FACTORS INFLUENCING VARIATIONS IN VAGINAL FLORA: THE ASSOCIATION BETWEEN DOUCHING, CONDOM USE, AND BACTERIAL VAGINOSIS (BV) IN THE GIFT STUDY

by

Katherine Helen Berger

BA, Whitman College, 2000

MSPH, Emory University, 2002

Submitted to the Graduate Faculty of

Department of Epidmiology

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2006

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

This dissertation was presented

by

Katherine H. Berger

It was defended on

July 27, 2006

and approved by

Dissertation Advisor:
Roberta B. Ness, MD, MPH
Professor and Chair
Department of Epidemiology
Graduate School of Public Health
Professor of Medicine, Obstetrics and Gynecology
Department of Medicine
School of Medicine
University of Pittsburgh

Kevin Kip, PhD
Assistant Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Catherine Haggerty, PhD, MPH
Assistant Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Robert Cook, MD
Associate Professor
Department of Medicine
School of Medicine
Department of Behavioral and Community Health Sciences
Graduate School of Public Health
University of Pittsburgh

FACTORS INFLUENCING VARIATIONS IN VAGINAL FLORA:
THE ASSOCIATION BETWEEN DOUCHING, CONDOM USE, AND BACTERIAL VAGINOSIS (BV) IN THE GIFT STUDY

Katherine H Berger, PhD

University of Pittsburgh, 2006

Bacterial vaginosis (BV) is one of the most prevalent diseases in women of reproductive age; however, the natural history of BV is poorly understood. We characterized variations in vaginal flora by assessing factors that influence the persistence of BV and BV-associated organisms. In addition, we evaluated the potential impact that prior infection may have on the relationship between douching and BV, and assessed whether condom use may protect against BV. A total of 1199 women enrolled in the Gyn. Infections Follow-through Study were utilized for this study. Women were followed for a median of 3 years, and vaginal microbiology samples were obtained for Gram-stain diagnosis of BV and culture of microflora at baseline and every 6 to 12 months thereafter. After adjusting for confounding factors, only black race (adjusted RR 1.47, 95% CI 1.09, 1.98) and a baseline Gram-stain of BV (adjusted RR 6.60, 95% CI 4.41, 9.87) increased the risk of persistent BV. Other factors, commonly associated with BV in cross-sectional analyses were not associated with persistent BV. In cross-sectional analyses, douching at least once per month was associated with BV among women who had a history of BV, but not among women without prior experience of BV. In prospective analyses, douching only increased the risk of acquisition for BV among women with intermediate flora at baseline (adj. HR 1.5, 95% CI 1.1-2.4), suggesting that douching may lead to BV among women with abnormal flora. Consistent condom use (10 out 10 sexual encounters) was associated with a

decreased frequency of BV in case-crossover analyses (adjusted OR = 0.68, 95% CI = 0.49-0.94, p for trend = 0.047). Similar results were seen for carriage of *M. hominis* (adjusted OR=0.61, 95% CI: 0.41-0.93) and anaerobic Gram-negative pigmented rods (OR=0.65, 95% CI: 0.47-0.91). These results identify women at high risk for persistent infection, and among women with a history of BV douching should be avoided. This study also provided evidence that condoms are protective against BV. Given the high proportion of women with BV, the identification of protective factors is of significant public health importance for reducing the prevalence of BV.

DEDICATION

I dedicate this work to my parents, Douglas and Cheryl Berger, who have always encouraged and supported me in every aspect of my life. They provided me with the dedication and drive to pursue my goals and I will forever be grateful for all they have done. And I also dedicate this to Matthew Hutchinson, who was and always will be there for me.

TABLE OF CONTENTS

LIST OF TABLES

PREFACE

Over the course of my graduate education I have been fortunate in the quality of education, mentorship, guidance, and training opportunities that I have received within the field of public health. The Epidemiology program at the University of Pittsburgh has provided me with a thorough understanding of the fundamentals of epidemiology and has given me the necessary skills to succeed. This dissertation challenged me to expand my understanding of epidemiology in order to analyze complex questions of significant public health importance. My education has taught me to question our understanding of diseases so that improvements in how we meet public health challenges might be found. Overall, I have gain a strong foundation in epidemiologic methods that will help me to be an effective and productive researcher in the field of public health, for which I am grateful.

