounting for the highest incidence in 2020. Antimicrobial resistance (AMR), which refers to the ability of bacteria, including N. gonorrhoeae, to resist antimicrobial drugs, is widely acknowledged as a significant global public health issue that may impact Africa more profoundly, as the region had the highest number of deaths attributed to AMR in 2019. While doxyPEP represents one intervention to prevent bacterial STIs, the broader implications of doxyPEP, such as unintended consequences on non-target pathogens and its potential impact on AMR patterns, require further exploration. This study examined antimicrobial resistance patterns in *N. gonorrhoeae* among cisgender women participating in the same doxyPEP trial and identified associated demographic, behavioral, and clinical factors. Analysis of gonorrhea-positive samples revealed universal tetracycline resistance (100%) and high fluoroquinolone resistance (90.5%), while cephalosporin resistance remained low (2.6%), and no macrolide resistance was detected. No differences in resistance patterns were observed between study groups, although doxycycline use was low among those randomized to doxyPEP. These findings underscore the importance of ongoing surveillance as doxyPEP implementation expands globally.

The third study shifted focus to examine the complex relationship between vaginal health and cervical inflammation. Bacterial vaginosis (BV), a condition characterized by a marked shift in vaginal microbiota from a *Lactobacillus*-dominated community to a more diverse community comprised of anaerobic and Gram-negative species, is the most common cause of vaginitis in cisgender women. While BV has been associated with various reproductive health complications, its relationship with cervicitis, a cervical inflammatory condition often caused by sexually transmitted pathogens, has been the subject of conflicting research findings, particularly regarding intermediate vaginal microbiota, a transitional state between normal microbiota and BV. Understanding this relationship is critical for developing nuanced therapeutic interventions to improve vaginal health and reduce cervicitis-related complications. This study examined the relationship between vaginal Nugent score categories and cervicitis among cisgender women engaged in transactional sex in Mombasa, Kenya. The intermediate vaginal microbiota demonstrated a significantly higher risk of cervicitis (adjusted RR=1.32, 95% CI: 1.18-1.48) compared to normal microbiota, a risk surpassing that associated with BV (adjusted RR=1.12, 95% CI: 1.01-1.25). This novel finding challenges conventional binary views of vaginal health and suggests that intermediate microbiota represents a distinct pathological state requiring targeted clinical attention. Furthermore, the study highlighted a progressive increase in cervicitis risk associated with the presence of both non–H_2O_2–producing lactobacilli and H_2O_2-producing lactobacilli, indicating a complex interplay between protective and potentially pathogenic vaginal bacterial species. These findings advocate for a nuanced understanding of vaginal microbiota and highlight the need for future mechanistic studies exploring the specific cytokine and chemokine profiles associated with intermediate vaginal microbiota states.

The three studies analyzed in this dissertation collectively provide important insights into STI prevention among Kenyan cisgender women, investigating the efficacy of doxyPEP against *T. vaginalis*, documenting antimicrobial resistance in *N. gonorrhoeae*, and revealing complex relationships between vaginal microbiota and cervical inflammation. The findings underscore the need for multifaceted interventions that address vaginal health, antimicrobial stewardship, and pathogen-specific prevention strategies. Future research should explore microbiota-inflammation interactions, conduct long-term AMR surveillance in populations using prophylactic biomedical interventions, and develop innovative approaches tailored to diverse STI pathogen.

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ACKNOWLEDGMENTS

This dissertation is the culmination of years of hard work, dedication, and unwavering support from many individuals. I am deeply grateful to all those who have contributed to this journey.

First and foremost, I want to express my sincere gratitude to the women of Kenya who participated in these studies. Your willingness to share your experiences made this research possible, and I am humbled by your strength and resilience.

I am profoundly thankful to my chair, Jen Balkus, for her exceptional mentorship, unwavering support, and patient guidance throughout my doctoral journey. Your expertise, thoughtful feedback, and constant encouragement have been invaluable. To my committee members - Jenell Stewart, Elizabeth Bukusi, Scott McClelland, Olusegun Soge, and Holly Janes - thank you for your countless hours of support, insightful perspectives, and steadfast guidance. Your collective wisdom, constructive feedback, and genuine dedication to my growth as a researcher have shaped not only this dissertation but also my development as a scientist.

My heartfelt gratitude goes to my mother, Christiana, whose love, prayers, and belief in me have been a constant source of strength. I am equally grateful to my father, Albert Sesay, and his wife (Aunty Sylvia), along with my siblings, for their encouragement and steadfast support throughout my academic journey.

My most profound appreciation goes to my husband and daughter. Your love, patience, and understanding have been my anchor, and I am eternally grateful for your sacrifices and unwavering belief in me.

I am incredibly thankful for the love and support of my family and friends, both on my side and my husband's, across the globe. Your encouragement has been invaluable throughout this process.

To my sisters from Sierra Leone – Edna, Dawn, Hawanatu, Ayo, and Mary – thank you for your constant love and support from afar.

I would also like to express my sincere gratitude to Jeanette, my host family, for providing a welcoming and supportive home away from home.

To the Sillah family – Arthur, Mr. and Mrs. Sillah – thank you for your kindness and support. Moreover, to Alfred and Kadija John, your support and friendship have been truly appreciated.

I am grateful for the sense of community and support I found within the Seattle Fambul group, as well as the support system I found with Fatima, Temi, and Aunty Jan.

To my close friends from UW, Ramya, Mariama, and Kidist, thank you for your camaraderie and encouragement and for sharing in the ups and downs of this journey.

I sincerely thank the Epidemiology cohort for their support and intellectual stimulation.

Thank you all from the bottom of my heart.

DEDICATION

To my daughter

Telina, my little co-author. As I nurtured this dissertation, you were on this journey with me, and your strength became my own. You were my quiet cheerleader through every challenging chapter and my calm in the final, hectic days. We crossed the finish line together, and I will be forever proud that our first great adventure was completing this work.

CHAPTER 1: Introduction

Global and Regional Burden of Sexually Transmitted Infections

Sexually transmitted infections (STIs) represent a major global public health challenge, with more than one million new cases occurring daily worldwide among individuals aged 15-49.[1-3] Of particular concern are four curable infections – chlamydia (*Chlamydia trachomatis*), gonorrhea (*Neisseria gonorrhoeae*), syphilis (*Treponema pallidum*), and trichomoniasis (*Trichomonas vaginalis*) – which collectively resulted in an estimated 374 million new infections in 2020.[4,5] The global response has been inadequate, characterized by insufficient visibility, funding, and implementation support.[4] The limited availability of diagnostic tools has led to widespread reliance on syndromic management as the standard of care in resource-limited settings, where treatment is based on clinical symptoms rather than laboratory confirmation—often resulting in suboptimal outcomes and ongoing transmission (discussed in detail below). Data from the World Health Organization (WHO) shows that apart from modest reductions in congenital syphilis, STI rates have largely plateaued, with no global targets for 2020 being met.[1,4] The burden is particularly pronounced in Africa, which bears approximately 40% of global STI cases and only 19% of the world's population.[1]

Sexually Transmitted Infections: Landscape in Kenya

Kenya, like other African countries, faces a high burden of STIs. Studies have shown varying prevalence depending on the population and specific STI. Adolescent girls and young women (AGYW) are disproportionately impacted by STIs, with research consistently showing higher infection rates among young women relative to their male peers.[6-8] Studies across different regions in Kenya highlight this burden. Among adolescent females attending school in rural western Kenya, reproductive tract infections affected more than one-quarter (28%) of those studied. The most prevalent condition was bacterial vaginosis, found in 18% of participants, followed by *Candida albicans* (9%), while *C. trachomatis, T. vaginalis*, and *N. gonorrhoeae* were detected in 3%, 3%, and 1% of participants, respectively.[9] Similarly, among AGYW in Mombasa, the most common infections were *C. trachomatis* (3.6%), followed by *N. gonorrhoeae* (1.6%), and *T. vaginalis* (0.7%).[10] Additional research in Eldoret found that among adolescents, 56% of females tested positive for at least one STI, with 14.8% testing positive for HIV.[8]

Female sex workers (FSWs) are recognized as a key population for STI research and surveillance due to their 30-fold higher HIV risk compared to other women and their critical role as bridging populations that facilitate STI transmission between high-risk networks and the general population.[11]

In Mombasa, one study reported HIV-1 seroprevalence of 30.6%, along with chlamydia (4.2%), gonorrhea (1.8%), and syphilis (2.0%).[12] Similarly, among 596 female sex workers in Nairobi, HIV prevalence was 29.5% (95% CI: 24.7–34.9), while bacterial STI prevalence was 3.1% for chlamydia, 1.1% for gonorrhea, and 0.9% for syphilis.[13] Longitudinal data covering 2009–2021 found that the HIV incidence rate among FSWs in Nairobi was 0.8 cases per 100 person-years, with a general decline in infections until 2020 and 2021, when rates increased again.[14] *Trichomonas vaginalis* infection remains consistently elevated, with 9.2% of FSWs in Nairobi testing positive at baseline and approximately 9% annual incidence among this population.[15] These findings demonstrate the substantial STI burden in Kenya, particularly among young women and those engaged in sex work, emphasizing the importance of comprehensive prevention and treatment strategies.

Health and Socioeconomic Impact of STIs

Sexually transmitted infections contribute to substantial morbidity and mortality. The impact on women's health is especially severe, with World Health Organization estimates indicating that STIs, including HIV, claim approximately one million women's lives annually across the continent.[16,17] Four curable sexually transmitted infections, chlamydia, gonorrhea, syphilis, and trichomoniasis, represent major causes of preventable morbidity and mortality globally, with particularly severe consequences for women's reproductive health.[18] While this dissertation focuses on the four curable bacterial and parasitic STIs, it is important to acknowledge that viral STIs such as HIV, HPV, and HSV also cause significant morbidity and mortality. However, the four curable STIs warrant particular attention due to their treatability and the potential for prevention through targeted interventions. Among women of reproductive age (15-44 years), STIs (excluding HIV) represent the second leading cause of healthy life years lost, accounting for about 17% of the total disease burden according to World Bank data.[16,17] The long-term effects of STIs could cause severe sexual and reproductive complications for cisgender women, including infertility, cancer, HIV acquisition, and pelvic inflammatory disease.[2,19-21] Research also demonstrates that individuals with STIs often experience mental health challenges, particularly depression and anxiety, mainly due to stigma, shame associated with symptoms, fear of disclosure to partners, and concerns about long-term reproductive consequences,,.[22] The consequences of STIs extend beyond individual health outcomes, impacting public health and straining healthcare systems. Sexually transmitted infection epidemics can overwhelm laboratory diagnostic capacity, require specialized clinical expertise for the management of complications, and necessitate extensive contact tracing and partner notification programs.[18] Healthcare systems must also address the increased HIV transmission risk associated with untreated STIs, as genital ulceration and inflammation facilitate HIV acquisition and transmission.[21,23] Further, the costs associated with STI management include diagnosis, treatment, and long-term care for complications, with approximately 17% of economic losses due to STIs occurring because of ill health and healthcare expenditures, particularly affecting adolescents, young adults and pregnant women.[24]Additionally, STIs perpetuate health disparities, disproportionately affecting marginalized populations and individuals with limited access to healthcare services.[25,26]

Challenges in STI Prevention and Control in the African context

Many STIs remain asymptomatic, meaning individuals may be infected without showing any symptoms, creating significant challenges for public health control efforts.[1,4] This challenge is particularly pronounced in African countries, where the STI burden is higher and access to diagnostic testing is limited. While countries with advanced economies have access to timely diagnostic testing and rapid treatment, resource-limited settings often rely on syndromic management as the standard of care.[27] This approach, which relies on treating visible symptoms rather than laboratory confirmation, has inferior sensitivity (<20%) compared to etiologic testing28 and a low positive predictive value (approximately 50%).[28,29] Consequently, asymptomatic infections frequently go undetected, potentially leading to prolonged periods without treatment and increasing the risk of complications and ongoing transmission.[30] The limitations of syndromic management, combined with the high prevalence of asymptomatic infections, highlight critical gaps in current prevention and control efforts and point toward the need for innovative, low-cost interventions to reduce the STI burden.

Introduction to Key Research Areas: Exploring Novel Prevention Approaches, Vaginal Health Dynamics, and Antimicrobial Resistance

Doxycycline post-exposure prophylaxis (doxyPEP) has emerged as the latest evidence-based STI prevention intervention, with proven efficacy in preventing bacterial sexually transmitted infections. The approach has been rigorously evaluated through randomized controlled trials and has demonstrated effectiveness in preventing chlamydia, syphilis, and gonorrhea in men who have sex with men (MSM) and transgender women.[31,32] The concept of STI prophylaxis using antibiotics has a long history, with several studies on single-dose or monthly antibiotics among female sex workers in Asia and Africa demonstrating reduced disease burden.[33-39] A study in France among gay men found a 47% reduction in new bacterial STIs among HIV pre-exposure prophylaxis (PrEP)users who took doxycycline after each sexual encounter.[40] A study conducted in the United States demonstrated that doxyPEP reduced the combined incidence of gonorrhea, chlamydia, and syphilis by two-thirds in MSM and transgender women, either on HIV pre-exposure prophylaxis (PrEP) or living with HIV. This approach was particularly effective in individuals with recent bacterial STIs, although some cases of tetracycline-resistant gonorrhea were observed.[32] Following these promising results in MSM and transgender women, doxyPEP has been approved for use in the United States and is now being implemented in clinical practice. However, the effectiveness of doxyPEP had not been evaluated among cisgender women. A separate randomized controlled trial was conducted to assess doxyPEP efficacy, specifically in cisgender women in Kenya, which found that doxyPEP did not prevent gonorrhea or chlamydia in this population. According to hair-sample analysis, the use of doxycycline PEP among those assigned to receive it was low, which likely contributed to the lack of observed efficacy.[41] Data from the Kenyan doxyPEP trial among cisgender women serve as the basis for two of the chapters of this dissertation.

There is a dire need to investigate and strengthen prevention strategies to curb STI transmission and reduce the substantial health burden among women in resource-limited settings.

Beyond individual prevention strategies, understanding the complex dynamics of vaginal health has become increasingly important. The interplay between different vaginal conditions may have significant implications for women's reproductive health outcomes.[42,43] The relationship between bacterial vaginosis (BV) and cervicitis, two common conditions affecting women's health, remains poorly understood despite their high prevalence.[44-46] Bacterial vaginosis affects approximately 23-29% of women of reproductive age globally,[47-49], while cervicitis has been reported in 20-40% of women attending reproductive health clinics, with rates varying by population and diagnostic criteria.[50-52]

Bacterial vaginosis, characterized by disruption of the healthy vaginal microbiota, and cervicitis, defined as inflammation of the cervix, may interact through complex immunological pathways, as changes in vaginal bacterial communities could influence cervical inflammation.[53,54] Cervicitis is particularly concerning as it can lead to serious reproductive health complications, including pelvic inflammatory disease, increased risk of HIV and other sexually transmitted infection acquisition, infertility, and adverse pregnancy outcomes such as preterm birth and low birth weight.[52,55,56] Additionally, cervicitis often serves as a gateway for ascending infections that can cause long-term damage to the reproductive tract. Understanding their independent relationship is crucial, as it could inform clinical practice and public health interventions. While previous research has examined the relationship between BV and cervicitis, studies have yielded conflicting results, and important methodological limitations have prevented a clear understanding of this association. Most prior studies have used small sample sizes, lacked comprehensive STI testing, or failed to distinguish between different vaginal microbiota states beyond a simple normal versus BV classification.[44,45,57,58]Further research is needed to explore the underlying mechanisms between these conditions and advance our approaches to protecting women's reproductive health.

Antimicrobial resistance in *Neisseria gonorrhoeae* has emerged as one of the most pressing challenges in global infectious disease control.[59,60] Since the introduction of antimicrobial therapy, *N. gonorrhoeae* has demonstrated a remarkable ability to develop resistance to virtually every antibiotic used for treatment, from sulfonamides in the 1940s to the current concerns about reduced susceptibility to extended-spectrum cephalosporins.[61-63] This pattern of emerging resistance poses a significant threat to public health, as untreatable gonorrhea infections can have severe, long-term consequences for reproductive and overall health.[64,65]

The World Health Organization designated *N. gonorrhoeae* as a "high priority" pathogen for research and development of new antibiotics, highlighting the urgent need to understand and address its resistance patterns.[66] Rising antimicrobial resistance threatens our ability to effectively treat these infections, making it crucial to monitor resistance patterns as we implement new prevention approaches, including doxyPEP.

Rationale for this Dissertation

Despite significant progress in STI research and prevention, critical gaps remain in our understanding of several key areas. While doxycycline post-exposure prophylaxis has shown promise in preventing bacterial STIs in specific populations, its efficacy against *T. vaginalis* remains unknown - a crucial question given both the high burden of *T. vaginalis* among cisgender women and increasing resistance to standard metronidazole treatment.[67-69] Although in vitro studies suggest doxycycline's potential as a treatment option,[70] its preventive efficacy has yet to be explored. Similarly, the association between bacterial vaginosis and cervicitis is not well understood despite both conditions being highly prevalent. The biological mechanisms linking them are not well understood, and the role of BV in contributing to cervical inflammation remains unclear. Further investigation into this association could clarify etiologic pathways and enhance clinical management strategies for women presenting with genital tract symptoms. Understanding this relationship could inform targeted interventions for reproductive health. Equally important is our limited understanding of antimicrobial resistance (AMR) patterns in *N. gonorrhoeae* within the African context, particularly in relation to doxyPEP. Examining AMR profiles both at baseline and following preventive interventions (for example, doxyPEP) is crucial for understanding how these patterns might evolve and for developing effective treatment and prevention approaches.

The convergence of prevention needs, vaginal health concerns, and AMR threats creates both challenges and opportunities. While new prevention tools offer hope for reducing infection rates, their implementation requires careful evaluation across multiple domains to ensure they provide a net benefit to individual and public health. This dissertation explores the complex landscape of STIs and vaginal health in Africa, with a particular focus on Kenya. The research examines four reproductive tract conditions of significant public health concern: *T. vaginalis*, *BV*, cervicitis, and *N. gonorrhoeae*. These conditions significantly impact women's reproductive health, contributing to a range of complications and posing substantial public health challenges. To address these gaps, this dissertation leverages data from the doxycycline PEP clinical trial in Kenya and the Mombasa Cohort Study.

Each chapter of this dissertation focuses on a specific research aim.

Aim 1: Evaluating the Impact of doxyPEP on Trichomonas vaginalis

Doxycycline PEP is a novel strategy to reduce bacterial STIs, but its impact on protozoal infections, such as *T. vaginalis*, remains unknown. This aim examines whether doxyPEP reduces incident *T. vaginalis* infections among cisgender women in Kenya. The study employs a secondary analysis of data from a randomized controlled trial, utilizing modified Poisson regression models to assess the risk of infection.

Aim 2: Characterizing AMR Patterns in *Neisseria gonorrhoeae*

Antimicrobial resistance in *N. gonorrhoeae* is a growing concern, with increasing resistance to first-line antibiotics. This aim examines the prevalence of genetic markers associated with resistance to tetracycline, macrolide, fluoroquinolone, and cephalosporin antimicrobial classes in *N. gonorrhoeae*-positive samples from Kenyan cisgender women. Using molecular approaches, the study investigates whether demographic, behavioral, and clinical factors, including doxyPEP use, are associated with the presence of these resistance markers.

Aim 3: Assessing the Association Between BV and Cervicitis

The relationship between BV and cervicitis remains ambiguous, with inconsistent findings in previous studies. This aim utilizes longitudinal data from the Mombasa Cohort Study to evaluate whether BV is independently associated with cervicitis, controlling for confounders such as STIs and sexual behaviors. The analysis employs generalized estimating equations (GEE) to account for repeated measures.