In particular, I would like to acknowledge Roberta Ness, MD, MPH for all her support, mentorship, guidance, and encouragement during the three years of my training. As my advisor, Dr. Ness has continually challenged me and pushed me to go beyond the expected, and has worked to ensure the scientific rigor of this work. Dr. Ness has been an advocate for my success and I am thankful for the opportunity to work with and learn from her. I would also like to acknowledge Kevin Kip, PhD for his support and help with understanding all of the methods required to complete this dissertation. Dr. Kip was always available and extremely helpful throughout the entire development of this project, and was willing to answer every question I

came up with. Dr. Kip's support of this work has helped me to be become a stronger researcher and epidemiologist and I am grateful for all he has done. I would also like to thank Cathy Haggerty, PhD, MPH and Robert Cook, MD for their help with reviewing the project and revising each manuscript. Their comments challenged me to approach this project from multiple perspectives, significantly improved this work, and helped me to gain a better understanding of this research and the pathophysiology of BV. Also, this project would not have been possible without the many collaborators, staff, and participants involved with the Gyn. Infections Follow-through Study (GIFT Study), and I would like to express my appreciation for all their hard work to make the study successful.

In addition, I would like to acknowledge the support of Roger Rochat, MD at the School of Public Health, Emory University for his never-ending belief in me. His guidance has helped to get to this point and has been invaluable for my development as both an epidemiologist and public health worker. I will always be grateful for the doors that have been opened for me. Also, I would like to thank James Russo, PhD at Whitman College for his support as my advisor and for offering the course on infectious diseases. That course opened my eyes to a whole new career choice, and without that, I might never have entered into public health.

My friends and colleagues have also been integral to my success. To everyone at 3602 5th Ave., thank you for supporting me throughout my three years. I have enjoyed the talks and friendship, and thank you for always listening. Also, the Bryan's (especially Lindsey) and Jen E. have been integral to keeping me sane. I would particularly like to express my love and gratitude to Matthew Hutchinson. His encouragement, love, and support helped to steady me during the hardest parts. The future will be quite an adventure and I am so grateful that Matt will be with me on the journey.

Finally, I would like to express my gratitude, appreciation, and love for my parents, Douglas and Cheryl Berger, who have always encouraged me in every dream. You have been made it possible for me to reach this goal. Your support has meant so much to me and I am where I am today because of you.

1.0 INTRODUCTION

1.1 SPECIFIC AIMS

Bacterial vaginosis (BV) is one of the most prevalent diseases in women of reproductive age (1). However, the natural history of BV infection is poorly understood (2). Numerous risk factors for BV have been identified, yet it is unclear how these factors influence acquisition, persistence, or resolution of BV. Douching significantly alters the vaginal flora (3) and despite the increasing evidence for harmful effects (4), douching is a common practice among women in the US (5). Numerous studies have shown an association between douching and BV; however, the question of whether douching precedes or follows BV acquisition is unknown, and it is unclear what effect douching has on the long-term maintenance of healthy vaginal flora. Additionally, very little is known about factors leading to the acquisition of BV, and evidence linking BV to sexual transmission remains controversial (6). Studies investigating the association between male colonization of BV-associated organisms have not supported sexual transmission of BV (6); however, whether or not condoms may prevent BV has not been determined.

The GIFT study is a large prospective study which examined the relationship between douching and the outcome of pelvic inflammatory disease (PID). Women were followed every 6 months for approximately three years and vaginal microbiology samples, which assessed the presence of bacterial vaginosis and BV-associated organisms, were obtained at five time points

during follow-up (baseline, 6 months, 12 months, 24 months, and 36 months). The longitudinal nature of the study with multiple measures on vaginal flora will allow for the assessment of factors associated with increased risk of bacterial vaginosis, in addition to factors associated with short and long-term variations in vaginal flora.

Thus, we propose to characterize time-dependent variations in vaginal flora by assessing factors that influence the persistence and increased risk of bacterial vaginosis. We hypothesize that women with persistent infections with BV will be different from women with variability in their infection status. In addition, we hypothesize that a history of BV will interact with douching to increase the risk of current infection with BV. Finally, we hypothesize that consistent condom use will significantly decrease the risk of bacterial vaginosis and provide evidence for sexual transmission of BV.

In this study we utilized data from the GIFT study to evaluate the following specific objectives and associated hypotheses:

1. To evaluate whether characteristics of women who maintain consistent vaginal flora (either consistently high or low with regards to bacterial vaginosis) are significantly different from women who show variable BV scores over the study period. *We hypothesize that women who show variable transitions in BV scores with resolution of BV will exhibit different risk factor profiles than women who maintain consistently high BV scores.*

2. To evaluate whether a history of BV or the presence of abnormal flora impacts the association between douching and the development of BV. *We hypothesize that women who have a history of BV or who have abnormal flora will be at increased risk for*

acquiring BV following douching, whereas among women who have normal flora, douching will not impact the acquisition of BV.

3. To evaluate whether condom use is associated with the presence of bacterial vaginosis. *We hypothesize that condom use during the prior two months is associated with a decreased risk for current bacterial vaginosis*

It is important to evaluate these hypotheses in order to determine whether modifiable factors, such as douching or condom use, significantly impact variations in BV status. Douching is a common practice among US women and findings from this study may help to enhance awareness regarding potential harmful effects of this practice. Additionally, evaluating the effectiveness of condoms will enhance our understanding of whether BV may result from sexual transmission. Understanding how sexual activity relates to BV is necessary for addressing the significant public health challenge posed by BV.

1.2 BACKGROUND AND SIGNIFICANCE

1.2.1 Introduction

Bacterial vaginosis (BV) is the one of the most prevalent and least understood diseases in women of reproductive age (1). BV results from an overgrowth of anaerobic and facultative aerobic bacteria at the expense of the normally dominant vaginal lactobacilli (7). Bacterial vaginosis is clinically recognized by increased vaginal discharge with discoloration (grey or yellow), a malodorous vaginal smell (often fishy), abdominal pain, intermenstrual bleeding, or prolonged menses (6, 8). However, the disease is asymptomatic in half of women with BV, underdiagnosed by clinicians, and poorly understood by women (9). Nearly 64% of the women

in a 1993 Gallup survey had never heard of BV and 80% in a 1998 survey among women with a prior vaginal infection in the past 3 years could not identify symptoms of bacterial vaginosis (9). Despite the lack of knowledge regarding BV and the general lack of severe acute symptoms, research over the last two decades has indicated that BV is a large public health problem. BV is associated with a number of adverse sequelae, including preterm delivery, pelvic inflammatory disease, infertility, cervical cancer, increased acquisition of STDs, pre-term birth, and intrapartum and postpartum infections (2, 6, 9), which underscores the need for further research regarding the etiology of bacterial vaginosis.

1.2.1.1 Definition and diagnosis of BV

BV is characterized by complex alterations of the normal vaginal flora (10). Normal vaginal lactobacilli are replaced by an overgrowth of anaerobic and facultative aerobic bacteria (11). The etiology of bacterial vaginosis is poorly understood, and BV occurs as an acute, chronic, or recurrent condition (2). Additionally, BV may spontaneously resolve with the natural ebb and flow of the menstrual cycle (6). The common clinical criteria for diagnosing BV follow Amsel's criteria, which defines BV as the presence of any three of the four following criteria (12, 13):

1) homogeneous vaginal discharge,
2) vaginal pH greater than 4.5,
3) positive "whiff" test or release of amine odor with the addition of a base, and
4) presence of 'clue' cells (bacteria adhering to epithelial cells) on a wet mount of the vaginal fluid.