Looking Forward: Translational Potential of Dissertation Research

This dissertation takes a multi-faceted approach to advancing STI prevention by examining three interconnected areas. It advances the field by examining critical questions at the intersection of prevention, antimicrobial resistance, and vaginal health in Kenya. Through the evaluation of doxycycline as post-exposure prophylaxis, investigation of the bacterial vaginosis-cervicitis relationship, and investigation of gonococcal AMR patterns, this work provides insights crucial for developing more effective and evidence-based approaches to STI prevention that consider multiple factors simultaneously including novel prevention methods, host factors that influence infection risk, and resistance patterns that may limit treatment options. By examining these interconnected elements, this research contributes to understanding how prevention interventions perform in real-world settings, how vaginal health conditions may predispose women to STI acquisition, and how antimicrobial resistance patterns evolve in the context of new prevention strategies. These three projects, while distinct, inform each other in important ways. Together, these studies contribute to a more comprehensive understanding of STI prevention. By examining multiple aspects simultaneously - from direct prevention effects to vaginal health impacts to resistance implications - this work helps build a foundation for more effective and sustainable approaches to reducing STI burden.

The findings from this dissertation will inform multiple aspects of STI prevention and care in Kenya and similar settings. Evaluating the effectiveness of doxyPEP against *T. vaginalis* can inform guidelines on its use for broader STI prevention. Insights into the bacterial vaginosis-cervicitis relationship may lead to more effective approaches for maintaining vaginal health, while documentation of AMR will enhance our understanding of AMR patterns in this setting and inform both treatment and prevention strategies. This work provides data directly relevant to public health programming in high-burden settings by generating evidence in populations most affected by STIs. These findings may help identify optimal strategies for different populations, guide the implementation of new interventions, and inform future research directions in STI prevention and control.

CHAPTER 2

Title: *Trichomonas vaginalis* Among Cisgender Women Taking Doxycycline Postexposure Prophylaxis

ABSTRACT

Background

Trichomonas vaginalis, the most prevalent curable sexually transmitted infection (STI), disproportionately affects cisgender women, leading to reproductive complications and increased HIV acquisition risk. In vitro studies suggest that doxycycline may be a potential treatment; however, no study has explored the ability of doxycycline to prevent *T. vaginalis* infection.

Methods

We conducted an open-label randomized trial among 449 women (18-30 years) taking oral HIV preexposure prophylaxis (PrEP) in Kisumu, Kenya. Participants were randomized to doxycycline postexposure prophylaxis (dPEP), 200mg within 72 hours of condomless sex, or standard of care (SOC), quarterly STI screening and treatment. All participants were followed for 12 months with quarterly visits for STI testing, including *T. vaginalis* testing (Cepheid GeneXpert) and data collection on various health and behavioral parameters. The trial had over 90% power to detect a 50% reduction in incident *T. vaginalis* infections. An intention-to-treat analysis utilizing generalized estimating equations was performed.

Results

Baseline characteristics were similar in the dPEP (n=224) and SOC groups (n=225). Overall, participants had a median age of 24 years, reported a median of 2 sexual partners, and 36.7% engaged in transactional sex. Retention in our study was high at 97% of expected scheduled visits. There were 56 incident *T. vaginalis* infections: 27 in the dPEP group and 29 in the SOC group, contributed by 46 participants. There was no significant decrease in *T. vaginalis* incidence when comparing the dPEP group to the SOC group (RR: 0.96, 95% CI: 0.54-1.73, p=0.9). In an analysis censoring participants upon pregnancy (n=80), there was no notable decrease in incident *T. vaginalis* infection (RR: 1.07, 95% CI: 0.58-1.97). Subgroup analyses by age groups, hormonal contraceptive use, transactional sex, and STI detected at baseline revealed similar findings. In a subset of 50 participants assigned to dPEP, doxycycline was found in all quarterly hair samples for only 29% of participants.

Conclusions

Randomization to the doxycycline PEP group was not associated with a decreased incidence of *T. vaginalis* infections among cisgender women taking oral PrEP in Kenya. Low use of doxycycline may have contributed to this null result, highlighting the need to explore its role in preventing *T. vaginalis* in future research.

BACKGROUND

Trichomonas vaginalis is the most prevalent curable sexually transmitted infection (STI) globally, with an estimated incidence of 156 million cases worldwide in 2020.[67] The prevalence of *T. vaginalis* is higher in sub-Saharan Africa compared to other regions globally.[1] *T. vaginalis* frequently causes symptoms in the vagina and not other sites of exposure.[71] Infections can lead to complications such as preterm birth, low birth weight, and pelvic inflammatory disease (PID)[71] and may increase HIV susceptibility and infectivity.[72]

T. vaginalis is treated with 5-nitroimidazole class of medications, e.g., metronidazole and tinidazole. Increasing reports of metronidazole resistance pose challenges for successful treatment, leading to potential treatment failures and prolonged infections.[73] Increased *T. vaginalis* resistance to 5-nitroimidazole drugs prompted the revision of treatment guidelines to recommend extended therapy,[69,74] highlighting the need for novel treatment and prevention strategies. One in vitro study suggests that doxycycline, a tetracycline antibiotic, may treat *T. vaginalis* by inhibiting aminoacyl-tRNA synthetases and ferredoxin, thereby disrupting hydrogenosomal metabolism and inducing oxidative stress that leads to cell death.[70] Doxycycline postexposure prophylaxis (doxyPEP) using 200mg of doxycycline hyclate taken orally within 72 hours of condomless sex reduces incident bacterial STIs (*Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum*) among people who were assigned male sex at birth. [32,40]

The doxyPEP Kenya Study (dPEP Kenya) was conducted to assess the efficacy of doxyPEP in preventing *N. gonorrhoeae, C. trachomatis, and T. pallidum* infections among cisgender women. The study found no significant difference in incident infections between the doxyPEP and standard of care (SOC) groups in the setting of low use of doxycycline.[41] Nested in the doxyPEP Kenya Study, the present analysis is the first to evaluate the efficacy of doxyPEP against *T. vaginalis*, an important area of investigation beyond the trial's initial focus on bacterial STIs.

METHODS

We conducted an open-label randomized clinical trial in Kisumu, Kenya, between February 2020 and November 2022 (ClinicalTrials.gov number, NCT04050540). Methods for the trial were previously published.[41,75] Participants were between 18 and 30 years old, assigned female sex at birth, and had a current prescription for HIV pre-exposure prophylaxis (PrEP) according to the Kenya National Guidelines. Participants were excluded if they were pregnant, breastfeeding, allergic to tetracycline-class antibiotics, on medications that could interact with doxycycline, had used prolonged antibiotics the month before enrolment, or had active medical or psychiatric conditions that could interfere with study participation. The Kenya Medical Research Institute and the University of Washington Institutional Review Board approved the study. All participants provided written informed consent.

The doxyPEP group participants were instructed to take 200mg of doxycycline hyclate within 72 hours following each condomless sex act, not exceeding 200mg daily. Participants in the SOC group received quarterly STI screening and treatment only. All participants were followed for 12 months with quarterly STI testing and treatment if indicated, with doxyPEP discontinued during pregnancy and breastfeeding. Provider-collected vaginal swabs were tested for *T. vaginalis* using nucleic acid amplification testing (NAAT) using GeneXpert (Cepheid) or Aptima (Hologic Panther) systems. We collected data on concurrent medications, including metronidazole, utilized both as a standard treatment for *T. vaginalis* and for syndromic management of vaginitis symptoms while awaiting *T. vaginalis* testing results.

We conducted an intention-to-treat (ITT) analysis to compare the frequency of incident *T. vaginalis* infections over the 12-month study period among participants randomized to doxyPEP versus SOC. For this analysis, a subsequent *T. vaginalis* positive result was only considered a new infection if prior treatment was documented. We used a modified Poisson regression with generalized estimating equations and an exchangeable correlation structure to account for potential repeat infections and correlation of outcomes within individuals. We used the Wald test to assess the null hypothesis of no difference in *T. vaginalis* infection frequency over time between the doxyPEP and SOC group, considering a p-value < 0.05 statistically significant.

We conducted a survival analysis to examine the time to first *T. vaginalis* infection by study group employing Kaplan-Meier estimators and log-rank tests, maintaining the ITT approach. A sensitivity analysis was performed by censoring person-time following a positive pregnancy test. We also performed sub-group analyses to explore whether the intervention effect differed by age (18-24 years versus 25-30 years), hormonal contraceptive use, transactional sex, and STI detected at baseline. Lastly, we conducted an exploratory analysis examining the relationship between self-reported doxyPEP adherence and incident infections among participants assigned to doxyPEP who were not on medication hold. Adherence was based on responses to "*In the last 30 days, how many times did you miss taking doxyPEP as directed?*" (within 72 hours of sex) and categorized as either missing at least one dose or no missed doses. All analyses were performed using R software version 4.3.2.

RESULTS

The doxyPEP Kenya Study enrolled 449 participants and randomized 224 to the doxyPEP group and 225 to the SOC group. The study population has been described in detail.[41] In brief, the median age for both groups was 24 years, with 360 (80.2%) participants achieving a secondary school education or higher. Participants reported a median of 2 sexual partners in both groups at baseline. Overall, 165 (36.7%) participants were engaged in transactional sex.

The prevalence of *T. vaginalis* at baseline was 25 (5.6%). Retention was high, with 97% of scheduled visits completed as expected. Seven participants did not attend any follow-up visits and were excluded from the ITT analysis.

We observed 56 incident *T. vaginalis* infections (27 doxyPEP, 29 SOC) among 46 participants, occurring at 13.9 per 100 person-years. The percentage of participants with new infections in both groups ranged from 0.9% to 5.9% across quarters (Figure 1). Thirty-seven participants experienced one *T. vaginalis* infection, eight had two infections, and one acquired three. Three participants in the doxyPEP group and six in the SOC group experienced repeat infections.

There was no difference in *T. vaginalis* incidence in the doxyPEP group compared to the SOC group (Relative Risk [RR] = 0.96; 95% Confidence Interval [CI] = 0.54-1.73) (Table 1). There was no significant difference in time to the first *T. vaginalis* infection between the doxyPEP and SOC study groups (log-rank test p=0.7). During the study, 80 pregnancies were reported, with 44 occurring in the doxyPEP group and 36 in the SOC group. In the doxyPEP group, the medication was withheld during pregnancy, which accounted for 10.1% of follow-up visits. Pregnancies occurred during 6.0% of follow-up visits in the SOC group. A sensitivity analysis censoring post-pregnancy follow-up time showed no difference in incident *T. vaginalis* infection (RR = 1.07; 95% CI 0.58-1.97). Subgroup analyses across age, hormonal contraceptive use, transactional sex, and STI detected at baseline showed no significant differences in the incidence of *T. vaginalis* infections between the study groups.

As previously reported, in a subset of 50 participants assigned to doxyPEP, doxycycline was detected in quarterly hair samples for 29% (58/200) of visits.[41] In our analysis of self-reported adherence among all participants assigned to doxyPEP who were not on medication hold, most participants reported no missed doses (629/746, 84.3% of visits). We found no difference in infection rates between those who reported no missed doses and those who missed at least one dose (RR: 0.91, 95% CI: 0.30-2.73) (Table 1).

DISCUSSION

DoxyPEP for preventing protozoal infection of *T. vaginalis* is a novel application, as doxycycline is not an established treatment for *T. vaginalis*. Our study revealed no difference in incident *T. vaginalis* between cisgender women randomized to receive doxyPEP versus SOC. This finding persisted across multiple sub-group and sensitivity analyses.

The ineffectiveness of doxyPEP in preventing *T. vaginalis* may be explained by low use, as reported in the primary doxyPEP analysis.[41] Alternatively, doxyPEP may be ineffective in preventing *T. vaginalis* infections despite plausible biological mechanisms. The biological mechanisms by which doxycycline could potentially prevent *T. vaginalis* infections are not fully understood. While an in vitro study suggested that doxycycline may lead to *T. vaginalis* cell death, these findings may not translate to in vivo efficacy. Further, the 200mg doxyPEP dosage used in this study may not be efficacious at preventing the protozoal pathogen *T. vaginalis*. Higher dosages than those used for bacterial STIs may be required to prevent *T. vaginalis* infections, as the dosage needed for primary prevention remains unknown. Some studies suggest a possible reduced efficacy of doxycycline against *T. vaginalis*. For instance, a study found no therapeutic effect when treating *T. vaginalis* with doxycycline in three non-pregnant women.[76] While anecdotal, given the small sample size, this suggests limited doxycycline effectiveness against this pathogen at the administered dosage. Doxycycline, a key component of PID treatment, demonstrated limited efficacy against *M. genitalium* and *T. vaginalis* in a trial where women were administered the PID regimen and followed up for 90 days.[77] These limited clinical findings suggest that doxycycline may lack clinically meaningful activity against *T. vaginalis*. It is important to acknowledge that the dosage requirements for preventing *T. vaginalis* infections may differ from those used for treatment. Although prophylactic doxycycline has shown promise against bacterial STIs in other contexts—for example, a recent pilot study of daily doxycycline pre-exposure prophylaxis among Japanese female sex workers showed a two-thirds reduction in bacterial STIs without affecting rates of bacterial vaginosis or candidiasis[78]—these outcomes do not necessarily extrapolate to prophylaxis for other pathogens. This highlights that different organisms may require different prevention strategies and underscores the need for further research into *T. vaginalis* prophylaxis. Future research should explore the conflicting findings between in vitro activity and clinical outcomes and whether different dosing strategies could influence doxycycline's prophylactic potential against *T. vaginalis*.

Self-reported adherence data in our analysis indicated high use, with 84.3% of visits reporting no missed doses, though these measures can be subject to recall and social desirability bias. The event-driven nature of doxyPEP presents unique challenges for monitoring and evaluating adherence, as medication use should align with specific sexual behaviors rather than following a fixed schedule. This complexity in measuring adherence is illustrated by findings from the primary doxyPEP Kenya trial,[41] where, among a small subset of 50 participants assigned to doxyPEP, doxycycline was detected in only 29% of hair samples. While low detection may reflect periods without the need for medication, it could also indicate challenges in implementing event-driven prevention strategies. The current recommendations to withhold doxycycline during pregnancy due to lack of data highlight the need for future research to explore alternative or personalized preventive strategies that cater to the unique needs of people who might become pregnant.

Our study had several strengths, including the randomized controlled trial design, systematic NAAT testing for *T. vaginalis* detection, high retention rate, and comprehensive follow-up of participants over 12 months. Our results should be interpreted in the context of several limitations. First, the study did not have test-of-cure visits and relied on documented treatment to determine the incidence of *T. vaginalis* infections. This is particularly important for the ten participants who tested positive for *T. vaginalis* multiple times, as we cannot definitively distinguish between incident and persistent infections, potentially affecting our understanding of the true infection rates. Second, we relied on self-reported adherence data, which may be subject to social desirability bias and could lead to overestimation of medication use. This limitation impacts our ability to fully assess the relationship between adherence and infection outcomes. Finally, while our study provides important insights for cisgender women aged 18-30 using PrEP in Kisumu, Kenya, future studies should include people assigned female sex at birth across the gender spectrum who need STI prevention, as well as different age groups and geographic settings, to better understand the broader applicability of doxyPEP for STI prevention.

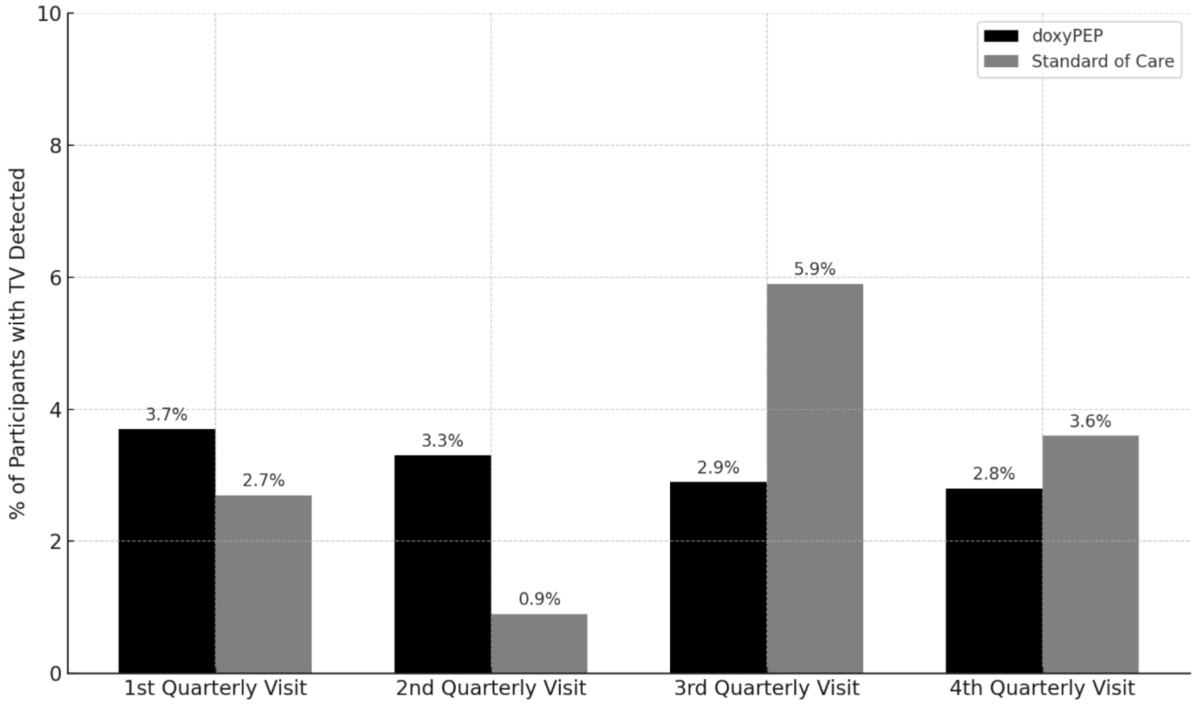
In conclusion, our study extends the ongoing exploration of doxyPEP for STI prevention. The evaluation of doxycycline, as well as other prevention strategies, against *T. vaginalis* remains an important area of investigation, given the high prevalence and significant health consequences associated with this often underrecognized STI. Developing novel prevention strategies is crucial in light of the growing resistance to current treatment options. Our findings highlight the critical need to optimize dosing strategies and explore alternative preventive approaches to reduce the global burden of this underrecognized yet consequential STI.

Table 1. Efficacy of doxyPEP on incident *T. vaginalis* in the intention-to-treat population and select sub-groups[a]

Analysis/subgroup	Incident infections in doxyPEP group		Incident infections Standard of Care arm		Relative Risk (95% CI)
Intention-to-Treat	27/849	(3.18%)	29/883	(3.28%)	0.96 (0.54-1.73)
Follow-up censored at first pregnancy [b]	24/741	(3.24%)	24/804	(2.99%)	1.07 (0.58-1.98)
STI at Baseline[C,d,e]					
None	18/658	(2.74%)	19/692	(2.75%)	0.99 (0.47-2.07)
STI at Baseline	9/191	(4.71%)	10/191	(5.24%)	0.90 (0.35-2.32)
Age Group					
18-24 years	20/512	(3.91%)	20/494	(4.05%)	0.96 (0.50-1.86)
25-30 years	7/337	(2.08%)	9/389	(2.31%)	0.89 (0.27-2.95)
Transactional Sex [c,e]					
None	9/367	(2.45%)	13/448	(2.90%)	0.84 (0.32-2.26)
Transactional Sex	9/335	(2.69%)	14/299	(4.68%)	0.57 (0.23-1.41)
Contraceptive use[c]					
None	6/286	(2.10%)	13/355	(3.66%)	0.56 (0.20-1.58)
Hormonal contraceptives[f]	21/563	(3.73%)	16/528	(3.03%)	1.23 (0.60-2.51)
Self-reported adherence (doxyPEP arm only)[g]					
Missed at least one dose	4/117	(3.42%)	**	**	1.00 (ref)
Did not miss any doses	19/629	(3.02%)	**	**	0.91 (0.30-2.73)

CI = Confidence Interval; doxyPEP = Doxycycline postexposure prophylaxis.
a The denominator represents the number of quarterly visits with test results.
b For the 'Pregnancy time censored' population, person-time stops at a positive pregnancy test, with no re-entry for later negative tests
c At baseline
d STI includes *T.vaginalis*, *C. trachomatis, N. gonorrhoeae, T. pallidum*
e Missing baseline data: Transactional sex (41 doxyPEP, 34 SOC), STI testing (0 doxyPEP, 1 SOC for *T. vaginalis, C. trachomatis, and N. gonorrhoeae*)
f Contraception includes IUD, implant, DMPA, and oral contraceptive pills
g Analysis restricted to doxyPEP arm participants during periods without medication hold. The referent group is "missed at least one dose"
** indicates not applicable as the analysis was conducted only in the doxyPEP arm

Figure 1. Quarterly distribution of incident *T. Vaginalis* by study group



CHAPTER 3

Title: *Neisseria gonorrhoeae* Antimicrobial Resistance Among Kenyan Women Enrolled in the Doxycycline Post-Exposure Prophylaxis Study.

ABSTRACT

Background

Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* poses a significant challenge globally. Data on AMR patterns in the context of doxycycline post-exposure prophylaxis (doxyPEP) in African settings are limited. We determine the prevalence of genetic markers associated with tetracycline, fluoroquinolone, and macrolide resistance in *N. gonorrhoeae* and identify associated demographic, behavioral, and clinical factors among cisgender women in the doxyPEP Kenya Study.

Methods

We conducted a cross-sectional analysis of 42 *N. gonorrhoeae*-positive endocervical swabs from 37 cisgender women in a randomized trial, comparing doxyPEP to the standard of care (SOC). Samples underwent genomic DNA extraction and PCR assays to detect genetic markers associated with resistance to tetracyclines (*tet*(M)), fluoroquinolones (*gyrA* S91F), macrolides (23S rRNA mutations, C2611T and A2059G), and decreased susceptibility to cephalosporins (*penA* N513Y). Modified Poisson regression with generalized estimating equations was used to assess factors associated with fluoroquinolone resistance.

Results

The *tet*(M) gene conferring high-level tetracycline resistance was detected in 100% of samples (95% CI: 92-100%), while fluoroquinolone resistance was present in 91% (95% CI: 77-97%). One sample demonstrated decreased susceptibility to cephalosporin, and no low-level and high-level macrolide resistance was detected. Resistance to two or more antimicrobial classes was observed in 91% of samples, with co-resistance to fluoroquinolones and tetracyclines being the most common pattern. No significant differences in resistance patterns were observed between doxyPEP and SOC groups (p=1.00, Fisher's Exact Test). We found no significant

associations between fluoroquinolone resistance and age, recent antibiotic use, number of sexual partners, or condomless sex.

Conclusion

The high prevalence of tetracycline and fluoroquinolone resistance in this population is consistent with other Kenyan studies. Despite universal tetracycline resistance, decreased cephalosporin susceptibility was minimal, and macrolide resistance was absent. No differences in resistance patterns were observed between study groups, although doxycycline use was low among those randomized to doxyPEP. Ongoing surveillance remains essential to monitor AMR trends in *N. gonorrhoeae*.

INTRODUCTION

The African continent bears the highest burden of incident *Neisseria gonorrhoeae* infection in the world.[1,66] Cisgender women in Africa have a higher prevalence (1.6%) of gonorrhea than men (1.2%), and the prevalence is even higher among women living with HIV.[1,79,80] Earlier studies in Kenya reported an *N. gonorrhoeae* prevalence of up to 6% among cisgender women.[12,81-83]Gonorrhea infections could lead to severe health consequences and significant economic burdens related to lost productivity and quality of life.[64,84]

Antimicrobial resistance (AMR), which refers to the resistance of bacteria, including *N. gonorrhoeae*, to antimicrobial drugs, is widely acknowledged as a global public health issue.[59,60,85] Ceftriaxone, an injectable antibiotic that belongs to the cephalosporin antimicrobial class, is currently the primary treatment for *N. gonorrhoeae* in most settings due to the widespread resistance of *N. gonorrhoeae* to oral antimicrobials previously recommended for gonorrhea treatment.[61,86,87] While cephalosporins such as ceftriaxone are still highly effective in treating *N. gonorrhoeae*, reports of reduced susceptibility and increasing concerns about the rise of ceftriaxone-resistant strains have been reported globally.[62,63,88] The actual extent of this problem is likely underestimated due to challenges with gonococcal antimicrobial susceptibility testing in many regions, making it an urgent threat.[89] While AMR is a worldwide concern, its effects could be more severe in Africa. In 2019, the continent experienced the highest number of fatalities related to AMR.[90] This may be due to the high burden of infectious diseases, under-resourced healthcare systems, limited diagnostic capacity, and challenges in regulating and monitoring the use of antimicrobial drugs.[90]

Data on *N. gonorrhoeae* AMR in Africa is scarce, mainly due to the absence of active surveillance for gonococcal AMR.[91,92] Research on factors associated with AMR in African contexts remains limited, though studies have identified associations with age, concurrent STIs, antibiotic usage, and sexual behaviors..[93-98] This knowledge gap is especially pronounced regarding the potential impact of emerging preventive intervention strategies (like doxyPEP) on resistance patterns across African populations.

In Kenya, instances of *N. gonorrhoeae* quinolone resistance have been reported, as well as tetracycline resistance.[99,100] In Kenya, as in many global settings, ceftriaxone and cefixime remain the empirical treatment options for *N. gonorrhoeae* infections. Continued monitoring of resistance patterns is essential, as resistant strains often exhibit cross-resistance mechanisms that affect multiple antimicrobial classes. For example, strains with resistance to one class of antibiotics frequently show elevated minimum inhibitory concentrations (MICs) to cephalosporins,[101] underscoring the importance of comprehensive resistance monitoring across antimicrobial classes even when certain drugs are no longer used for empirical treatment.

Doxycycline post-exposure prophylaxis (doxyPEP) is a new preventive strategy against bacterial STIs (chlamydia, syphilis, and gonorrhea). However, the increased use of antibiotics in populations can lead to heightened selection pressure on bacteria, promoting the emergence of resistant strains.[102] In the context of doxyPEP, the use of doxycycline to prevent *N. gonorrhoeae* infections among highly sexually active populations might inadvertently encourage the development of multidrug-resistant *N. gonorrhoeae* strains, making gonococcal infections more challenging to treat as resistance mechanisms can sometimes confer cross-resistance to other antimicrobial classes.[103,104]

The WHO emphasizes the potential of molecular methods for improving antimicrobial resistance surveillance in *N. gonorrhoeae* infections.[105] This study employs molecular methods to detect genetic markers associated with resistance to various antimicrobials, including tetracycline, macrolides, fluoroquinolones, and cephalosporins. It aims to provide a comprehensive understanding of the resistance profiles among the gonorrhea-positive samples collected from the Kenyan women enrolled in the Doxycycline Post-Exposure Prophylaxis (dPEP) Study.[41]

Our work aims to address important aspects of *N. gonorrhoeae* infections by determining the prevalence of AMR-associated genetic markers and identifying demographic, behavioral, and clinical factors that may be linked to these genetic markers. Our analysis also focuses on whether doxyPEP use influences AMR patterns in *N. gonorrhoeae* by comparing resistance prevalence between the doxyPEP and standard of care (SOC) groups across multiple time points. These data could potentially inform future strategies for surveillance, prevention, and management of drug-resistant gonorrhea, particularly in the context of increasing doxyPEP adoption.

METHODS

We conducted a cross-sectional analysis of *N. gonorrhoeae-positive* endocervical swab samples and data collected from the doxyPEP Kenya Study. Information about the population and intervention has been detailed in Chapter Two. The study population for this research consisted of cisgender women participating in the doxyPEP clinical trial who tested positive for *N. gonorrhoeae* at a quarterly screening test over the one-year study period. Eligibility was determined based on the detection of *N. gonorrhoeae* in endocervical swab samples by *Chlamydia trachomatis*/*N. gonorrhoeae* NAAT (GeneXpert, Cepheid).[106] Only samples with sufficient DNA quality and quantity for molecular testing were included in the analysis to ensure data integrity. Ultimately, 42 *N. gonorrhoeae-positive* endocervical swab samples met the eligibility criteria and were available for molecular analysis.

Each *N. gonorrhoeae-positive* sample identified during quarterly study visits was treated as an independent observation for analysis purposes. Given the structured testing schedule with three-month intervals between assessments, we considered each detected *N. gonorrhoeae* infection as a distinct event for prevalence purposes, as standard clinical protocols would lead to treatment upon detection, further reducing the possibility of persistent infections.

High-level tetracycline resistance was assessed by detecting the *tet*(M) gene using previously described PCR and cycling parameters.[107,108] Single-nucleotide polymorphisms (SNPs) associated with reduced susceptibility and resistance to cephalosporins (*penA* N513Y) and macrolide resistance (23S rRNA A2059G, C2611T) were detected using TaqMan™-based real-time PCRs..[109,110] and the commercially available ResistancePlus GC assay was used for detection of fluoroquinolone resistance (*gyrA* S91F). [111] Well-characterized positive and negative controls were included in all PCR and real-time PCR assays to ensure the validity of the results.[112]

Data used in this analysis included the study participants' demographics and surveys on sexual behavior and clinical care. Demographic factors included age, education level, marital status, employment status, independent income, and recent history of transactional sex. Behavioral surveys included the number of sexual partners in the past three months, frequency of sexual intercourse, and recent condom use. Clinical variables included recent antibiotic use in the past three months, group assignment (doxyPEP vs. SOC), and STI status at baseline. Molecular data consisted of the presence of genetic markers associated with *N. gonorrhoeae* AMR.

The primary outcome of interest was the presence of genetic markers associated with *N. gonorrhoeae* AMR across four antimicrobial classes. Resistance to tetracycline was defined by the presence of the *tet(*M) gene. Decreased susceptibility to cephalosporins was identified through single-nucleotide polymorphisms (SNPs) in the *penA* gene, specifically N513Y mutations. Fluoroquinolone resistance was determined by detecting the *gyrA* S91F mutation, while macrolide resistance was assessed by identifying mutations in the 23S rRNA gene at positions A2059G conferring high-level azithromycin resistance and C2611T mediating low-level azithromycin resistance. Each of these outcomes was treated as a binary variable, coded as zero if the resistance marker was absent and one if present.

Based on a review of prior literature[93,94,96-98] and considering our sample size constraints, we selected key demographic, behavioral, and clinical factors potentially associated with *N. gonorrhoeae* AMR as exposures of interest. These included age, condom use, number of sexual partners in the past three months, and recent antibiotic use, all of which have been previously linked to resistant *N. gonorrhoeae* strains in other populations.

Descriptive statistics were used to summarize the study participants' demographic, behavioral, and clinical characteristics. Categorical variables were presented as frequency distributions, while continuous variables were summarized using medians and interquartile ranges (IQR). The prevalence of each AMR genetic marker among the *N. gonorrhoeae*-positive samples was calculated. Given the small sample size, exact (Clopper-Pearson) 95% confidence intervals (CIs) were estimated for prevalence measures. We determined the presence of multiple resistance markers associated with at least two different antibiotic classes within the same sample, and its prevalence was also calculated. Due to the universal presence of tetracycline resistance markers (tet(M)) and the limited detection of other resistance patterns, we focused all subsequent analyses on fluoroquinolone resistance as our primary outcome, specifically the gyrA S91F mutation, which was highly prevalent in our samples.

Given the small sample size, Fisher's exact test was used to examine potential differences in the prevalence of genetic markers between participants in the doxyPEP and SOC groups. Comparisons were made at both baseline and follow-up, where follow-up was defined as any data collected after the baseline assessment. Additionally, a visualization approach was used to compare AMR prevalence at baseline and the last quarter of the study period by generating line plots stratified by doxyPEP and SOC groups.

The association between demographic, behavioral, and clinical factors and the presence of AMR genetic markers was assessed using modified Poisson regression with Generalized Estimating Equations (GEE) to account for within-participant clustering due to repeat infections. We specified an exchangeable correlation structure as our primary interest was population-averaged effects. In the bivariate analysis, unadjusted risk ratios (RRs) and 95% confidence intervals were estimated for each exposure variable, including age group (≤24 years vs. >24 years), condomless vaginal intercourse in the past three months (yes vs. no), number of sexual partners in the past three months (≤1 vs. >1), and recent antibiotic use in the past three months (yes vs. no). Given the small sample size, binary categorization of exposure variables was done to simplify interpretation and minimize overfitting. Our outcome of interest was the presence of *gyrA* S91F mutation, indicating fluoroquinolone resistance as a binary yes/no variable.

A multivariate analysis was performed to estimate adjusted associations while controlling for potential confounders. Despite our initial concerns about model stability, given the sample size, we were able to include all four exposure variables in the final model. The multivariate model estimated adjusted RRs and 95% confidence intervals for age group, condomless vaginal intercourse, number of sexual partners, and recent antibiotic use, with statistical significance defined as a p-value less than 0.05. All statistical analyses were conducted using R version 4.3.2 (R Core Team, 2023).

RESULTS

The study included a total of 37 participants who contributed *N. gonorrhoeae* samples for resistance testing. The median age was 25 years (IQR: 23-28) (Table 1). Most participants had completed secondary education (54.1%) or primary education (37.8%). The majority (67.6%) earned income, with 96.0% of employed participants working in the formal sector. Most participants (81.1%) reported having a primary sexual partner, and 54.8% reported engaging in transactional sex in the past three months. Nearly two-thirds (63.6%) reported not using condoms during their last vaginal sex act, and 73.0% were using contraception. At baseline, 21.6% of participants tested positive for *C. trachomatis*, 43.2% for *N. gonorrhoeae*, and 10.8% for *T. vaginalis*. No participants tested positive for *Treponema pallidum*.

We analyzed 42 *N. gonorrhoeae*-positive samples for genetic markers associated with antimicrobial resistance and resistance markers varied by antimicrobial class (Table 2). Fluoroquinolone resistance (*gyrA* S91F mutation) was detected in 90.5% (38/42, 95% CI: 77.4%–97.3%) of samples, indicating a high prevalence of ciprofloxacin resistance. In contrast, no samples tested positive for macrolide resistance markers (23S rRNA A2059G or rRNA C2611T mutations), suggesting no detectable low-level and high-level azithromycin resistance in the study population. One sample (2.63%, 95% CI: 0.1-13.8%) showed the *penA* 513Y mutation indicating decreased cephalosporin susceptibility. Notably, tetracycline resistance (*tet*(M) gene) was present in all 42 samples (100%, 95% CI: 92%–100%), reflecting widespread high-level tetracycline resistance.

Resistance to two or more antimicrobial classes was common. While all samples (100%) demonstrated tetracycline resistance through the presence of the *tet*(M) gene, only 9.5% of samples (95% CI: 2.7%–22.6%) showed resistance exclusively to tetracyclines without additional resistance markers. The majority (88.1%) exhibited resistance to both tetracyclines and one additional antimicrobial class (95% CI: 74.4%–96.0%) (Table 3). Only one sample (2.4%) showed resistance to three antibiotics, and no samples demonstrated resistance to all four antimicrobial classes. Co-occurrence of resistance markers was observed, with the most frequently observed co-occurrence being fluoroquinolone and tetracycline resistance, which was found in 90.5% of samples (Table 4).

Analysis of resistance markers across quarterly visits revealed that the prevalence of antimicrobial resistance markers was predominantly high throughout the study period, though resistance patterns differed by antibiotic class (Figure 1). Tetracycline resistance, indicated by *tet*(M), was detected in 100% of samples across all quarters, showing no variation over time. Fluoroquinolone resistance, measured by the *gyrA* S91F mutation, remained consistently high with slight fluctuation, ranging from 93.3% at baseline (Q0) to 85.7% in Quarter 3 before increasing to 100% in Quarter 4. In contrast, cephalosporin resistance, marked by the *penA* 513Y mutation, was detected only in Quarter 3, at a prevalence of 14.3%, with no cases observed in other quarters.

When comparing AMR prevalence between the doxyPEP and SOC groups, no significant differences were observed at either baseline or follow-up. For fluoroquinolone resistance (*gyrA* S91F mutation), prevalence at baseline was 88.9% in the doxyPEP group and 100% in the SOC group ($p = 1.000$) (Table 5). At follow-up, fluoroquinolone resistance remained stable at 88.9% in both groups. Similarly, for tetracycline resistance (*tet*(M) gene), 100% of samples in both groups were resistant at both baseline and follow-up, precluding statistical comparison (Table 6). The results were unremarkable when specifically examining fluoroquinolone resistance at baseline and the final quarter for the two study groups.

All participants with confirmed *N. gonorrhoeae* infections were treated according to the current guidelines with extended-spectrum cephalosporins. Follow-up testing after treatment demonstrated successful clearance of the infection in all cases, with 100% of participants having negative test-of-cure results (NAAT).

In bivariate analyses, age over 24 years showed a borderline association with lower prevalence of the *gyrA* S91F mutation (RR: 0.86, 95% CI: 0.75-1.00, $p=0.05$), while antibiotic use in the past three months showed a borderline association with higher prevalence (RR: 1.12, 95% CI:

1.00-1.25, p=0.05) (Table 6). Having multiple sexual partners (RR: 1.15, 95% CI: 0.92-1.44, p=0.22) and engaging in condomless sex (RR: 1.12, 95% CI: 0.82-1.52, p=0.48) showed positive but non-significant associations with fluoroquinolone resistance.

In the multivariate analysis adjusting for the same variables examined in the bivariate analysis (age, number of sexual partners, condomless sex, and antibiotic use), all associations with fluoroquinolone resistance were attenuated and not statistically significant. Age over 24 years maintained an inverse association (adjusted RR: 0.86, 95% CI: 0.73-1.02, p=0.08) while having multiple partners (adjusted RR: 1.14, 95% CI: 0.90-1.44, p=0.28), condomless sex (adjusted RR: 1.10, 95% CI: 0.82-1.47, p=0.53), and antibiotic use (adjusted RR: 1.15, 95% CI: 0.90-1.45, p=0.26) showed positive but non-significant associations with fluoroquinolone resistance (Table 6).

DISCUSSION

Our findings show important patterns of antimicrobial resistance in *N. gonorrhoeae* among cisgender women in Kenya enrolled in a doxyPEP trial. We observed a high prevalence of fluoroquinolone (90.5%) and tetracycline (100%) resistance among *N. gonorrhoeae*-positive samples. Resistance to macrolides was absent, and cephalosporin resistance was rare (2.6%). Despite minor temporal variations in AMR patterns, we observed a high burden of fluoroquinolone and tetracycline resistance at enrollment and throughout the study period, with only one sample exhibiting cephalosporin molecular resistance. All participants were successfully treated with cephalosporins on the test of cure, which indicated likely phenotypic susceptibility to cephalosporin. Co-resistance to fluoroquinolones and tetracyclines was common. There were no differences in resistance prevalence between the doxyPEP and SOC groups at either baseline or follow-up. We found no significant associations between fluoroquinolone resistance and age, recent antibiotic use, number of sexual partners, or condomless sex.

The universal presence of tetracycline resistance (100%) in our samples corroborates recent findings documenting predominant high-level tetracycline resistance in western Kenya,[100], as well as findings from other studies in the region.[92,113,114] This pattern appears consistent across Africa, with systematic reviews showing that tetracycline resistance in *N. gonorrhoeae* frequently exceeds 80% and can reach 100% in some settings.[114,115] According to a 2024 systematic review, there is a significant difference in the weighted pooled prevalence estimate of tetracycline resistance across regions ($p < 0.01$). Africa's rate (86%) far exceeds those in Asia (64%), South America (57%), and Europe/North America (26%), suggesting distinct regional resistance patterns potentially driven by differences in antimicrobial usage and control measures.[114] The detection of the *tet*(M) gene in all samples reinforces the magnitude of the problem in the Kenyan context and.[116]

The impact of doxyPEP on *N. gonorrhoeae* resistance is the most immediate and well-documented AMR concern. Other trials that have researched the effectiveness of doxyPEP have reported results on AMR in their studies. In the US DoxyPEP study, tetracycline resistance testing was available for only a small portion (17.2%) of gonorrhea diagnoses due to challenges with culture collection and recovery of *N. gonorrhoeae*, particularly with extragenital samples.[32] At baseline, tetracycline resistance was found in 27% (4/15) of *N. gonorrhoeae* isolates. Following randomization, tetracycline resistance was observed in 38% (5/13) of isolates from participants receiving doxycycline prophylaxis compared to 12% (2/16) in the SOC group. While the sample size is limited, these findings suggest the possibility that doxyPEP may select for tetracycline-resistant strains of *N. gonorrhoeae.*

In the French DOXYVAC trial, similar to our parent trial, baseline testing revealed tetracycline resistance in all *N. gonorrhoeae* isolates (n = 7).[117] During the study period, similar complete tetracycline resistance was observed across both arms, with all isolates from both the doxyPEP arm (n=21) and no-PEP arm (n=37) displaying tetracycline resistance. However, the proportion of isolates with high-level tetracycline resistance (MIC > 8 mg/L) was higher in the doxy-PEP group (33.3%) compared to the no-PEP group (18.9%).