Additional diagnostic methods, including Nugent's, Hay-Ison, and Schmidts's, which are based upon Gram-stains of vaginal smears, have also been developed for the diagnosis of BV

(14, 15). These systems assign scores based upon the relative presence of *Lactobacillus* spp, *Gardnerella* spp, and other morphotypes (*Mobiluncus*, *Streptococcus, and Staphylococcus*) (14, 15). Not all scoring systems include the same criteria for observing vaginal flora, and Schmidt's criteria does not include characterization of *Mobiluncus* spp (15). Nugent's method (Table 1) is the gold standard for diagnosis (13); however, the specialized training required for reading the slide makes the method difficult to employ in a clinical setting. Compared to Amsel's criteria, which is primarily used in the clinical setting, Nugent's method has been shown to have 89% sensitivity and 83% sensitivity (14). In the clinical setting a combination of scoring systems or modifications of the current systems are commonly employed (14), which may lead to variations in treatment and/or prevalence estimates.

Table 1: Nugent's criteria for diagnosing bacterial vaginosis - sum of three scores.

Score	Lactobacilli morphotype/ field	Gardnerella morphotype/ field	Curved bacteria (Mobiluncus)/field
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	
4	0	>30	
BV: score 7-10, Intermediate flora: score 4-6, Normal flora: score 0-3.			

1.2.1.2 Treatment of BV

A number of treatment options are widely available for women with BV: Oral metronidazole (2 doses daily for 1 week), 2% clindamycin vaginal cream (once daily for 1 week), or 0.75% metronidazole gel (vaginally once daily for 5 days) (17). All regimens have approximately similar cure rates at one week (>90%), and at 3 months (~70-80%) (10, 17). Current CDC recommendations suggest that symptomatic with BV be treated with any of the above options (18, 19). Due to a lack of convincing evidence general screening and treatment of

women with asymptomatic BV is not currently recommended, except possibly for women considered at high-risk for a premature birth (20).

1.2.2 Epidemiology of BV

1.2.2.1 Prevalence of BV

Prevalence estimates among reproductive aged women range from 3.6% to over 40% (Table 2), depending on the setting and diagnostic criteria (21). A Dutch national screening for cervical screening found a prevalence estimate of 3.6% among women aged 30 to 60 (22). However, women with multiple infections or a predominance of *Gardnerella* bacteria were not included in the estimate, which likely caused an underestimation of the population-based prevalence. Other estimates of prevalence largely stem from specific populations: those attending fertility clinics, STD clinics, primary care clinics, genitourinary clinics, and gynecology clinics (9, 17, 21, 23-25). Estimates tend to be highest in high-risk populations, such as women attending STD clinics (17). While estimates vary widely, most studies report prevalence estimates over 10% indicating that, irrespective of the variance in prevalence, BV is a significant public health concern for all women.

Table 2: Prevalence estimates of bacterial vaginosis in select populations (modified from Priestly (25)).

Population	Prevalence
College students (25)	4-25%
Family planning clinics (25)	9-23%
Primary care clinics (21)	16%
STD clinics (9, 23, 25)	24-40%
Gynecology clinics (9, 23, 25, 26)	9-23%
Genitourinary clinics (25)	12-61%
Pregnant women (9, 21, 23, 25)	10-20%
Women attending fertility clinics (17)	30%
Lesbians (25, 27, 28)	6-13%
Women seeking abortions (21, 24)	24-33%
Female sex workers (21)	33-40%
HIV sero-positive women (21)	47%
Adolescent virgins (25, 29)	12%
Sexually active adolescents (29)	15%

1.2.2.2 Risk factors for BV

Numerous risk factors have been associated with BV, however, it is still unclear as to whether BV results from endogenous infection, exogenous influences, or both (21). Smoking, black race, older age, douching, and use of IUDs have all been associated with an increased risk of bacterial vaginosis (7, 30). Additionally, a number factors related to sexual activity have been associated with BV, including increasing number of sexual partners, new sexual partners, early age at first intercourse, and history of sexually transmitted diseases (31, 32). Black women have a three-fold increased risk of BV compared to white women (19), however the reason for the discrepancy is not understood and risk factor differences between black and white women do not explain the observed racial disparity (33). Douching often disrupts and alters the vaginal flora, increasing the vaginal pH to >7.0, and thus, women with unstable vaginal flora may then be unable to restore normal vaginal flora (2, 8). While the increased risk with older age is not understood, a longer sexual history and increased number of partners may play a role (34). Additionally, researchers have suggested that the tail of an IUD may favor the growth of BV

associated organisms (35). The consistent association between BV and sexual activity has also raised the possibility that BV may be sexually transmitted; however, evidence for sexual transmission remains sparse and conflicting (See section on transmission below) (6, 31).