In contrast to gonorrhea, the current risk of clinically significant doxycycline resistance emerging in *C. trachomatis* or *T. pallidum appears* low.[41,117] Doxycycline remains a highly effective treatment for chlamydia and syphilis, and resistance is considered rare or has not been reliably documented in clinical settings to date.[118,119] Studies analyzing isolates from doxyPEP trials have not detected resistance; for instance, no resistance was found in tested *C. trachomatis* strains from DOXYVAC or doxyPEP Kenya.[41,117] The differential impact of doxyPEP on resistance highlights that the AMR risk profile is not uniform across all target bacteria. The immediate and significant concern revolves around *N. gonorrhoeae,* whereas the risk for *C. trachomatis* and *T. pallidum* appears substantially lower based on current evidence.

The high prevalence of fluoroquinolone resistance (90.5%), as demonstrated by the presence of the gyrA S91F mutation, aligns with the continuing upward trend in resistance reported across East Africa. Our findings corroborate earlier work by Lagace-Wiens et al., who documented the emergence of fluoroquinolone resistance in Kenya, with rates ranging from 47.7% to 64.7% across different regions.[99] Other studies, including more recent surveillance data, suggest that this upward trajectory has continued, with resistance rates now exceeding 90% in Kenya.[120,121] The sustained high prevalence of fluoroquinolone resistance in our study likely reflects continued selective pressure from historical fluoroquinolone use in the region, as well as the potential spread of resistant strains through sexual networks.[99,122-124] Our findings support the current clinical recommendation against using fluoroquinolones for empiric therapy of gonorrhea[66] and highlight the ongoing need to monitor AMR patterns to guide local treatment approaches.

This study did not detect any macrolide resistance, which is encouraging. This finding is somewhat reassuring, as macrolides (most notably azithromycin) have remained a cornerstone of dual therapy for gonorrhea management in many regions worldwide.[20,66,125] However, it is important to note that macrolide resistance is emerging in other regions.. .[126] Our findings are consistent with previous research from Kenya, where prior studies have not detected azithromycin resistance,[92,99,115,127-129] though it should be noted that other investigations have identified limited resistance, with one[130] reporting 1.8% resistance and another[121] documenting low-level resistance to azithromycin (MICs> 0.05-2 mg/L) in approximately one-third of isolates, while another observed concerning increases in azithromycin MICs over time.

Our study revealed minimal evidence of cephalosporin resistance, which aligns with several previous Kenyan studies that have consistently reported low or absent resistance to ceftriaxone and cefixime.[92,99,113,121,127,129] Multiple investigations between 2002-2010 found no resistance to these agents,[99,127] though one documented concerning increases in MICs over time,[127] and a recent study identified low levels of resistance (0.9%) and intermediate resistance (2.7%) to ceftriaxone in western Kenya.[130] The low prevalence of cephalosporin resistance markers in our samples offers a measure of short-term reassurance, as extended-spectrum cephalosporins (particularly ceftriaxone) remain the primary recommended treatment for gonorrhea in most guidelines.[66,74] However, this finding should not diminish vigilance; the global threat of cephalosporin-resistant gonorrhea demands continued close surveillance. Even though our results suggest largely preserved cephalosporin susceptibility in this population, the potential emergence of even a single resistant isolate warrants ongoing monitoring, especially considering reports of increasing resistance in other regions worldwide.

Given the universal tetracycline resistance observed, the absence of macrolide resistance and minimal cephalosporin resistance in our samples is particularly reassuring. This finding suggests that despite widespread high-level tetracycline resistance conferred by the *tet*(M) gene, cross-resistance mechanisms have not significantly compromised the effectiveness of other critical antimicrobial classes in this population. [131] This compartmentalization of resistance is encouraging for current treatment approaches that rely on cephalosporins and, in some regions, macrolides. The preservation of susceptibility to these vital antimicrobial classes, even in the context of complete tetracycline resistance, provides valuable reassurance for current therapeutic strategies while emphasizing the importance of continued antimicrobial stewardship to maintain these treatment options.

The high prevalence of resistance to more than one class of antibiotics in our study (88.1%), particularly co-resistance to fluoroquinolones and tetracyclines, adds to the growing body of evidence documenting multidrug-resistant *N. gonorrhoeae* strains globally.[120,132,133] Co-resistance in *N. gonorrhoeae* can evolve through horizontal gene transfer and the accumulation of mutations, rendering many first-line antibiotics obsolete.[59,86] Such patterns of resistance complicate clinical management should cephalosporin resistance increase. These findings underscore the ongoing need to prioritize antibiotic stewardship and promote the judicious use of antibiotics.

There were no significant differences in resistance prevalence detected between participants randomized to the (doxyPEP) arm and those receiving SOC. However, this finding must be interpreted in the context of low objective use of doxycycline in a subset of participants in the parent doxyPEP trial. In addition, baseline resistance to doxycycline was 100%. Ongoing concerns persist about the potential long-term AMR implications of doxyPEP if implemented and adhered to more widely. [134,135] No macrolide resistance was detected, and only one instance of decreased cephalosporin susceptibility (detected in the doxyPEP group). However, given this single case, its clinical significance remains unclear. Importantly, the single case of decreased cephalosporin susceptibility detected was successfully treated with the standard cephalosporin regimen, with documented clearance of infection on the test of cure. This clinical outcome aligns with our molecular findings of minimal cephalosporin resistance and supports the continued effectiveness of cephalosporins as the recommended first-line treatment for gonorrhea in this population. Meanwhile, tetracycline resistance (*tet*(M)) was universal at baseline across both groups and remained unchanged throughout the study. Recent findings, however, demonstrate a concerning trend: tetracycline resistance in *N. gonorrhoeae* rose dramatically from 27% to 70% among men who have sex with men in King County, Washington, following the implementation of doxyPEP guidelines, with high-level tetracycline resistance increasing from 2% to 65% between 2021-2024. Moreover, taking more than three doses of doxyPEP per month was significantly associated with both tetracycline and high-level tetracycline resistance. [104] There remains a potential risk that the widespread use of prophylactic antibiotics could contribute to increased antimicrobial resistance over the long term. .[102,103] Larger-scale surveillance and longitudinal studies beyond our one-year timeframe will be necessary to fully assess any longer-term impact of prophylactic regimens on gonococcal resistance dynamics.

We observed a slightly decreased association with fluoroquinolone resistance in individuals over 24 years old, which contrasts with existing studies that mainly reported higher resistance in individuals 25 years and older, including those with fluoroquinolone resistance.[93,94,136,137] Similar to our study, these age-related associations were often attenuated after adjusting for other factors. Recent studies have shown a complex relationship between antibiotic use and antimicrobial resistance. While most investigations have found an association between recent antibiotic use and increased antimicrobial resistance in *N. gonorrhoeae*,[95-97] some studies have produced inconsistent results.[98] Our study showed a borderline significant association in bivariate analysis, but this relationship was attenuated after adjusting for other factors, similar to the varied findings in the existing literature, where associations often diminished in multivariable analyses.[97]

This study has several limitations that should be considered when interpreting the findings. First, our small sample size (n = 42) may have limited our ability to detect subtle differences in resistance patterns or weak associations with risk factors and may reduce generalizability. Second, our molecular approach to resistance detection, while highly specific for known genetic markers, cannot detect novel resistance mechanisms or phenotypic resistance without corresponding genetic determinants.[138,139] We evaluated the impact of a single penA mutation (N513Y) on predicting reduced susceptibility to cephalosporins in *N. gonorrhoeae*. However, the prediction of *N. gonorrhoeae* cephalosporin resistance or reduced susceptibility remains challenging due to the complex interplay of multiple resistance. While penA mutations are a key factor in decreased susceptibility across many strains, recent findings highlight the significant roles of other contributors, including mutations in the mtr promoter, loss-of-function changes in mtrC and mtrR, and alterations in RNA polymerase genes. Nonetheless, our study offers several notable strengths. First, we employed robust molecular methods in line with WHO recommendations to detect genetic markers of AMR, providing greater specificity than traditional phenotypic approaches. Second, we incorporated rigorous clinical trial procedures for specimen collection from a well-characterized population of cisgender women in Kenya, a region where AMR data for *N. gonorrhoeae* remains limited. Third, we captured a comprehensive snapshot of the resistance landscape by examining resistance markers across multiple antimicrobial classes.

This study provides valuable insights into the AMR landscape of *N. gonorrhoeae* in a Kenyan population participating in a doxyPEP trial. The widespread resistance to fluoroquinolones and tetracyclines highlights the importance of antibiotic stewardship at the population level, including the judicious use of antimicrobials and continued monitoring. While ceftriaxone and azithromycin remain highly susceptible, vigilant surveillance is essential to detect emerging resistance. We recommend implementing routine AMR monitoring with molecular screening for resistance determinants, particularly as interventions like doxyPEP expand. Future research priorities should include surveillance across diverse populations (including different age groups, genders, and socioeconomic contexts), investigation of novel resistance mechanisms, and development of alternative prevention and treatment strategies. As AMR continues to evolve, successful gonorrhea control hinges on strategic balance—expanding innovative prevention approaches while strengthening surveillance systems and promoting judicious antimicrobial use to preserve our most effective treatment options.

Table 1: Demographic and clinical characteristics of study participants

Characteristic	**N = 37**[1]
Age (median; IQR)	25 (23, 28)
Highest Level of Education (n, %)	
No Schooling	1 (2.7%)
Primary School	14 (37.8%)
Secondary School	20 (54.1%)
Post-Secondary School	2 (5.4%)
Earns Income (n, %)	25 (67.6%)
Employment Status (n, %)	
employment (formal)	24 (96.0%)
other	1 (4.0%)
(Missing)	12
Marital Status (n, %)	
Married	8 (21.6%)
Never married	20 (54.1%)
Previously married	9 (24.3%)
Has a Primary Sexual Partner (n, %)	30 (81.1%)
New Sex Partner (in the Past 3 Months) (n, %)	15 (40.5%)
History of Transactional Sex (in the Past 3 Months) (n, %)	17 (54.8%)
Condom Usage During Last Vaginal Sex (n, %)	12 (36.4%)
Contraceptive Use[a] **(n, %)**	27 (73.0%)
C. trachomatis **(n, %)**	8 (21.6%)
N. gonorrhoeae **(n, %)**	16 (43.2%)
T. pallidum **(n, %)**	0
T. vaginalis (n, %)	4 (10.8%)
[1]n (%)	

a Contraceptive methods include implants, oral birth control pills, depot medroxyprogesterone acetate (DMPA), and intrauterine devices (IUDs)

Table 2: Prevalence of genetic markers associated with *Neisseria gonorrhoeae* antimicrobial resistance [a,b]

Marker	Marker Description	Positive Samples	Negative Samples	Total Tested	Prevalence	95% CI
gyrA_S91F	(Ciprofloxacin Resistance)	38	4	42	90.5%	77.4-97.3
rRNA_C2611T	(Low-level Azithromycin Resistance)	0	42	42	0%	0-8.4
rRNA_A2059G	(High-level Azithromycin Resistance)	0	41	41	0%	0-8.6
penA_513Y	(decreased cephalosporin susceptibility)	1	37	38	2.6%	0.1-13.8
tet(M)	high-level tetracycline-resistance	42	0	42	100%	92-100

[a] Confidence intervals were calculated using the exact (Clopper-Pearson) method, which is particularly suitable for small sample sizes and provides conservative bounds for binomial proportions.
[b] Indeterminate results were excluded from prevalence calculations. The number of indeterminate results for each marker were: rRNA_A2059G (n=1) and penA_513Y (n=4).

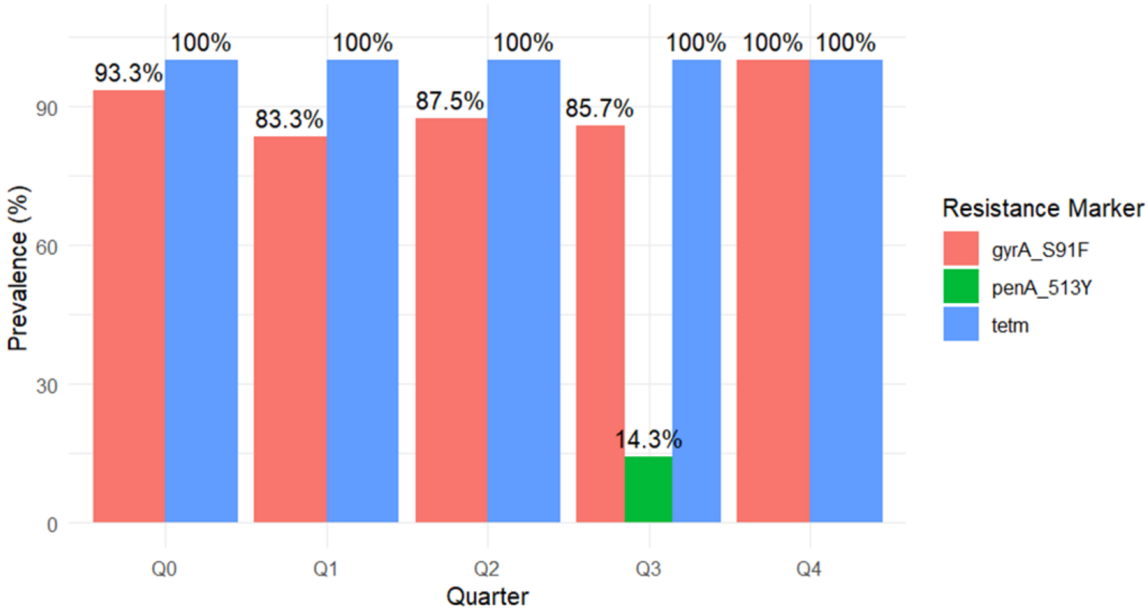
Table 3: Prevalence of multidrug resistance/decreased susceptibility(n=42)

Category	Number of Samples	Prevalence	95% CI
Resistance/ Decreased Susceptibility to 1 class	4	9.5%	2.7-22.6
Resistance/Decreased Susceptibility/ to 2 classes	37	88.1%	74.4-96.0
Resistance/Decreased Susceptibility/ to 3 classes	1	2.4%	0.1-12.6
Resistance/Decreased Susceptibility/ to 4 classes	0	0%	0-8.4

Table 4: Co-occurrence of resistance markers across antibiotic classes (counts)

Resistance_Class	Cephalosporin (penA)	Fluoroquinolone (gyrA S91F)	Macrolide (23S rRNA)	Tetracycline (tetM)
Cephalosporin (penA)	1	1	0	1
Fluoroquinolone (gyrA S91F)	1	38	0	38
Macrolide (23S rRNA)	0	0	0	0
Tetracycline (tetM)	1	38	0	42

Figure 1: Prevalence of resistance markers over quarterly visits[1]



[1] Q0 represents baseline measurements, while Q1-Q4 represent consecutive quarterly follow-up visits.

Table 5: Comparison of prevalence of fluoroquinolone (gyra s91f) resistance marker between doxycycline post-exposure prophylaxis and standard of care groups at baseline and follow-up

Time Point	Group	Count/Total	Prevalence (%)	P-value[a]
Baseline	Doxycycline Post-Exposure Prophylaxis	8/9	88.9	1.0
	Standard of Care	6/6	100	
Follow-up[b]	Doxycycline Post-Exposure Prophylaxis	16/18	88.9	1.0
	Standard of Care	8/9	88.9	

a Fisher's exact test
b Follow-up includes all visits post-baseline

Table 6: Comparison of prevalence of tetracycline (tet(m)) resistance marker between doxycycline post-exposure prophylaxis and standard of care groups at baseline and follow-up

Time Point	doxyPEP Group Count/Total	doxyPEP Group Prevalence (%)	SOC Group Count/Total	SOC Group Prevalence (%)	P-value[a]
Baseline	9/9	100	6/6	100	NA
Follow-up	18/18	100	9/9	100	NA

a Fisher's exact test

Table 6: Bivariate associations between exposure variables and the presence of fluoroquinolone (gyra s91f) resistance marker

Variable	Number of AMR Positive Samples / Total Samples	Relative Risk (95% CI)	P-value
Age			
≤24 years (Reference)	13/13 (100%)	1.00	-
>24 years	25/29 (86.21%)	0.86 (0.75 – 1.00)	0.05
Condomless vaginal sex (past 3 months)			
No (Reference)	9/11 (81.82%)	1.00	-
Yes	21/23 (91.30%)	1.12 (0.82 - 1.52)	0.48
Number of Sexual Partners (past 3 months)			
≤1 partner (Reference)	15/18 (83.33%)	1.00	-
>1 partner	23/24 (95.83%)	1.15 (0.92 - 1.44)	0.22
Any antibiotic use (past 3 months)[a]			
No (Reference)	34/38 (89.47%)	1.00	-
Yes	4/4 (100%)	1.12 (1 - 1.25)	0.05

a Excluding day of the visit

Table 7: Multivariate associations between exposure variables and presence of fluoroquinolone (gyra s91f) resistance marker

Variable	Relative Risk (95% CI)	P-value
Age		
≤24 years (Reference)	1.00	-
>24 years	0.86 (0.73 - 1.02)	0.08
Condomless vaginal sex (past 3 months)		
No (Reference)	1.00	-
Yes	1.10 (0.82 - 1.47)	0.53
Number of Sexual Partners (past 3 months)		
≤1 partner (Reference)	1.00	-
>1 partner	1.14 (0.90 - 1.44)	0.28
Any antibiotic use (past 3 months)[a]		
No (Reference)	1.00	-
Yes	1.15 (0.90 - 1.45)	0.26

a Excluding day of the visit

CHAPTER 4

Title: Association Between Vaginal Gram Stain Categories and Cervicitis in Kenyan Women Who Engage in Sex Work

ABSTRACT

Background

Cervicitis, an inflammatory condition of the cervix, is often caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC), but its etiology remains unexplained in approximately 50% of cases. Studies examining the relationship between the vaginal microbiota and cervicitis have yielded conflicting results.

Methods

Data were analyzed from an open cohort study of cisgender women who engage in sex work in Mombasa, Kenya (2004-2023). The primary aim was to examine associations between vaginal Gram stain categories and cervicitis. Vaginal microbiota was categorized using Nugent and Hillier criteria as normal (0-3), intermediate (4-6), or BV (7-10), and cervicitis as ≥30 polymorphonuclear cells per high-power field on cervical Gram stain. The association between *Lactobacillus* status (none detected, non-H_2O_2-producing, H_2O_2-producing) and cervicitis was also evaluated. Modified Poisson regression with generalized estimating equations was used to assess associations while adjusting for known or suspected confounders.

Results

There were 1,615 participants, contributing 25,322 visits. Cervicitis was present in 5.8% of visits with normal microbiota, compared to 9.1% with intermediate microbiota (relative risk [RR], 1.39 [95% confidence interval [140,141], 1.24–1.56]) and 6.8% with BV (RR, 1.14 [95% CI, 1.02–1.26]). Additionally, cervicitis was present in 5.1% of visits with no *Lactobacillus* detected, compared to 8.5% with non-H_2O_2-producing *Lactobacillus* (RR, 1.33 [95% CI, 1.18–1.50]) and 11.9% with H_2O_2-producing *Lactobacillus* (RR, 1.70 [95% CI, 1.52–1.90]). Results were similar in multivariable analyses and remained consistent across multiple sensitivity analyses.

Conclusion:

The findings from this study show that intermediate microbiota was associated with the highest risk of cervicitis, and a progressive increase in cervicitis risk was observed from women with no *Lactobacillus* to those with H_2O_2-producing *Lactobacillus*. Prior studies have shown that a *Lactobacillus*-dominated vaginal microbiota is associated with higher concentrations of CXCL10, while BV is associated with higher concentrations of a group of proinflammatory cytokines (but not CXCL10). Intermediate microbiota may be a state in which the combination of higher CXCL10 and other proinflammatory cytokines leads to more cervical inflammation. This hypothesis should be explored in future studies.

BACKGROUND

Bacterial vaginosis (BV) is the most common vaginal infection among cisgender women of reproductive age.[142] It is associated with multiple obstetric and gynecologic complications, including preterm birth,[143,144] spontaneous abortion,[145] sexually transmitted infections (STIs),[146] pelvic inflammatory disease (PID),[147,148] and human immunodeficiency virus (HIV) infection.[149,150] Bacterial vaginosis is characterized by a depletion of lactobacilli, responsible for maintaining a healthy acidic environment, and their replacement with predominantly anaerobic bacteria.[151,152] The presence of BV has consistently been associated with increased concentrations of the proinflammatory cytokine IL-1 beta.[153-155] Elevations of proinflammatory cytokines are concerning because they indicate active immune activation and inflammation in the genital tract, which can compromise epithelial barrier function, recruit inflammatory cells that may facilitate pathogen transmission, and create a local environment that increases susceptibility to STI acquisition.[30,140,156] The chronic inflammatory state associated with elevated IL-1 beta may also contribute to tissue damage and the various adverse reproductive health outcomes linked to BV.

While vaginal lactobacilli are generally considered beneficial, their relationship with vaginal health exists on a spectrum. *Lactobacillus crispatus* has been consistently associated with vaginal health,[157-159], whereas *Lactobacillus iners* has been less consistently associated with protective effects despite being common in women with a healthy vaginal microbiota.[160-162] A *Lactobacillus*-dominated vaginal environment is associated with increased concentrations of the proinflammatory chemokine interferon gamma-induced protein 10 (IP-10).[154,163-166]

Cervicitis is often caused by sexually transmitted infections (STIs), including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. However, the cause is unknown in 50% of cases.[45,167-169] Bacterial vaginosis and cervicitis are often detected concurrently, [45,57], and the relationship between these conditions has been examined in multiple studies.[44,45,57,58,170] A number of studies have explored the relationship between BV and cervicitis with mixed results. One study of women attending an STI clinic in the United Kingdom found that BV diagnosed by Amsel's criteria was associated with mucopurulent cervicitis, defined as visible yellow cervical secretions and >10 polymorphonuclear leukocytes (PMNs) per high-power field, even after adjusting for the presence of *C. trachomatis and N. gonorrhoeae*.[58] This finding suggests that BV may contribute to cervical inflammation through mechanisms independent of conventional STI pathogens. Additional evidence comes from a randomized trial conducted in Birmingham, Alabama, examining women with both BV (Amsel's criteria) and mucopurulent cervicitis attending an STI clinic. This study found that adding metronidazole gel to the standard doxycycline and ofloxacin treatment regimen was associated with higher cervicitis resolution than placebo.[45] On the other hand, some studies have not shown significant associations between BV and cervicitis. A study of women attending an STI clinic and a student health clinic in Seattle, Washington, found that while BV diagnosed by Amsel's criteria was initially associated with mucopurulent cervicitis, this association was no longer statistically significant after adjusting for *C. trachomatis.*[57] Similarly, a study of women engaged in sex work in Peru found only a modest, non-significant association between BV, defined based on positive Amsel's criteria combined with a positive Nugent score, and cervicitis, based on the presence of mucopus and friability.[170] Some other studies have also failed to find a significant association between BV and cervicitis.[169,171]

While previous studies have provided important insights into the relationship between vaginal Gram stain categories and cervicitis, these studies are limited by several factors. First, many previous studies have included modest sample sizes, making it difficult to comprehensively investigate the independent association between BV and cervical inflammation. Second, some early studies were conducted before the availability of nucleic acid amplification testing, raising the possibility that undetected STIs are contributing to the observed relationships. Third, most studies have approached vaginal microbiota as a binary comparison between "normal" and "BV" states, overlooking the potential importance of intermediate microbiota. The objective of the present analysis was to test the hypothesis that intermediate vaginal microbiota and BV are both independently associated with cervicitis in an extensive, carefully controlled, prospective cohort study.

METHODS

Study Design and Population

This study used data from the Mombasa Cohort, a prospective open cohort study of risk factors for HIV and STI acquisition and transmission among cisgender women engaged in transactional sex in Mombasa, Kenya.[172] The cohort enrolled women who are 16-60 years old, able to provide informed consent, self-identify as exchanging sex for cash or in-kind payment, and reside within commuting distance of the clinic. The study received ethical approval from the Kenyatta National Hospital Ethics and Research Committee and the University of Washington Human Subjects Review Committee. All participants provided written informed consent.

While the cohort was initiated in 1993, this analysis included data beginning in November 2004, corresponding to the implementation of nucleic acid amplification testing (NAAT) for improved STI diagnosis. Visits where women were pregnant, breastfeeding, living with HIV, or had undergone hysterectomy, were excluded, as these conditions could influence the main study variables of interest.

Data Collection

At enrollment and monthly follow-up visits, study nurses collected data on demographic characteristics, sexual behaviors, pregnancy history, contraceptive use, and medical history using standardized case report forms. Blood was collected for HIV testing according to Kenyan guidelines. A general physical examination, including a pelvic examination using a speculum, was performed. Signs and symptoms of genital tract infections were noted. Study clinicians collected cervical and vaginal fluid specimens, and diagnostic testing for STIs was performed. While participants were invited for monthly visits, NAATs were performed quarterly to detect *N. gonorrhoeae* and *C. trachomatis*. Syndromic management for STIs was provided as needed at each study visit. In addition, pathogen-directed treatment was provided for STIs detected during testing if they had not been covered under a syndromic approach.

Laboratory Assessments

Vaginal swabs were collected, heat-fixed, Gram-stained, and evaluated under microscopy according to the criteria of Nugent and Hillier.[173] Similarly, cervical swabs were collected, prepared, and examined microscopically by counting the number of PMNs in three non-adjacent high-power fields. Cervical or vaginal specimens for *N. gonorrhoeae* and *C. trachomatis* detection were analyzed using the Hologic APTIMA Combo-2 Assay.

For *Lactobacillus* assessment, vaginal samples were cultured on Rogosa selective medium to isolate *Lactobacillus* species. Isolates were subsequently tested for H_2O_2 production using tetramethylbenzidine agar containing horseradish peroxidase.

Exposure and Outcome Definitions

The primary exposure for this analysis was vaginal Gram stain categories: normal microbiota (0-3), intermediate microbiota (4-6), and BV (7-10). The three-category classification was chosen because it provides a more granular understanding of the vaginal microbiota rather than assuming that intermediate microbiota can be lumped with either the normal or BV category.

Lactobacillus status was evaluated as an additional exposure of interest, categorized into three levels based on laboratory assessments: no lactobacilli detected, non-H_2O_2-producing lactobacilli present, and H_2O_2-producing lactobacilli present. This classification allowed for the specific evaluation of the association between distinct *Lactobacillus* phenotypes and cervicitis.

The primary outcome was cervicitis, defined as the presence of ≥30 PMNs per high-power field in Gram-stained cervical smears. The ≥30 PMN cutoff has been widely used in previous research and was chosen because it represents a substantial inflammatory response consistent with the pathophysiology of cervicitis.

Statistical Analysis

Descriptive statistics were used to summarize and present the demographic and behavioral data, providing an overview of the study population's key characteristics and behaviors. The analysis examined vaginal Gram stain and cervicitis measurements taken at the same visit while leveraging repeated measures from multiple visits per participant to provide a comprehensive view of these conditions. This analysis also examined the relationship between vaginal *Lactobacillus* status and cervicitis.

Modified Poisson regression models with generalized estimating equations (GEE) were used to evaluate the relationship between vaginal Gram stain categories and cervicitis. This approach allowed the estimation of relative risks while accounting for correlated data from participants who contributed multiple visits. An exchangeable correlation structure was specified to handle repeated measurements.

The primary analysis included all available data, assuming negative results for *N. gonorrhoeae* and *C. trachomatis* when missing due to quarterly testing schedules. The model included the vaginal Gram stain category as the primary exposure (normal, intermediate, BV). The primary outcome was cervicitis (≥30 vs. <30 PMNs). A multivariable model was constructed to adjust for known or suspected potential confounders identified a priori including age (<25, 25-29, 30-34, 35-39, ≥40 years), educational level (≤8 vs.>8 years), workplace (bar, nightclub, other), number of sex partners in past week (≤1 vs >1), frequency of sex in past week (≤1 vs >1), condomless vaginal sex (yes/no), contraceptive use (none/condoms only, combined oral contraceptive pills, depot medroxyprogesterone acetate, IUD, tubal ligation, Norplant, other), vaginal washing (none, water only, soap or other substance), presence of *C. trachomatis,* and presence of *N. gonorrhoeae*. The same analytical approach was applied to evaluate the relationship between vaginal *Lactobacillus* status and cervicitis. A complete case analysis was performed since all variables had less than 5% missing data.

Three sensitivity analyses were conducted to assess the robustness of findings under different approaches to handling missing STI data: (1) creating analysis windows of ±45 days around each visit and imputing missing *N. gonorrhoeae* and C. *trachomatis* results based on the most recent available results, (2) restricting the analysis to visits with available *N. gonorrhoeae* and *C. trachomatis results*, and (3) further restricting to visits with available and negative *N. gonorrhoeae* and *C. trachomatis* results. All analyses were conducted using R statistical software version 4.3.1

RESULTS

Study Profile and Participant Demographics

Between November 1, 2004, and September 30, 2022, 1,615 participants contributed 25,500 visits to the Mombasa Cohort. At enrollment, the median age was 28 years, with an interquartile range (IQR) of 24–34 years, and the median number of years of education completed was 8 (IQR, 7–12 years). Most participants, 880 (55%), worked in bars, while 511 (32%) worked in nightclubs, with a median duration since their first experience of sex work of 2.0 years (IQR, 0.5–4.0 years) at the time of enrollment. Most participants were either widowed or divorced (55%) or had never married (43%). (Table 1).

Prevalence of Vaginal Gram Stain Categories, Cervicitis, and STIs

Overall, vaginal Gram stains identified normal microbiota at 54.3% (13,840/25,500), intermediate microbiota at 15.6% (3,967/25,500), and BV at 30.2% (7,693/25,500) of visits. Cervicitis was present at 6.7% (1,696/25,500) of visits. The visit-level prevalence of concurrent STIs was relatively low in this population, which receives regular screening and treatment every three months. *Chlamydia trachomatis* was detected at 3.2% (368/11,410), and *N. gonorrhoeae* at 1.9% (214/11,410) of quarterly visits.

Association Between Vaginal Gram Stain Categories and Cervicitis

Figure 1 illustrates the unadjusted and adjusted association between vaginal Gram stain category and cervicitis across 25,322 visits. This reflects our complete case analysis approach, which excluded visits with missing data for key variables in the analytical model. Cervicitis was present in 5.8% of visits with normal microbiota (796/13,739), compared to 9.1% with intermediate microbiota (359/3,946; relative risk [RR], 1.39 [95% confidence interval [174], 1.24–1.56]; $P < .001$) and 6.8% with BV (522/7,637; RR, 1.14 [95% CI, 1.02–1.26]; $P = .020$). Results were similar in multivariable analyses adjusted for age, education, workplace, recent sexual behavior, condom use, contraceptive method, vaginal washing practices, and the presence of *C. trachomatis* and *N. gonorrhoeae*.

Results were consistent across three sensitivity analyses conducted to evaluate the robustness of these findings: 1) imputing *C. trachomatis* and *N. gonorrhoeae* data from visits where testing was performed to visits without testing within a ±45-day window (*Table 2*); 2) restricting the sample to visits with complete *C. trachomatis* and *N. gonorrhoeae* results (*Table 3*); and 3) further restricting to visits with negative results for both infections (*Table 4*).

Association Between *Lactobacillus* Status and Cervicitis

Figure 2 shows the unadjusted and adjusted association between cultivable *Lactobacillus* status and cervicitis. Cervicitis was present in 5.1% (916/17,872) of visits with no *Lactobacillus* detected, compared to 8.5% (308/3,635) of visits with non–H_2O_2-producing *Lactobacillus* (RR, 1.33 [95% CI, 1.18–1.50]; $P < .001$), and 11.9% (440/3,685) of visits with H_2O_2-producing *Lactobacillus* present (RR, 1.70 [95% CI, 1.52–1.90]; $P < .001$). Results were similar in multivariable analyses adjusted for the same covariates described above.

As with the vaginal Gram stain categories, sensitivity analyses were performed to evaluate the robustness of the findings of an association between *Lactobacillus* culture status and cervicitis. Again, the results were similar to the primary analysis (*Tables 5–7*).

DISCUSSION

This study revealed a distinctive relationship between vaginal Gram stain categories and cervicitis among women in Kenya. While BV has previously been linked to cervicitis, the findings from this study make a unique contribution by demonstrating that women with intermediate microbiota had the highest risk of cervicitis, exceeding even those with BV. Equally striking was the progressive, step-wise increase in cervicitis risk when comparing women with no *Lactobacillus* to women with non-H2O2-producing *Lactobacillus* and H2O2-producing *Lactobacillus* detected by culture. Both relationships were robust in multiple sensitivity analyses, which used different methods to account for STI data. Collectively, these findings challenge conventional assumptions about the relationship between vaginal microbiota and cervical inflammation, suggesting a more complex interplay between bacteria traditionally associated with vaginal health and those linked to dysbiosis.

Previous investigations into the BV-cervicitis relationship have yielded inconsistent results.[45,58,170,171,175] Methodological differences in defining conditions may have contributed to conflicting findings in different studies. As described above, earlier studies from the United Kingdom, the U.S., and Peru primarily relied on Amsel's criteria for BV diagnosis.[57,58,170] While the study conducted in Peru also incorporated vaginal Gram stain scores, it did not isolate intermediate microbiota as a distinct category.[170] This methodological difference makes it difficult to compare directly to the present study. Only a few studies have examined cervicitis prevalence across all three vaginal Gram stain categories. One study of HIV-negative, non-pregnant cisgender women in India found that the prevalence of cervicitis (defined as mucopurulent discharge, easily induced endocervical bleeding, and >30 PMNs per HPF) was 44.1% in women with normal microbiota, 51.1% in those with intermediate microbiota, and 57.8% in women with BV.[176] However, these differences were not statistically significant. Our findings parallel those of another study from India.[177] Among women presenting with gynecological complaints, the frequency of cervicitis was 7.1% in women with normal microbiota, 10.9% in women with intermediate microbiota, and 7.7% in women with BV.

Previous research on the relationship between the presence of *Lactobacillus* species and cervicitis has produced mixed results. One found that among women aged 18-45 years seeking healthcare at women's health clinics and sexually transmitted disease clinics in Pittsburgh and Seattle, with BV (defined using a vaginal Gram stain score ≥4), the absence of H_2O_2-producing lactobacilli was a risk factor for cervicitis.[44] However, the study population included women with a more broadly defined BV category and did not include a group with normal microbiota, making direct comparison with the present findings challenging. Another study of women at a sexual health clinic in Australia demonstrated that women with STI-negative cervicitis are more likely to exhibit *Lactobacillus*-deficient vaginal community state types categorized by 16S rRNA gene sequencing,[178] but again, these results are difficult to compare to the present findings. There have also been case reports of lactobacillary endocervicitis, where overgrowth of usually beneficial strains (e.g., *L. gasseri*, *L. johnsonii*, *L. rhamnosus*) in the endocervical canal triggered inflammation.[179] In a case series from Switzerland, cervicitis resolved in 15 of 16 women following antibiotic treatment to suppress these lactobacilli, suggesting that even commensal bacteria can act as opportunistic pathogens when present in the 'wrong' location.

One possible explanation for why intermediate vaginal microbiota is associated with the highest risk of cervicitis is the combined proinflammatory stimuli produced by *Lactobacillus* species and anaerobic bacteria associated with vaginal dysbiosis. *Lactobacillus* species are associated with elevated levels of the proinflammatory chemokine IP-10.[154,163-166] In contrast, vaginal dysbiosis has been associated with increased levels of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α.[164,180,181] Future research should evaluate the cytokine and chemokine milieu associated with the intermediate microbiota category, which could provide further insight into the mechanism leading to cervical inflammation in this group.

This study offers several strengths. First, the large sample size provided substantial statistical power to differentiate the effects of normal, intermediate, and BV vaginal Gram stain categories. Second, multiple sensitivity analyses were conducted, demonstrating the robustness of the findings when varying the assumptions used to handle STI data. Third, NAATs were employed for STI diagnosis, offering much higher sensitivity than detection methods used in several earlier studies, making bias due to undetected STIs less likely in this analysis. Notably, analyses restricted to visits with no detectable STIs based on NAAT testing confirmed the relationship between vaginal Gram stain categories and cervicitis, a distinction not adequately addressed in studies that only adjusted for the presence of STIs as a confounding variable.

This study also had a number of limitations. First, this study design does not establish a causal relationship despite the consistent associations between intermediate microbiota, BV, and cervicitis. It remains plausible that cervicitis influenced the vaginal microbiota and that a third, unmeasured factor influenced both variables. Second, the use of microscopy and Lactobacillus culture alone to evaluate the vaginal microbiota did not allow for a detailed investigation of the specific bacteria associated with cervicitis. Third, while the Mombasa Cohort provides valuable insights into women engaged in transactional sex in Kenya, the findings may not be generalizable to all populations.

This study provides novel evidence that intermediate vaginal microbiota may confer a greater risk of cervicitis than BV and that cervicitis risk increases progressively with the presence of non–H_2O_2- and H_2O_2-producing lactobacilli. These findings challenge traditional assumptions about the protective role of lactobacilli and the inflammatory potential of BV, offering new insight into the complex relationship between the vaginal microbiota and cervical inflammation. By highlighting intermediate microbiota as a distinct and clinically relevant state, our results contribute to a growing body of literature calling for more nuanced models of cervicitis pathogenesis. Future research should aim to clarify the mechanisms underpinning this association and may ultimately inform targeted interventions to reduce cervicitis and its downstream sequelae.