1.2.2.3 Public health impact of BV

30-40% of high-risk women and 10% of the general population are estimated to have BV at any given time (17, 21). Nearly all women will get BV at some point in their lives (8), suggesting that the public health impact, while currently unknown, may be large. Additionally, BV has been associated with acute and short-term adverse outcomes, such as post-partum endometritis, miscarriage, preterm birth, post-abortion endometritis, and urinary tract infections (8, 19, 36, 37). Evidence, while controversial, also suggests that BV may be associated with long-term outcomes such as non-chlamydial/non-gonococcal pelvic inflammatory disease and endometritis, and the acquisition of STDs, including Chlamydia, gonorrhea, and HIV (19).

Because the short and long-term consequences of BV remain controversial, the cost of BV is difficult to estimate. Over 1 million PID cases occur each year, costing an estimated $10 billion per year (38). Up to 70% of PID cases have a nongonococcal/nonchlamydial etiology (39); thus BV may play a substantial role in the disease and contribute substantially to the cost of PID. Estimates also suggest that nearly 30% of the racial difference in premature birth could be attributable to BV at a cost of nearly 1 billion each year (19). Adding to the burden associated with BV are the potential costs, days lost, and morbidity associated with other adverse sequelae including post-surgery infection, preterm birth, and miscarriage.

1.2.3 Pathogenesis of BV

1.2.3.1 Healthy vaginal flora and the development of BV

Healthy vaginal flora is characterized by a dominance of hydrogen peroxide (H_2O_2) producing *Lactobacillus* species (21, 40). The presence of lactobacilli help to maintain the acidic environment of the healthy vagina (pH <4.5) (40). The acidic environment also is protective against the growth of other organisms (40). In addition to the production of lactic acid and hydrogen peroxide by lactobacilli, the low pH of the vagina is also attributable to the conversion of glycogen to lactic acid by vaginal epithelial cells (7); thus, the physiology of the vagina favors the growth of acidic tolerant lactobacilli (40). The low pH favors the growth and adherence of lactobacilli to epithelial cells and inhibits the growth of potentially pathogenic organisms such as *G. vaginalis* and other anaerobic bacteria (2, 7). While, anaerobic and facultative aerobic bacteria are often present in a healthy vagina, they occur in low concentrations (10^2 to 10^5 per gm of vaginal fluid) (2) and are often transient (41).

BV results from a dramatic decrease in lactobacilli, an increase in pH, and an increase in other mixed flora in which anaerobic and facultative aerobic bacteria dominate (21). The shift in flora is also characterized by a 100 to 1000-fold increase in overall bacterial growth (10^8 to 10^{11} CFU/gm of fluid) (2, 19). It is not known whether the change in flora results from an initial decrease or lack of H_2O_2+ lactobacilli or whether the lack of H_2O_2+ lactobacilli follows colonization by BV associated organisms (2). Hillier et al. (42) showed that women lacking high quantities of H_2O_2 producing lactobacilli had a greater risk of developing BV and relapsing after treatment than women with H_2O_2 producing lactobacilli, suggesting that the loss of H_2O_2 producing lactobacilli may precede the growth of BV organisms. However, the presence of *G. vaginalis*, a primary factor in BV, has also shown to inhibit the growth of lactobacilli (40); thus,

the temporal relationship between the loss of lactobacilli and the over-growth of anaerobic bacteria remains controversial.