Table 1: Demographic and baseline characteristics of study participants

Characteristic	**N = 1,615**[1]
Age (years)	
Median (IQR)	28 (24, 34)
Education (years)	
Median (IQR)	8 (7, 12)
Duration of sex work (years)	
Median (IQR)	2 (0.5, 4)
Number of Pregnancies	
Median (IQR)	2 (1, 3)
Average number of different partners (per week)	
Median (IQR)	3 (1, 5)
Frequency of sexual intercourse (per week)	
Median (IQR)	3 (2, 5)
Marital status	
never married	684 (42.7%)
currently married	40 (2.5%)
widowed/divorced	878(54.8%)
Workplace	
bar	880 (54.5%)
nightclub	511 (31.7%)
other	223 (13.8%)
Contraceptive use	
None/Condoms only	890 (55.2%)
Combined oral contraceptive pills	114 (7.1%)
Depot medroxyprogesterone acetate	340(21.1%)
IUD	38(2.4%)
Tubal ligation	15(0.9%)
Norplant	211 (13.1%)
Other	4(0.2%)
Vaginal washing	
none	118 (7.3%)
soap or other	881 (54.6%)
water only	616(38.1%)
Alcohol use (any)	
no	466 (28.9%)
yes	1,148 (71.1%)
Cigarette smoking (any)	
no	1,348 (83.8%)
yes	260 (16.2%)
Any condomless sex act (per week)	
no	1,259 (78.0%)
yes	356 (22.0%)
[1]n (%)	

Figure 1. Univariate and multivariate analysis of the association between vaginal gram stain categories and cervicitis

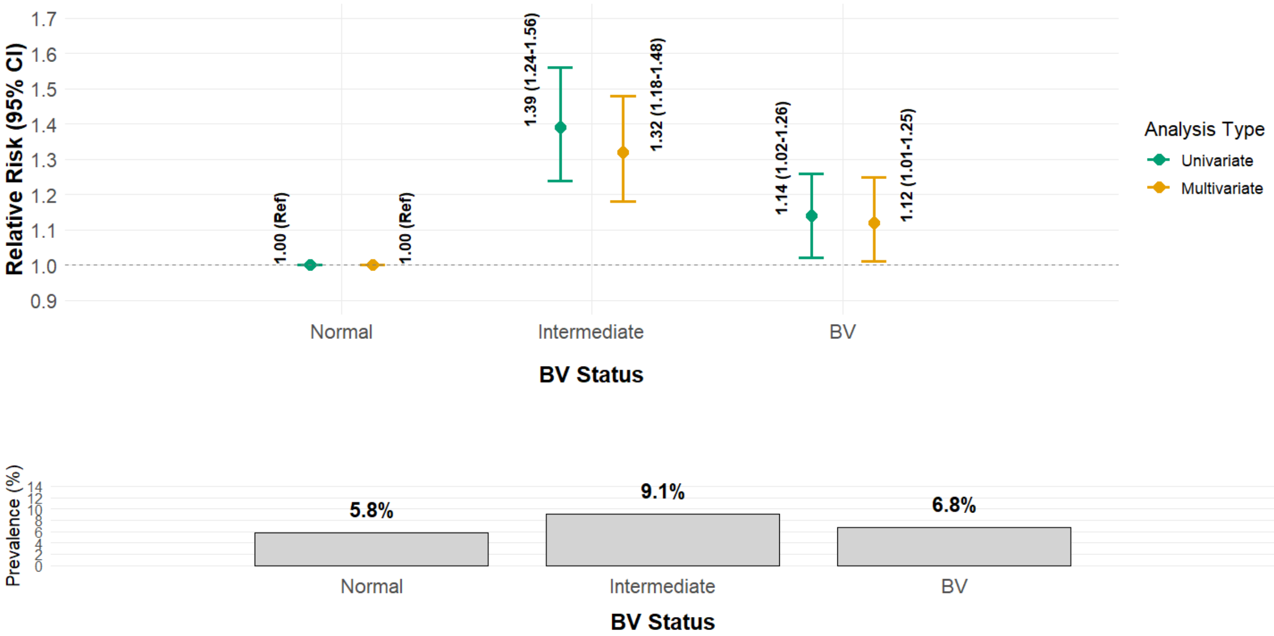


Figure 2. Univariate and multivariate analysis of the association between lactobacilli and cervicitis

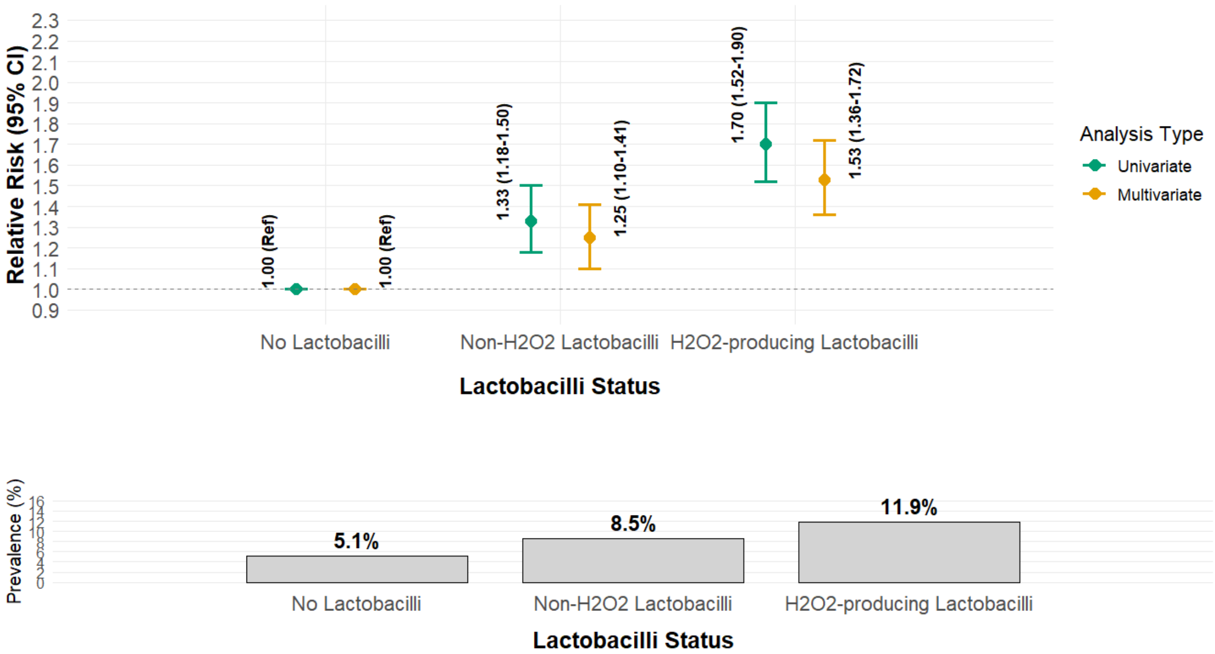


Table 2. Sensitivity analysis of the relationship between vaginal gram stain categories and cervicitis[a]

Variable	Number of Outcome Events/Visits(%)	Univariate RR (95% CI)	Univariate P-value	Multivariate RR (95% CI)	Multivariate P-value
BV status					
Normal (Reference)	648/10393 (6.2%)	1.00	-	1.00	-
Intermediate	316/3108 (10.2%)	1.42 (1.25-1.60)	<0.001	1.35 (1.19-1.53)	<0.001
BV	427/5717 (7.5%)	1.13 (1.01-1.27)	0.04	1.12 (1.00-1.26)	0.06
Age					
<25 (reference)	74/1087 (6.8%)	1.00	-	1.00	-
25-29	204/2823 (7.2%)	1.00 (0.70-1.42)	>0.99	1.03 (0.73-1.44)	0.87
30-34	279/3619 (7.7%)	1.25 (0.87-1.79)	0.23	1.30 (0.92-1.82)	0.14
35-39	306/3704 (8.3%)	1.51 (1.05-2.19)	0.03	1.61 (1.13-2.29)	<0.01
≥40	528/7985 (6.6%)	1.57 (1.10-2.25)	0.01	1.83 (1.30-2.56)	<0.001
Educational level (years)					
≤8 (reference)	417/7237 (5.8%)	1.00	-	1.00	-
>8	974/11981 (8.1%)	1.27 (1.04-1.54)	0.02	1.28 (1.07-1.54)	<0.01
Workplace					
Bar (reference)	757/10442 (7.2%)	1.00	-	1.00	-
Nightclub	443/6424 (6.9%)	0.80 (0.66-0.97)	0.03	0.82 (0.68-0.99)	0.04
Other	191/2352 (8.1%)	1.00 (0.75-1.32)	0.97	0.92 (0.70-1.20)	0.54
Number of sex partners					
≤1 (reference)	491/10234 (4.8%)	1.00	-	1.00	-
>1	900/8984 (10%)	1.61 (1.39-1.87)	<0.001	1.47 (1.23-1.77)	<0.001
Sex frequency					
≤1 (reference)	368/7880 (4.7%)	1.00	-	1.00	-
>1	1023/11338 (9%)	1.46 (1.28-1.67)	<0.001	1.10 (0.92-1.31)	0.28
Condomless vaginal sex					

No (reference)	1094/14999 (7.3%)	1.00	-	1.00	-
Yes	297/4219 (7%)	0.98 (0.86-1.10)	0.70	0.97 (0.85-1.11)	0.67
Contraceptive use					
None/Condoms only (reference)	773/12371 (6.2%)	1.00	-	1.00	-
OCP	52/810 (6.4%)	1.00 (0.72-1.37)	0.98	1.02 (0.75-1.40)	0.90
DMPA	266/3148 (8.4%)	1.24 (1.03-1.48)	0.02	1.27 (1.06-1.52)	<0.01
IUD	36/371 (9.7%)	1.43 (0.91-2.23)	0.12	1.46 (1.00-2.13)	0.05
Tubal ligation	9/495 (1.8%)	0.27 (0.11-0.69)	<0.01	0.30 (0.12-0.72)	<0.01
Norplant	252/1985 (12.7%)	1.67 (1.37-2.03)	<0.001	1.57 (1.31-1.88)	<0.001
Other	3/38 (7.9%)	1.30 (0.49-3.44)	0.60	1.33 (0.51-3.47)	0.56
Vaginal washing					
None (reference)	115/2833 (4.1%)	1.00	-	1.00	-
Water only	1003/11331 (8.9%)	1.69 (1.45-1.97)	<0.001	1.63 (1.38-1.94)	<0.001
Soap or other substance	273/5054 (5.4%)	1.09 (0.90-1.32)	0.37	1.11 (0.90-1.37)	0.35
C. trachomatis					
Negative (reference)	1315/18625 (7.1%)	1.00	-	1.00	-
Positive	76/593 (12.8%)	1.49 (1.17-1.90)	<0.01	1.50 (1.17-1.92)	<0.01
N. gonorrhoeae					
Negative (reference)	1355/18850 (7.2%)	1.00	-	1.00	-
Positive	36/368 (9.8%)	1.23 (0.93-1.63)	0.16	1.21 (0.92-1.60)	0.18

RR= relative risk; CI = confidence interval; OCP oral contraceptive pill; DMPA= depot medroxyprogesterone acetate; IUD intrauterine device.
a= All available data, imputing *N. gonorrhoeae* and *C. trachomatis* results within +/- 45 days when missing, and there are available test results within this window. (N=19,218 visits)

Table 3. Sensitivity analysis of the relationship between vaginal gram stain categories and cervicitis[b]

Variable	Number of Outcome Events/Visits	Univariate RR (95% CI)	Univariate P-value	Multivariate RR (95% CI)	Multivariate P-value
BV status					
Normal (Reference)	304/6005 (5.1%)	1.00	-	1.00	-
Intermediate	164/1820 (9%)	1.64 (1.38-1.95)	<0.001	1.53 (1.28-1.83)	<0.001
BV	230/3504 (6.6%)	1.26 (1.08-1.47)	<0.01	1.23 (1.05-1.45)	0.01
Age					
<25 (reference)	35/678 (5.2%)	1.00	-	1.00	-
25-29	99/1765 (5.6%)	1.08 (0.71-1.65)	0.72	1.15 (0.76-1.74)	0.51
30-34	143/2215 (6.5%)	1.31 (0.85-2.01)	0.22	1.45 (0.95-2.21)	0.08
35-39	174/2248 (7.7%)	1.69 (1.09-2.60)	0.02	1.94 (1.26-2.98)	<0.01
≥40	247/4423 (5.6%)	1.51 (0.99-2.30)	0.05	1.99 (1.31-3.01)	<0.01
Educational level (years)					
≤8 (reference)	205/4203 (4.9%)	1.00	-	1.00	-
>8	493/7126 (6.9%)	1.29 (1.03-1.61)	0.03	1.30 (1.05-1.61)	0.01
Workplace					
Bar (reference)	386/6204 (6.2%)	1.00	-	1.00	-
Nightclub	214/3759 (5.7%)	0.81 (0.65-1.01)	0.06	0.81 (0.65-1.01)	0.06
Other	98/1366 (7.2%)	1.02 (0.75-1.40)	0.89	0.92 (0.68-1.25)	0.60
Number of sex partners					
≤1 (reference)	243/6086 (4%)	1.00	-	1.00	-
>1	455/5243 (8.7%)	1.79 (1.51-2.13)	<0.001	1.69 (1.31-2.19)	<0.001
Sex frequency					
≤1 (reference)	184/4664 (3.9%)	1.00	-	1.00	-
>1	514/6665 (7.7%)	1.58 (1.33-1.87)	<0.001	1.03 (0.79-1.35)	0.81
Condomless vaginal sex					

No (reference)	541/8778 (6.2%)	1.00	-	1.00	-
Yes	157/2551 (6.2%)	1.00 (0.85-1.17)	0.96	1.01 (0.85-1.20)	0.93
Contraceptive use					
None/Condoms only (reference)	389/7388 (5.3%)	1.00	-	1.00	-
OCP	28/502 (5.6%)	1.03 (0.71-1.50)	0.87	1.06 (0.73-1.55)	0.76
DMPA	129/1857 (6.9%)	1.21 (0.96-1.53)	0.11	1.22 (0.97-1.53)	0.09
IUD	20/222 (9%)	1.53 (0.92-2.55)	0.10	1.47 (0.90-2.39)	0.12
Tubal ligation	4/246 (1.6%)	0.33 (0.09-1.18)	0.09	0.38 (0.12-1.22)	0.10
Norplant	126/1090 (11.6%)	1.84 (1.44-2.34)	<0.001	1.63 (1.30-2.06)	<0.001
Other	2/24 (8.3%)	1.40 (0.34-5.67)	0.64	1.34 (0.39-4.57)	0.64
Vaginal washing					
None (reference)	51/1421 (3.6%)	1.00	-	1.00	-
Water only	488/6665 (7.3%)	1.74 (1.40-2.15)	<0.001	1.62 (1.26-2.09)	<0.001
Soap or other substance	159/3243 (4.9%)	1.18 (0.92-1.53)	0.20	1.18 (0.88-1.58)	0.27
C. trachomatis					
Negative (reference)	650/10964 (5.9%)	1.00	-	1.00	-
Positive	48/365 (13.2%)	1.92 (1.46-2.53)	<0.001	1.87 (1.41-2.47)	<0.001
N. gonorrhoeae					
Negative (reference)	674/11117 (6.1%)	1.00	-	1.00	-
Positive	24/212 (11.3%)	1.79 (1.28-2.50)	<0.001	1.68 (1.20-2.37)	<0.01

RR= relative risk; CI = confidence interval; OCP oral contraceptive pill; DMPA= depot medroxyprogesterone acetate; IUD intrauterine device.

b= Limited to visits with available *N. gonorrhoeae* and *C. trachomatis* results (N=11,329 visits)

Table 4. Sensitivity analysis of the relationship between vaginal gram stain categories and cervicitis[c]

Variable	Number of Outcome Events/Visits	Univariate RR (95% CI)	Univariate P-value	Multivariate RR (95% CI)	Multivariate P-value
BV status					
Normal (Reference)	282/5758 (4.9%)	1.00	-	1.00	-
Intermediate	147/1726 (8.5%)	1.62 (1.35-1.93)	<0.001	1.53 (1.27-1.83)	<0.001
BV	201/3292 (6.1%)	1.22 (1.04-1.44)	0.02	1.21 (1.02-1.43)	0.03
Age					
<25 (reference)	30/599 (5%)	1.00	-	1.00	-
25-29	77/1623 (4.7%)	0.95 (0.60-1.50)	0.83	1.00 (0.63-1.58)	0.99
30-34	127/2087 (6.1%)	1.26 (0.79-2.00)	0.33	1.35 (0.85-2.13)	0.20
35-39	162/2144 (7.6%)	1.69 (1.06-2.70)	0.03	1.88 (1.18-2.98)	<0.01
≥40	234/4323 (5.4%)	1.51 (0.96-2.37)	0.07	1.87 (1.20-2.92)	<0.01
Educational level (years)					
≤8 (reference)	185/4034 (4.6%)	1.00	-	1.00	-
>8	445/6742 (6.6%)	1.29 (1.02-1.64)	0.03	1.30 (1.03-1.63)	0.02
Workplace					
Bar (reference)	345/5933 (5.8%)	1.00	-	1.00	-
Nightclub	199/3549 (5.6%)	0.86 (0.68-1.09)	0.21	0.86 (0.68-1.08)	0.19
Other	86/1294 (6.6%)	1.03 (0.73-1.44)	0.88	0.91 (0.65-1.26)	0.56
Number of sex partners					
≤1 (reference)	224/5857 (3.8%)	1.00	-	1.00	-
>1	406/4919 (8.3%)	1.77 (1.48-2.13)	<0.001	1.66 (1.28-2.17)	<0.001
Sex frequency					
≤1 (reference)	170/4496 (3.8%)	1.00	-	1.00	-
>1	460/6280 (7.3%)	1.57 (1.32-1.88)	<0.001	1.06 (0.81-1.40)	0.66
Condomless vaginal sex					
No (reference)	493/8357 (5.9%)	1.00	-	1.00	-
Yes	137/2419 (5.7%)	0.96 (0.81-1.14)	0.64	0.97 (0.80-1.16)	0.71

Contraceptive use					
None/Condoms only (reference)	358/7091 (5%)	1.00	-	1.00	-
OCP	28/483 (5.8%)	1.13 (0.77-1.64)	0.54	1.14 (0.79-1.66)	0.48
DMPA	112/1736 (6.5%)	1.17 (0.91-1.49)	0.21	1.20 (0.94-1.53)	0.14
IUD	19/210 (9%)	1.56 (0.92-2.66)	0.10	1.55 (0.94-2.53)	0.08
Tubal ligation	3/240 (1.2%)	0.30 (0.07-1.29)	0.11	0.32 (0.07-1.34)	0.12
Norplant	109/994 (11%)	1.80 (1.39-2.35)	<0.001	1.65 (1.28-2.12)	<0.001
Other	1/22 (4.5%)	0.77 (0.10-6.11)	0.81	0.80 (0.13-4.95)	0.81
Vaginal washing					
None (reference)	49/1382 (3.5%)	1.00	-	1.00	-
Water only	442/6330 (7%)	1.68 (1.37-2.08)	<0.001	1.58 (1.24-2.01)	<0.001
Soap or other substance	139/3064 (4.5%)	1.12 (0.86-1.45)	0.40	1.13 (0.85-1.50)	0.42

RR= relative risk; CI = confidence interval; OCP oral contraceptive pill; DMPA= depot medroxyprogesterone acetate; IUD intrauterine device.

c= Restricted to visits with available and negative *N. gonorrhoeae* and *C. trachomatis* results(N=10,776)

Table 5. Sensitivity analysis of the relationship between lactobacilli and cervicitis[d]

Variable	Number of Outcome Events/Visits (%)	Univariate RR (95% CI)	Univariate P-value	Multivariate RR (95% CI)	Multivariate P-value
Lactobacilli					
No Lactobacilli (Reference)	739/13132 (5.6%)	1.00	-	1.00	-
Lactobacilli present	258/2948 (8.8%)	1.30 (1.14-1.48)	<0.001	1.23 (1.07-1.40)	<0.01
H202 producing Lactobacilli	381/3020 (12.6%)	1.68 (1.49-1.89)	<0.001	1.53 (1.35-1.73)	<0.001
Age					
<25 (reference)	73/1077 (6.8%)	1.00	-	1.00	-
25-29	204/2806 (7.3%)	1.01 (0.70-1.44)	0.97	1.06 (0.76-1.49)	0.73
30-34	275/3606 (7.6%)	1.23 (0.85-1.77)	0.27	1.28 (0.91-1.80)	0.16
35-39	304/3678 (8.3%)	1.51 (1.04-2.20)	0.03	1.59 (1.13-2.25)	<0.01
≥40	522/7933 (6.6%)	1.57 (1.09-2.25)	0.02	1.78 (1.27-2.48)	<0.001
Educational level (years)					
≤8 (reference)	414/7196 (5.8%)	1.00	-	1.00	-
>8	964/11904 (8.1%)	1.26 (1.04-1.53)	0.02	1.25 (1.04-1.49)	0.02
Workplace					
Bar (reference)	753/10394 (7.2%)	1.00	-	1.00	-
Nightclub	437/6374 (6.9%)	0.80 (0.66-0.98)	0.03	0.83 (0.70-1.00)	0.05
Other	188/2332 (8.1%)	1.00 (0.75-1.32)	0.98	0.91 (0.70-1.18)	0.48
Number of sex partners					
≤1 (reference)	486/10187 (4.8%)	1.00	-	1.00	-
>1	892/8913 (10.0%)	1.62 (1.40-1.87)	<0.001	1.44 (1.20-1.73)	<0.001
Sex frequency					
≤1 (reference)	365/7848 (4.7%)	1.00	-	1.00	-
>1	1013/11252 (9.0%)	1.47 (1.29-1.67)	<0.001	1.10 (0.92-1.31)	0.29
Condomless vaginal sex					