Hydrogen peroxide (H_2O_2), lactic acid, low pH, and other potential bacteriocins produced by lactobacilli have all been postulated to be the primary inhibitory factors of BV-associated organisms (40); however, cause-effect relationships and relative importance of mechanisms have yet to be fully determined. The majority of studies have focused on the role of hydrogen peroxide and pH (40). Vaginal pH has been shown to inhibit BV-associated organisms, and a low pH has been shown to be sufficient to inhibit the growth of some BV-associated organisms *in vitro* (40, 43). Hydrogen peroxide has also long been considered to play a role, and studies consistently show that women without BV harbor significantly more H_2O_2+ lactobacilli and women without H_2O_2-producing lactobacilli are nearly 4 times as likely to develop BV compared to women with H_2O_2+ lactobacilli (40, 44). However, a number of *in vivo* and *in vitro* studies have called into question the role of H_2O_2 in the prevention of BV (40, 45). Significant quantities of H_2O_2+ lactobacilli have been found in some women with BV, and laboratory tests with commercially available H_2O_2 did not inhibit the growth of anaerobic and facultative aerobic organisms involved in BV (40, 43). Additionally, the relative importance of H_2O_2 production and pH in the vaginal ecosystem in combination with other potential inhibitors has yet to be elucidated.

Hormonal factors may also play a significant role in the development of BV (41, 45, 46). A longitudinal study examining vaginal flora over the course of a menstrual cycle showed transient increases in anaerobic bacteria occurred during the first half of the menstrual cycle when estrogen concentrations rise (41) and these observations have also been observed in animal models (45). Additionally, oral contraceptives have been shown to be protective against BV, and

while the mechanism is not currently understood, oral contraceptives are known to increase the glycogen content in vaginal epithelial cells, which may increase lactic acid production by lactobacilli (47). Hormonal status may also impact the vaginal pH allowing the overgrowth of BV-associated organisms (40).

Numerous other exogenous and endogenous factors have been postulated to be important in changes leading to BV (48). Antibiotics, steroids, and other medications, immunosuppressive conditions, uncontrolled diabetes, exogenous objects (IUDs, douching, tampons), sperm, and spermicides have all been associated with changes in vaginal flora (48). Many of these factors, such as sperm, which has an alkaline pH, may significantly influence vaginal pH, while other factors, such as antibiotics or IUDs, may directly promote or decrease the growth of bacteria (40). Additionally, pathogenic organisms may be directly introduced into the vagina through sexual intercourse (6, 31).

Clinical symptoms of BV are a result of the massive overgrowth of vaginal anaerobes and the increased production of proteolytic carboxylase enzymes and cytotoxins (17, 45). The proteolytic carboxylase enzymes break down peptides to a number of amines and ammonia, including putresceine, cadaverine, and trimethylamine, which increase in pH and cause the characteristic 'fishy' smell (17, 45, 49). Further, the amines and cytotoxins cause vaginal epithelial damage and increase the rate of epithelial cell transudation and exfoliation resulting in the gray homogenous discharge. Additionally, the high pH favors bacterial adherence to vaginal epithelial cells creating the 'clue' cells used to diagnose BV according to Amsel's criteria (17, 49).

While bacterial vaginosis often has discernable symptoms, an estimated 50% of bacterial vaginosis cases are asymptomatic and unrecognized by the woman or the clinician (15). The

reason for the high prevalence of asymptomatic women remains unclear, although this may be in part due to poor symptom recognition or underlying differences in the host and/or BV pathology (50). A recent study by Schwebke (50) did not find a lack of symptom recognition among asymptomatic women. However, the study did find that only 46% of asymptomatic women responded to therapy (compared to 80-90% of symptomatic women), suggesting that there may be differences between symptomatic and asymptomatic women in the underlying pathology for the development of BV.

1.2.3.2 Vaginal immunity and BV

The primary mechanism involved in vaginal immunity against bacterial pathogens is the innate immune response in the vaginal mucosa (51, 52). Several bactericidal compounds, including lysozyme, polyamines, zinc, and lactoferrin are released into the vaginal mucosa providing a first line of defense (52). Additional protection results from the recruitment and activation of polymorphonuclear neutrophils and the activation of the inflammatory response. Secretions of IgA and IgG in the mucosal surfaces are also important lines of defense against vaginal pathogens (53).

Despite the appearance of characteristic clinical symptoms, BV does not produce a characteristic inflammatory response similar to other vaginal infections caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Candida* species (51) and approximately 50% of women with BV are asymptomatic. In contrast to other vaginal infections, neutrophil recruitment and the accompanying inflammatory response is generally absent with bacterial vaginosis (51). However, BV positive women do show significant increases in levels of interleukin-1β in vaginal secretions (51). IL-1β normally induces the production of IL-8, which in turn is a key factor in the recruitment and activation of neutrophils

and the accompanying inflammatory response; however, in women with BV, IL-8 levels in vaginal secretions are not increased (51). The lack of IL-8 may explain the general lack of inflammatory response. Women with BV have been shown to induce specific immunoglobulin responses to *G. vaginalis*; however, the role this plays in the pathology and development of BV is unclear (21, 51, 54).

1.2.3.3 Vaginal flora involved in BV

Numerous pathogenic bacterial organisms have the ability to colonize the vagina. *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Candida albicans*, and *Trichomonas vaginalis* are commonly recognized vaginal infections. Additionally, other potentially pathogenic colonizers of the vagina, such as *Escherichia coli* and Group B *streptococcus* (*Streptococcus agalactiae*), are associated with urinary tract infections and neonatal disease, respectively (55-57). Numerous anaerobic and facultative aerobic bacteria have been identified in women with BV (21, 58). *Gardnerella vaginalis*, mycoplasmas, including *Mycoplasma hominis* and *Ureaplasma urealyticum*, *Prevotella* species (Gram-negative anaerobic rods and also known as *Bacteroides*), *Mobiluncus* species (Gram-positive anaerobic rods), and *Peptostreptococcus* species, are often more commonly found in women with BV than women without BV (21, 58, 59). Fifty to seventy-five percent of women with BV harbor *M. hominis*, up to 95% of women harbor *G. vaginalis*, and 50% or more of women may carry *U. urealyticum* (21, 59-61). Additionally, approximately 40-80% of women with BV harbor *Mobiluncus* species (21, 62, 63). Numerous other anaerobic bacteria have also been isolated, including *Fusobacterium* species, *E. coli*, *Porphyromonas* species, *Eubacterium* species, and *Bifidobacterium* species. Additionally, recent improvements in molecular identification through PCR have resulted in the identification of previous unknown bacterial species in women with BV, including *Atopobium vaginae*, BVAB

1, 2, 3, and *Megasphaera elsdenii* (64, 113). This may help to further our understanding of the organisms involved in the pathogenesis of BV.

Causative associations have been commonly explored for *G. vaginalis*, mycoplasmas, and *Mobiluncus* species; however, controversy exists with regards to any specific organism's role in the pathogenesis of BV (60, 65), and no single organism has been identified as the causative agent (64). Evidence does suggest, however, that positive interactions among multiple organisms may contribute to the overall pathogenesis of BV (40, 66). Nearly all women with BV are colonized by more than one organism (58). Some evidence also suggests that the growth of *G. vaginalis* and *M. hominis* is increased in the presence of other anaerobic bacteria (40). Women with a combination of *G. vaginalis*, anaerobic bacteria, and *M. hominis* were significantly more likely to have BV than if women were colonized with any organism alone (60). Additionally, women with multiple organisms have the highest risk of related sequelae (40). Ness et al. (67) found that a cluster of BV-associated organisms (including *G. vaginalis*, *M. hominis*, anaerobic Gram-negative rods (pigmented and nonpigmented), *U. urealyticum*, and the absence of hydrogen peroxide-producing lactobacilli) was significantly associated with PID; the authors, however, did not find that BV diagnosed with Gram-staining was associated with PID (68). This may have been due to the dependence of the Gram-stain on a limited number of organisms (*G. vaginalis* and *Mobiluncus*), while the BV-associated cluster captured the presence of additional anaerobic bacteria (67).