No (reference)	1085/14907 (7.3%)	1.00	-	1.00	-
Yes	293/4193 (7.0%)	0.97 (0.86-1.10)	0.68	0.99 (0.87-1.13)	0.88
Contraceptive use					
None/Condoms only (reference)	763/12290 (6.2%)	1.00	-	1.00	-
Combined oral contraceptive pills	52/808 (6.4%)	1.00 (0.73-1.38)	0.98	1.04 (0.76-1.41)	0.83
DMPA	264/3132 (8.4%)	1.25 (1.04-1.49)	0.02	1.26 (1.06-1.50)	<0.01
IUD	36/370 (9.7%)	1.45 (0.93-2.26)	0.10	1.45 (1.02-2.06)	0.04
Tubal ligation	9/493 (1.8%)	0.27 (0.11-0.70)	<0.01	0.28 (0.12-0.68)	<0.01
Norplant	251/1969 (12.7%)	1.69 (1.39-2.05)	<0.001	1.53 (1.28-1.83)	<0.001
Other	3/38 (7.9%)	1.31 (0.49-3.46)	0.59	1.24 (0.46-3.35)	0.67
Vaginal washing					
None (reference)	114/2820 (4.0%)	1.00	-	1.00	-
Water only	993/11242 (8.8%)	1.68 (1.44-1.96)	<0.001	1.63 (1.38-1.94)	<0.001
Soap or other substance	271/5038 (5.4%)	1.08 (0.89-1.31)	0.43	1.12 (0.91-1.38)	0.30
C. trachomatis					
Negative (reference)	1302/18510 (7.0%)	1.00	-	1.00	-
Positive	76/590 (12.9%)	1.51 (1.18-1.92)	<0.001	1.53 (1.19-1.96)	<0.001
N. gonorrhoeae					
Negative (reference)	1342/18734 (7.2%)	1.00	-	1.00	-
Positive	36/366 (9.8%)	1.24 (0.93-1.65)	0.15	1.24 (0.93-1.66)	0.15

RR= relative risk; CI = confidence interval; OCP oral contraceptive pill; DMPA= depot medroxyprogesterone acetate; IUD intrauterine device.
d= All available data, imputing *N. gonorrhoeae* and *C. trachomatis* results within +/- 45 days when missing, and there are available test results within this window. (N=19,100 visits)

Table 6. Sensitivity analysis of the relationship between lactobacilli and cervicitis[e]

Variable	Number of Outcome Events/Visits (%)	Univariate RR (95% CI)	Univariate P-value	Multivariate RR (95% CI)	Multivariate P-value
Lactobacilli					
No Lactobacilli (Reference)	368/7820 (4.7%)	1.00	-	1.00	-
Lactobacilli present	145/1776 (8.2%)	1.44 (1.21-1.71)	<0.001	1.33 (1.11-1.59)	<0.01
H202 producing Lactobacilli	179/1680 (10.7%)	1.83 (1.55-2.16)	<0.001	1.64 (1.38-1.94)	<0.001
Age					
<25 (reference)	35/675 (5.2%)	1.00	-	1.00	-
25-29	99/1755 (5.6%)	1.08 (0.71-1.65)	0.72	1.17 (0.77-1.76)	0.47
30-34	141/2211 (6.4%)	1.29 (0.84-1.97)	0.25	1.41 (0.93-2.15)	0.11
35-39	173/2235 (7.7%)	1.67 (1.08-2.59)	0.02	1.92 (1.25-2.93)	<0.01
≥40	244/4400 (5.5%)	1.49 (0.98-2.27)	0.06	1.91 (1.26-2.89)	<0.01
Educational level (years)					
≤8 (reference)	204/4184 (4.9%)	1.00	-	1.00	-
>8	488/7092 (6.9%)	1.28 (1.02-1.61)	0.03	1.25 (1.01-1.55)	0.04
Workplace					
Bar (reference)	384/6180 (6.2%)	1.00	-	1.00	-
Nightclub	211/3737 (5.6%)	0.81 (0.64-1.01)	0.06	0.81 (0.65-1.00)	0.05
Other	97/1359 (7.1%)	1.02 (0.75-1.40)	0.89	0.91 (0.68-1.23)	0.55
Number of sex partners					
≤1 (reference)	241/6063 (4.0%)	1.00	-	1.00	-
>1	451/5213 (8.7%)	1.80 (1.51-2.14)	<0.001	1.65 (1.27-2.13)	<0.001
Sex frequency					
≤1 (reference)	183/4649 (3.9%)	1.00	-	1.00	-
>1	509/6627 (7.7%)	1.58 (1.34-1.88)	<0.001	1.03 (0.79-1.34)	0.83
Condomless vaginal sex					

No (reference)	537/8737 (6.1%)	1.00	-	1.00	-
Yes	155/2539 (6.1%)	1.00 (0.85-1.17)	0.95	1.03 (0.87-1.22)	0.72
Contraceptive use					
None/Condoms only (reference)	385/7351 (5.2%)	1.00	-	1.00	-
Combined oral contraceptive pills	28/501 (5.6%)	1.04 (0.71-1.51)	0.85	1.05 (0.73-1.53)	0.78
DMPA	128/1852 (6.9%)	1.21 (0.96-1.53)	0.10	1.19 (0.95-1.49)	0.13
IUD	20/221 (9.0%)	1.57 (0.94-2.60)	0.08	1.51 (0.95-2.42)	0.08
Tubal ligation	4/245 (1.6%)	0.33 (0.09-1.18)	0.09	0.37 (0.12-1.19)	0.10
Norplant	125/1082 (11.6%)	1.85 (1.45-2.36)	<0.001	1.57 (1.24-1.98)	<0.001
Other	2/24 (8.3%)	1.40 (0.35-5.69)	0.64	1.29 (0.35-4.72)	0.70
Vaginal washing					
None (reference)	50/1415 (3.5%)	1.00	-	1.00	-
Water only	484/6624 (7.3%)	1.75 (1.41-2.18)	<0.001	1.67 (1.29-2.17)	<0.001
Soap or other substance	158/3237 (4.9%)	1.19 (0.92-1.54)	0.19	1.25 (0.93-1.68)	0.14
C. trachomatis					
Negative (reference)	644/10911 (5.9%)	1.00	-	1.00	-
Positive	48/365 (13.2%)	1.93 (1.47-2.54)	<0.001	1.94 (1.46-2.58)	<0.001
N. gonorrhoeae					
Negative (reference)	668/11065 (6.0%)	1.00	-	1.00	-
Positive	24/211 (11.4%)	1.80 (1.29-2.52)	<0.001	1.70 (1.20-2.40)	<0.01

RR= relative risk; CI = confidence interval; OCP oral contraceptive pill; DMPA= depot medroxyprogesterone acetate; IUD intrauterine device.

e= Limited to visits with available *N. gonorrhoeae* and *C. trachomatis* results (N=11,276 visits)

Table 7. Sensitivity analysis of the relationship between lactobacilli and cervicitis[f]

Variable	Number of Outcome Events/Visits (%)	Univariate RR (95% CI)	Univariate P-value	Multivariate RR (95% CI)	Multivariate P-value
Lactobacilli					
No Lactobacilli (Reference)	320/7437 (4.3%)	1.00	-	1.00	-
Lactobacilli present	135/1686 (8.0%)	1.54 (1.28-1.85)	<0.001	1.41 (1.16-1.70)	<0.001
H202 producing Lactobacilli	169/1601 (10.6%)	1.98 (1.67-2.35)	<0.001	1.76 (1.48-2.10)	<0.001
Age					
<25 (reference)	30/596 (5.0%)	1.00	-	1.00	-
25-29	77/1614 (4.8%)	0.95 (0.60-1.50)	0.83	1.01 (0.64-1.60)	0.96
30-34	125/2083 (6.0%)	1.23 (0.77-1.95)	0.38	1.30 (0.83-2.05)	0.25
35-39	161/2131 (7.6%)	1.67 (1.05-2.68)	0.03	1.84 (1.17-2.91)	<0.01
≥40	231/4300 (5.4%)	1.49 (0.94-2.34)	0.09	1.78 (1.14-2.77)	0.01
Educational level (years)					
≤8 (reference)	184/4016 (4.6%)	1.00	-	1.00	-
>8	440/6708 (6.6%)	1.29 (1.01-1.63)	0.04	1.24 (0.99-1.55)	0.06
Workplace					
Bar (reference)	343/5909 (5.8%)	1.00	-	1.00	-
Nightclub	196/3528 (5.6%)	0.86 (0.68-1.08)	0.20	0.86 (0.69-1.07)	0.18
Other	85/1287 (6.6%)	1.03 (0.73-1.45)	0.88	0.90 (0.65-1.25)	0.53
Number of sex partners					
≤1 (reference)	222/5834 (3.8%)	1.00	-	1.00	-
>1	402/4890 (8.2%)	1.78 (1.48-2.14)	<0.001	1.60 (1.22-2.09)	<0.001
Sex frequency					
≤1 (reference)	169/4481 (3.8%)	1.00	-	1.00	-
>1	455/6243 (7.3%)	1.57 (1.32-1.88)	<0.001	1.06 (0.81-1.39)	0.68
Condomless vaginal sex					

No (reference)	489/8316 (5.9%)	1.00	-	1.00	-
Yes	135/2408 (5.6%)	0.96 (0.81-1.13)	0.63	1.00 (0.83-1.20)	0.96
Contraceptive use					
None/Condoms only (reference)	354/7055 (5.0%)	1.00	-	1.00	-
Combined oral contraceptive pills	28/482 (5.8%)	1.13 (0.78-1.65)	0.51	1.14 (0.79-1.65)	0.49
DMPA	111/1731 (6.4%)	1.17 (0.92-1.50)	0.20	1.16 (0.92-1.48)	0.21
IUD	19/209 (9.1%)	1.60 (0.95-2.72)	0.08	1.62 (1.02-2.56)	0.04
Tubal ligation	3/239 (1.3%)	0.30 (0.07-1.30)	0.11	0.31 (0.07-1.33)	0.11
Norplant	108/986 (11.0%)	1.82 (1.40-2.37)	<0.001	1.56 (1.21-2.01)	<0.001
Other	1/22 (4.5%)	0.78 (0.10-6.14)	0.81	0.73 (0.11-4.66)	0.74
Vaginal washing					
None (reference)	48/1376 (3.5%)	1.00	-	1.00	-
Water only	438/6290 (7.0%)	1.70 (1.37-2.10)	<0.001	1.63 (1.28-2.08)	<0.001
Soap or other substance	138/3058 (4.5%)	1.12 (0.86-1.46)	0.39	1.20 (0.90-1.60)	0.22

RR= relative risk; CI = confidence interval; OCP oral contraceptive pill; DMPA= depot medroxyprogesterone acetate; IUD intrauterine device.

f= Restricted to visits with available and negative *N. gonorrhoeae* and *C. trachomatis* results(N=10,724)

CHAPTER 5: Discussion

This dissertation examines three complementary studies addressing critical challenges in STI prevention, epidemiology, and treatment among cisgender women in Kenya. These studies span the potential role of doxyPEP in STI prevention, the complex relationship between vaginal microbiota and cervicitis, and the urgent threat of antimicrobial resistance in *Neisseria gonorrhoeae*. Collectively, they underscore the importance of ongoing surveillance, innovative prevention strategies, and a more comprehensive understanding of the interplay between host factors, pathogens, and interventions.

The first study, a secondary analysis of data from a randomized trial, evaluated doxyPEP against *Trichomonas vaginalis* infection and found no significant protective effect. This study also highlights substantial adherence challenges in implementing event-driven prophylaxis, as indicated by hair samples, which show lower doxycycline utilization among participants randomized to doxyPEP compared to self-reported adherence. This discrepancy highlights implementation barriers in real-world settings. The second study investigated antimicrobial resistance in *N. gonorrhoeae*, documenting universal tetracycline resistance alongside a high prevalence of fluoroquinolone resistance while susceptibility to extended-spectrum cephalosporins and macrolides remained largely preserved. These findings carry important public health and clinical implications for STI prevention strategies in women, reinforce current treatment guidelines, and underscore areas for future research and surveillance. The third study, a longitudinal cohort analysis of female sex workers, revealed an unexpected association between vaginal microbiota composition and cervical inflammation: women with intermediate vaginal microbiota had the highest risk of cervicitis, exceeding even the risk observed with bacterial vaginosis (BV). Intriguingly, the presence of hydrogen peroxide (H_2O_2)-producing *Lactobacillus* species – typically considered markers of vaginal health – was linked to the greatest risk of cervicitis.

Making a case for the under-recognized STI

As detailed in Chapter 2, *T. vaginalis* remains the most prevalent curable sexually transmitted infection globally, with an estimated 156 million new cases in 2020 alone—a burden exceeding both chlamydia and gonorrhea infections combined.[18] Globally, approximately 5% of women are infected with *T. vaginalis* at any time, with women affected ten times more frequently than men.[182,183] Sub-Saharan Africa bears the highest burden of *T. vaginalis*, with an estimated 42.8 million women carrying the parasite in this region alone.[182,183] Despite its association with serious adverse outcomes, including preterm birth, low birth weight, pelvic inflammatory disease, and an increased risk of HIV acquisition, *T. vaginalis* has been systematically neglected in global health initiatives.[71,72] Unlike HIV or Human Papillomavirus (HPV), trichomoniasis is frequently omitted from routine screening programs and is not a reportable condition in many countries.[184,185]. The CDC only recommends routine screening for women living with HIV, reflecting a historical pattern of underrecognition.[185] This neglect stems from policy decisions based on clinical, economic, and social factors that have systematically marginalized trichomoniasis in public health priorities. The primary barrier to universal screening is that up to 85% of *Trichomonas vaginalis* infections are asymptomatic, leading to the perception that it is not a serious health threat.[185] While trichomoniasis causes adverse pregnancy outcomes and increases HIV acquisition risk, these consequences have not elevated it to the same priority level as other STIs.[185-187] Cost-effectiveness analyses question population-wide screening for a largely asymptomatic, easily curable infection when public health resources are finite.[188] The CDC explicitly states there is insufficient evidence that screening asymptomatic individuals would significantly reduce adverse health events or community disease burden.[119] Arguments against making trichomoniasis reportable center on practical implementation challenges. Mandatory reporting would burden already strained public health departments with data management and follow-up responsibilities, while potentially increasing STI-related stigma that could deter individuals from seeking testing or treatment.[189,190] Public health officials argue that targeted screening in high-prevalence settings like STD clinics is more resource-efficient than establishing national surveillance systems.[119]

This systematic neglect reflects deeper structural inequalities. Trichomoniasis disproportionately affects marginalized communities, particularly Black women, who experience prevalence rates nearly ten times higher than white women.[189] The historical pattern of underinvestment in diseases affecting minority and low-income populations, combined with trichomoniasis's asymptomatic nature and perception as low-priority, has created a self-perpetuating cycle of underrecognition and underfunding that persists today.

While substantial progress has been made in preventive interventions for other STIs, research specifically targeting *T. vaginalis* prevention remains extremely limited. Control efforts for trichomoniasis have traditionally relied on case management – diagnosing and treating infected women and their partners – rather than proactive prophylaxis.[119,191,192] In contrast, for bacterial STIs like syphilis and chlamydia, recent studies have explored antibiotic prophylaxis to curb incidence. For example, doxyPEP has demonstrated efficacy in men who have sex with men (MSM) and has the potential to significantly reduce bacterial STI incidence in this population, with real-world data showing reductions.[32,40,174,193-195] Until recently, however, women – and protozoal STIs such as trichomoniasis – have been left out of this prevention research.[41,75] Our randomized open-label trial, a secondary analysis within a larger doxyPEP study among Kenyan women, is one of the first to evaluate a prophylactic intervention for *T. vaginalis*. By testing whether doxycycline taken after sex could prevent trichomoniasis, this study addresses a gap in the STI prevention literature. Even a null result provides valuable evidence: it directs the field toward what may not work and spurs consideration of continued research or alternative approaches. In a landscape with no prior *T. vaginalis* prevention trials using doxyPEP, our study's findings serve as a critical data point to inform future interventions. However, the low use of doxycycline from a subset of participants means we cannot rigorously evaluate whether doxyPEP itself is ineffective against *T. vaginalis* or whether the null result reflects implementation challenges with event-driven prophylaxis. This distinction is crucial for future research: our findings highlight both the need to explore alternative dosing strategies or prophylactic agents for *T. vaginalis* and the importance of addressing adherence barriers in event-driven prevention approaches. Our work also elevates the conversation about trichomoniasis in the public health community, highlighting that this neglected STI warrants the same innovative prevention research that has been applied to HIV and other STIs.

Adherence challenges with event-driven prophylaxis

A critical factor influencing our trial outcomes is adherence to the doxyPEP regimen. While self-reported adherence appeared promising—participants reported no missed doses at 84% of study visits—testing of hair samples to assess for the presence of doxycycline revealed that doxycycline was detectable in only 29% of specimens from a random participant subset. This stark discrepancy highlights the inherent challenges of implementing event-driven prophylaxis.

The event-contingent dosing schedule distinguishes doxyPEP fundamentally from daily prophylactic regimens, such as daily HIV PrEP, and introduces a unique set of challenges related to medication adherence. Unlike daily prevention strategies that can become routine, event-driven dosing requires participants to consistently recognize risk, remember medication, and take action after each sexual encounter. The cognitive burden of this approach, particularly for young women with potentially unpredictable sexual patterns, creates substantial implementation barriers. The null effect observed may reflect insufficient doxycycline coverage at critical post-exposure windows rather than an inherent lack of efficacy. Similar patterns emerged in HIV pre-exposure prophylaxis (PrEP) trials among African women, where adherence, not biologic effect, determined effectiveness.[196,197] The low use of doxycycline in a subset of participants observed in our study points to the need for better implementation strategies if event-driven prophylaxis is to be effective. Simply prescribing a medication is not enough – we must consider human behavior, context, and support systems.

Implementation strategies should address the unique challenges of post-exposure dosing to enhance adherence to event-driven doxycycline prophylaxis. Since doxyPEP is a relatively new approach, there is limited evidence on which adherence strategies are most effective in this context. However, lessons from daily PrEP and antiretroviral therapy offer valuable insights. Tolley et al. developed and psychometrically validated novel tools specifically designed to screen and monitor adherence in HIV prevention trials through a comprehensive process involving literature review and surveys of over 700 former trial participants.[198] These tools identified predictors of adherence that could be used both at screening to identify likely adherers and during follow-up to provide targeted support, offering inexpensive, multilingual options regardless of randomization assignment. Applying similar validated screening and monitoring tools in doxyPEP trials could improve participant selection and enable timely, personalized interventions.

On-demand or event-driven, HIV PrEP also provides a pertinent parallel to doxyPEP. Regimens such as the "2-1-1" schedule (two pills taken 2-24 hours before potential sexual exposure, followed by one pill 24 hours after the initial dose, and a fourth pill 48 hours after the initial dose) are designed for use only around periods of sexual activity.[199] Similar to doxyPEP, the effectiveness of on-demand PrEP, which can be comparable to daily PrEP, is critically dependent on strict adherence to this precise, multi-dose, time-sensitive dosing schedule. This model is considered most suitable for individuals who can reliably predict periods of sexual activity and meticulously follow the prescribed regimen.

However, achieving such adherence can be difficult. A clinical trial evaluating an intermittent PrEP regimen (fixed twice-weekly doses plus post-coital doses) among Kenyan MSM and FSW found markedly lower median adherence to the fixed intermittent dosing (55% via Medication Event Monitoring System - MEMS) compared to daily PrEP dosing (83%). Adherence to the post-coital doses was even lower, at a median of only 26%.[200] This study discussed several contributing factors:

- **Routine Disruption:** Daily pill-taking establishes a routine that is inherently easier to remember than less frequent, event-triggered dosing.[200]
- **Situational Barriers:** Changes in daily routines, travel, substance use (particularly alcohol around the time of sex), the stigma associated with taking medication in front of partners, and not having pills readily available during unplanned sexual encounters were all cited as challenges.[200]
- **Perceived Risk:** Adherence might fluctuate based on the individual's perceived HIV risk associated with a specific encounter or partner.[200]

These findings are highly relevant for doxyPEP, as they underscore that coitally-dependent dosing can be particularly challenging to maintain. The difficulties in accurately measuring adherence in event-driven regimens also present a critical lesson for the design of future doxyPEP trials.

Given the high discrepancy between self-reported adherence and biologically confirmed use in our study, real-time drug monitoring with participant feedback might foster more accurate reporting and improved adherence. For some women, daily doxycycline dosing (preexposure prophylaxis) might present a simpler alternative by eliminating per-exposure decision-making, as demonstrated in a pilot study with female sex workers that showed a reduction in bacterial STIs without significant vaginal microbiome disruption,[78] although this raises the concern of

AMR. Research into other dosing regimens that may fit better into participants' lives should be explored, just like how there are now many choices for HIV prevention. Finally, conducting qualitative follow-up studies with participants could provide crucial insights into context-specific barriers and facilitators to adherence, similar to the approaches taken after the VOICE and FEM-PrEP trials. In these trials, in-depth interviews with strategically selected participants, based on their drug concentration data, revealed valuable information about adherence challenges that could inform future trial designs.[201,202] Adherence considerations should be integral to doxyPEP trial design from the outset. Future doxyPEP trials should a priori incorporate adherence measurement protocols and consider embedding adherence support strategies or dedicated intervention arms.

Biological challenges and research priorities with *T. vaginalis*

Despite a promising in vitro study showing doxycycline could induce cell-death pathways in *T. vaginalis* cultures,[70] our null findings suggest that laboratory efficacy may not always translate to clinical prevention. This present study cannot draw definitive conclusions about doxyPEP's efficacy against *T. vaginalis*; however, we can speculate on potential explanations for our findings. The 200mg doxycycline dose used in this study may be insufficient for *T. vaginalis* prophylaxis, and the biological differences between prokaryotic bacteria (the primary targets of doxycycline) and eukaryotic protozoans like *T. vaginalis* likely contribute to this limited efficacy.[76,203] Alternative explanations for the limited efficacy include inadequate drug concentrations in the vaginal environment or pharmacokinetic limitations. However, one study showed that after a single 200 mg oral dose, doxycycline efficiently distributes to vaginal tissues, achieving concentrations exceeding the minimum inhibitory concentrations for certain bacterial sexually transmitted infections.[204] Nevertheless, these concentrations may still be insufficient to inhibit *T. vaginalis*, or the drug may not maintain effective levels for the necessary duration to prevent infection. These null results should not discourage future research but rather encourage a pivot toward more promising approaches. Alternative dosing strategies (higher doses or multi-day regimens), different prophylactic agents, or modifications to improve adherence all warrant investigation. Additionally, pregnant women who face significant trichomoniasis-related complications require specific attention, as they were excluded from our trial due to doxycycline's contraindication in pregnancy. Developing prophylactic strategies that are safe during pregnancy represents a critical research gap. Personalized approaches could also be considered, such as risk-based screening protocols tailored to women unable to use doxycycline. Most importantly, the persistent global burden of trichomoniasis, particularly in certain regions, emphasizes the continued need for effective preventive interventions even as we refine our understanding of which specific strategies hold the most promise.

The current landscape of antimicrobial resistance patterns in *N. gonorrhoeae* from the Kenya doxyPEP trial

In chapter three, our molecular detection of *N. gonorrhoeae* AMR among cisgender women in Kenya enrolled in the doxyPEP trial revealed a resistance profile consistent with established regional patterns: universal tetracycline resistance (100% of isolates) and very high fluoroquinolone resistance (~90%), confirming these older antibiotic classes are now ineffective for gonorrhea treatment in this population.[99,100] Encouragingly, we detected no resistance to macrolides (e.g., azithromycin) and minimal resistance to extended-spectrum cephalosporins (2.6%), suggesting these cornerstone drugs of current therapy remain viable options.[74,205,206] We observed a high prevalence of resistance to two or more drug classes (88.1%), mainly co-resistance to tetracyclines and fluoroquinolones. Notably, we found no significant differences in resistance patterns between participants in the doxyPEP group and those receiving standard care. However, this finding must be interpreted cautiously, given the documented low use of doxycycline in the doxyPEP in the trial,[41] which limits our ability to draw firm conclusions about the intervention's potential impact on antimicrobial resistance.

Universal tetracycline resistance and implications for doxyPEP

The universal tetracycline resistance observed in our study indicates a concerning yet established pattern in Kenya and the region. Most notably, despite this widespread tetracycline resistance, our samples showed no macrolide and minimal cephalosporin resistance, suggesting that tetracycline resistance does not necessarily predict resistance to these critical treatment options. This finding carries dual implications for doxyPEP. Widespread tetracycline resistance may limit doxyPEP's effectiveness against gonorrhea in this setting and could have contributed, alongside low adherence, to the lack of observed impact on overall STI incidence in women in the parent trial.[41] Additionally, the near-saturation of tetracycline resistance in local gonococcal strains suggests that introducing doxyPEP may not substantially worsen existing resistance patterns. Maintaining the efficacy of ceftriaxone is paramount, as it is the backbone of gonorrhea treatment worldwide.[119,205] Our findings support the continued use of ceftriaxone (usually combined with azithromycin per some guidelines)[66] in Kenya as first-line therapy, given the current profile. The absence of azithromycin resistance in our sample is similarly encouraging, especially as dual therapy with ceftriaxone+azithromycin was, until recently, a standard recommendation to delay cephalosporin resistance.[119,207]

Many *N. gonorrhoeae* isolates circulating globally already exhibit resistance to tetracyclines, often at high frequencies.[116,208,209] In some populations or regions, tetracycline resistance prevalence may approach or exceed 50-70%.[209] It can be argued that in such settings, the *additional* selective pressure exerted by doxycycline PEP might have a limited impact on the overall *prevalence* of tetracycline resistance if it is already widespread. On the other hand, even if prevalence does not increase dramatically, doxyPEP could still play a role in maintaining these high levels of resistance, preventing susceptible strains from regaining ground, or potentially shifting the relative frequency of different resistance mechanisms (chromosomal vs. plasmid-mediated), which has implications for cross-resistance.[116,210] However, our small sample size limits definitive conclusions about potential cross-resistance mechanisms, which remain an important theoretical concern.

DoxyPEP: Potential Reduction in Overall Antibiotic Use

A key argument in favor of doxyPEP is its potential to reduce the overall burden of bacterial STIs that require treatment.[31,32] By preventing a substantial number of syphilis, chlamydia, and potentially some gonorrhea infections, doxyPEP could lead to a net decrease in the use of therapeutic antibiotics, including those critical for gonorrhea treatment like ceftriaxone and azithromycin. This potential reduction in the use of first-line therapeutic agents must be carefully weighed against the risks associated with increased exposure to doxycycline. The net impact on overall antimicrobial pressure is complex and depends on the relative efficacy of doxyPEP against each STI, the baseline incidence of these infections, and the specific antibiotics used for treatment versus prophylaxis.

Looking ahead: doxyPEP and Antimicrobial Resistance

Our study provides valuable insights into the antimicrobial resistance landscape of *N. gonorrhoeae* in a Kenyan population participating in a doxyPEP trial. The resistance patterns documented have direct implications for clinical practice and public health strategies. The high background resistance to multiple antibiotics means that gonorrhea treatment protocols must rely on the few drugs that still work (ceftriaxone and potentially azithromycin in combination) and avoid those that have lost efficacy. Our findings reinforce the current clinical recommendations and treatment guidelines[119,206]. They support the continued use of extended-spectrum cephalosporins as first-line therapy while highlighting the need for ongoing vigilance and routine surveillance. They also suggest that macrolides remain a viable component of treatment regimens in this setting, though their use should be coupled with resistance monitoring, given the rapid emergence of macrolide resistance observed in other regions.[59,211,212] The minimal detection of resistance to cephalosporins and macrolides presents a critical window of opportunity to maintain effective treatment options in Kenya. This window can only remain open by proactively strengthening AMR surveillance and response systems. Much of Africa lacks robust gonococcal resistance monitoring infrastructure, creating urgent challenges for early detection and containment of emerging resistance patterns.[213,214]

Kenya already has some antimicrobial resistance (AMR) surveillance work for *N. gonorrhoeae* is already underway.[215-217] However, a pressing need exists to expand and strengthen these efforts across the country and throughout Africa.[215,216] Establishing sustainable gonococcal AMR surveillance programs through sentinel clinics or laboratories is critical for early detection and containment of emerging resistance patterns.

These programs should prioritize regular *N. gonorrhoeae* culture and antimicrobial susceptibility testing, complemented by molecular screening for resistance genes, to provide a comprehensive understanding of gonococcal AMR dynamics. Building the necessary infrastructure will require significant investment in laboratory capacity, personnel training, and resources to ensure evidence-based STI control.[215-217] Partnerships with international organizations, including WHO-EGASP, are essential in supporting these initiatives and providing technical guidance for regional surveillance systems.

Integrating AMR monitoring with STI prevention interventions becomes increasingly important as doxycycline prophylaxis expands. Any rollout of doxyPEP or similar STI prophylaxis requires comprehensive AMR monitoring plans, including periodic assessment of gonococcal isolates from individuals using prophylaxis to detect shifts in minimum inhibitory concentrations or the emergence of new resistance markers. These "surveillance-feedback loops" enable evidence-based policy adjustments if concerning resistance patterns emerge or support the safer expansion of prophylaxis if monitoring shows no negative impact.[218,219] This integrated approach balances STI prevention benefits with antimicrobial resistance risks.

Longitudinal and larger-scale molecular studies across diverse populations are needed to provide a more comprehensive understanding of resistance patterns and their evolution over time. The limitations of our analysis (42 isolates over one year) highlight the need for extended follow-up of resistance trends in populations using interventions like doxyPEP. Studies that track gonococcal infection outcomes and resistance patterns over multiple years with larger cohorts across multiple sites would improve statistical power to detect subtle changes in resistance prevalence. These investigations should assess how adherence levels and utilization impact resistance development and explore optimal dosing strategies or alternative prophylactic agents. The concerns surrounding doxyPEP underscore the fragility of current gonorrhea control strategies and highlight the urgent need for innovation, including renewed investment in novel antibiotics with different mechanisms of action and the development of effective gonococcal vaccines as a sustainable, resistance-sparing approach to prevention.

As *N. gonorrhoeae* AMR continues to evolve, successful gonorrhea control requires a strategic balance—expanding innovative prevention approaches while strengthening surveillance systems and promoting judicious antimicrobial use to preserve effective treatment options.

Challenging conventional wisdom: the paradoxical role of vaginal microbiota in cervicitis

In Chapter 4, we investigated the longitudinal relationship between vaginal microbiota states and cervicitis in the Mombasa Cohort. The key finding was that intermediate vaginal microbiota (Nugent score 4–6) conferred the highest risk of cervicitis, exceeding the risk observed with overt bacterial vaginosis (BV, Nugent 7–10), while normal lactobacillusdominated microbiota (Nugent 0–3) was associated with the lowest risk. This gradient is notable and somewhat counterintuitive. These findings challenge the conventional assumption that cervicitis risk increases monotonically with the degree of dysbiosis. Prior literature has focused mainly on BV as a risk factor for cervical inflammation[178] or attributed cervicitis in BV to the absence of protective lactobacilli.[44] Our results affirm that lactobacillus-deficient states are detrimental, but they suggest that a transitional ("intermediate") microbiome may be particularly proinflammatory, potentially even more so than established BV. This nuanced insight expands our understanding of cervicovaginal health beyond a simple normal/BV dichotomy. Importantly, we also observed an unexpected trend regarding hydrogen-peroxide-producing lactobacilli. In Chapter 3, cervicitis risk increased stepwise across categories defined by Lactobacillus presence and H_2O_2 production, and paradoxically, the highest risk was seen in women harboring H_2O_2-producing lactobacilli. This contradicts the prevailing understanding that H_2O_2-positive lactobacilli (typically *Lactobacillus crispatus* or *Lactobacillus. jensenii*) are uniformly protective against genital inflammation and infection.[220,221] Generally, lactobacilli help maintain an acidic pH and even secrete anti-inflammatory factors; for instance, recent research showed *L. crispatus* produces anti-inflammatory β-carboline compounds that can suppress inflammation.[222] Our paradoxical finding suggests that while H_2O_2-producing lactobacilli may benefit the vaginal environment in specific contexts, their relationship with cervical immunity may be more complex than previously appreciated—a nuance that could have significant implications for how we conceptualize and approach cervicovaginal health interventions. Mechanistically, these ostensibly beneficial bacteria can trigger proinflammatory cytokine production (e.g., TNF-α, IL-1α) and NF-κB activation in host cells.[223] Their metabolic byproduct, H_2O_2, while antimicrobial, may also cause oxidative damage to epithelial tissue and contribute to cytolysis in overgrowth conditions.[223-225] Our finding prompts a forward-looking reappraisal of vaginal health paradigms. In particular, immunologic studies are warranted to dissect how Lactobacillus-derived factors (including H_2O_2) modulate cervical cytokine profiles and epithelial integrity, and longitudinal microbiome studies could clarify the temporal dynamics between shifts in Lactobacillus communities and cervicitis development. Such efforts will help distinguish the protective versus other facets of H_2O_2-producing lactobacilli and inform more nuanced interventions for maintaining vaginal health.

Reframing the Intermediate Microbiota as a Distinct Clinical Entity

Intermediate Nugent scores are often regarded as a transient or equivocal state, and many studies have grouped them with either “normal” or “BV” categories or excluded them from analysis. Our results indicate that this state is not benign. In fact, they resonate with emerging evidence that even partial disturbances in the vaginal microbiome can have clinical consequences. Existing studies have reported that an intermediate vaginal microbiota can increase susceptibility to STIs, including *T. vaginalis* and *Mycoplasma genitalium*, which in turn are known causes of cervicitis.[177,226-228] Thus, our study adds to a growing literature suggesting that non-optimal” microbiota states short of BV can still provoke inflammation and infection risk. It challenges the prior focus on BV alone by spotlighting the intermediate state as a distinct entity worthy of attention. Our results highlight that intermediate microbiota may have unique clinical implications deserving of targeted research and interventions.

Our findings support conceptualizing the intermediate vaginal microbiota as a distinct microecological state with unique inflammatory properties. This "intermediate state" represents a dynamic community that is neither fully healthy nor severely dysbiotic but rather actively shifting and potentially more vulnerable to inflammatory triggers. The microbial instability within this community, characterized by constant flux and turnover, may disrupt the delicate balance between beneficial and potentially harmful bacteria. Research has documented that intermediate microbiota uniquely features both *Lactobacillus* species associated with optimal states and species like *G. vaginalis* associated with BV in similar concentrations, highlighting its transitional nature.[229] Longitudinal studies have further demonstrated that women with intermediate Nugent scores show variable trajectories, with some returning to normal microbiota while others progress to full BV.[144,229,230]

This dynamic state appears to create a distinct immunological environment. Evidence indicates that individual bacterial taxa, rather than BV as a whole condition, may differentially trigger specific inflammatory responses in the cervicovaginal environment.[231,232] For instance, certain species like *Mageeibacillus indolicus* have been specifically linked to cervicitis, independent of BV diagnosis.[233]In intermediate microbiota, researchers have identified increased *Gardnerella spp*. and *Bifidobacterium breve* (versus more diverse bacteria in BV), accompanied by simultaneous increases in both anti-inflammatory (IL-1a) and proinflammatory (IL-2) cytokines, plus decreased embryo growth factors (FGF-b, GM-CSF).[234] This suggests that the specific bacterial composition within intermediate microbiota, rather than simply the Nugent score category, may be the crucial determinant of cervicitis risk.

The complexity deepens when considering that while certain BV-associated bacteria (e.g., BVAB1, *Megasphaera spp., M. indolicus*) correlate with elevated cervicitis risk, higher tertiles *of L. iners* and *L. jensenii* have also been linked to cervicitis.[235] This apparent duality suggests that the intermediate state, where both protective lactobacilli and proinflammatory BV-associated taxa may coexist, could be particularly prone to inflammation, underscoring the complex interplay between different bacterial species in driving cervical inflammatory outcomes.

Implications for women's health and clinical practice

The findings from Chapter 2 have significant implications for women's health and clinical practice. Current treatment guidelines typically focus on BV while often leaving intermediate microbiota unaddressed.[119] Our research suggests this approach may overlook a population potentially at the highest risk for cervical inflammation and its sequelae. From a clinical standpoint, these findings raise important questions about how we should manage and monitor women with intermediate microbiota or recurrent microbiota fluctuations. Currently, intermediate Nugent scores are often not treated, but our study suggests that women in an intermediate state may be at appreciable risk for cervicitis and its potential consequences.

While we highlight intermediate dysbiosis as an at-risk state, we refrain from recommending routine treatment without evidence from intervention trials, as overtreating microbiota disturbances could disrupt beneficial flora and contribute to antibiotic resistance. Instead, our findings support a more nuanced clinical awareness: clinicians should recognize that a patient with "intermediate" vaginal microbiota, especially with risk factors or symptoms, may merit careful evaluation for cervicitis and counseling on STI prevention.

Conclusion

The three studies in this dissertation collectively advance our understanding of sexually transmitted infection prevention, vaginal health, and antimicrobial resistance among cisgender women in Kenya, offering important insights while highlighting critical areas for future research and intervention.

Our evaluation of doxycycline postexposure prophylaxis for *T. vaginalis* revealed no significant protective effect, suggesting the limitations of this approach for protozoal infections. This finding, coupled with observed adherence challenges, underscores the need for alternative prevention strategies for this neglected STI. Rather than closing the door on *T. vaginalis* prevention research, our work serves as a starting point for developing more effective approaches, potentially through exploring different antimicrobial agents, dosing strategies, or novel delivery methods.

Our assessment of antimicrobial resistance in *Neisseria gonorrhoeae* documented universal tetracycline resistance and high fluoroquinolone resistance, while susceptibility to extended-spectrum cephalosporins and macrolides remained largely preserved. This AMR profile reinforces current treatment guidelines while highlighting the need for vigilant surveillance to protect our remaining effective treatment options. The absence of detected cephalosporin and macrolide resistance presents a critical window of opportunity that can only be maintained by proactively strengthening antimicrobial resistance monitoring systems.

Our investigation into the relationship between vaginal microbiota and cervicitis revealed the unexpected finding that intermediate microbiota conferred the highest risk of cervical inflammation—higher than bacterial vaginosis—challenging the conventional understanding of vaginal dysbiosis. Similarly surprising was the positive association between hydrogen peroxide-producing lactobacilli and cervicitis, suggesting that microbes traditionally viewed as beneficial may have more complex roles in mucosal immunity. These findings underscore the importance of recognizing intermediate microbiota as a distinct clinical entity that warrants specific attention in research and practice.

Together, these studies highlight how addressing women's reproductive health requires multifaceted approaches spanning preventive interventions, microbiome research, and antimicrobial stewardship. They demonstrate the complexity of sexually transmitted infection management in resource-limited settings. They emphasize that effective solutions must be evidence-based, contextually appropriate, and focused on individual and population-level outcomes. As we continue to confront the global challenges of sexual and reproductive health, this work contributes valuable knowledge while pointing toward more nuanced approaches that consider the biological, behavioral, and structural factors influencing women's health in Kenya and similar contexts.

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