1.2.4 Variations, changes, and persistence of vaginal flora

While the changes that occur in the vaginal ecosystem with current BV infection are well characterized, the natural history of BV remains poorly understood (2). BV occurs as an acute,

chronic, or recurrent condition (2), and very little is understood regarding the cause for variation among women. Despite available treatment options, high recurrence rates and persistence of BV are common (69). Women treated for BV with oral metronidazole, the standard recommended CDC therapy, have a recurrence rate of up to 30% after 3 months following successful treatment (2). Similar recurrence rates have been reported with the commonly used vaginal clindamycin cream (2). A long term follow-up study by Boris et al. (69) showed that during 6 years of follow-up, approximately 52% had at least one recurrent episode of BV, which was highly correlated with new sexual contact. Cook et al (70) showed that persistence of even one abnormal BV associated factor was a significant predictor of BV recurrence, suggesting that some women treated for BV are unable to reestablish normal vaginal flora, which results in a chronic persistence of BV. It is unknown, however, whether the recurrence of BV is due to failure to successfully treat the infection (resulting in persistent colonization), re-infection, failure to treat an unidentified pathogen, or a combination of factors (71).

While BV has been shown to persist in a number of women (70), BV has also been shown to be extremely variable over short and long periods of time (41, 72). Keane et al. (41) followed women daily over the course of a menstrual cycle and found that 33% of the women underwent a transient change to intermediate flora or BV during the beginning of the cycle that resolved by the end of the cycle. Schwebke et al. (73) followed 51 women recruited from an STD clinic for 6 weeks to monitor daily changes in vaginal flora. Transient changes were observed in 78% of the women and 22% had normal flora throughout follow-up. With respect to assessment of variability in BV status over longer periods of time, only one study has been conducted (72). Ness et al. (72) assessed changes in BV scores for approximately 1200 women over 6 to 12 month periods. Nearly 40% of women with normal flora increased floral score at a

subsequent visit. Similarly, approximately 40% of women with BV decreased floral score, and 70% of women with intermediate flora either increased or decreased their score.

Factors associated with changes in vaginal flora are not well understood. Schwebke et al. (73) found that menses, vaginal medication, spermicide, number of partners during past year, and number of times of sex per month were associated with day-to-day shifts in vaginal flora; yet the reasons for the changes remain unclear. Similarly, Ness et al (72) found that history of BV, lack of monogamy at baseline, race, and education were found to be associated with three unit increases in microflora score. History of gonorrhea/chlamydia, chlamydial/gonococcal infection with in the past 6 to 12 months, and being a former smoker were associated with resolution of BV to normal vaginal flora.

While these studies do shed light on factors influencing changes in vaginal flora over time, studies have yet to address potential differences in women who show persistence of BV compared to women who exhibit transient changes in flora. Additionally, the variability in lactobacilli status and BV-associated organisms has not been characterized, and factors influencing long-term variability in BV status, such as douching, have yet to be elucidated.

1.2.5 Douching and vaginal flora

1.2.5.1 Epidemiology of douching

Vaginal douching is the process of using a liquid cleansing agent to clean in the vagina. Vaginal douching is a common practice among women in the United States and over one-fourth of reproductive aged women report douching regularly (5). Non-Hispanic black women report the highest prevalence of regular douching (55.3%), while 33.4% of Hispanics and 20.8% of non-Hispanic white women report regular douching (3, 5). Additionally, up to 73% of women

report having douched at some point in their life (74). Women generally report douching as long-term practice that is encouraged and initiated from the woman's family and social support system (75). Women report douching for a number of reasons: general hygiene, to cleanse after menses or before/after sex, reduce or prevent vaginal odor, treat vaginal symptoms such as discharge or itching, bleeding between menses, prevent pregnancy, prevent sexually transmitted diseases (3, 75). Studies indicate that women primarily douche on average once per month (75, 84); however, douching frequency of once per week or greater is common (69). Increased douching frequency has been associated with low educational status, increased risk for STDs, and higher likelihood of vaginal symptoms indicative of infection (74).

Recent evidence also suggests that douching may be harmful and increase the risk of many adverse health outcomes, including pelvic inflammatory disease, bacterial vaginosis, acquisition of sexually transmitted diseases, cervical cancer, preterm birth, ectopic pregnancy, and infertility (3, 4, 76). However, the data regarding adverse outcomes is sparse and conflicting in many cases. One of the primary questionable aspects of douching is the temporal relationship to disease outcomes (3). Many women report douching due to vaginal symptoms (76) and factors that increase the risk of sexually transmitted infections are more common among women who douche (74, 84). Thus, douching may be a response to vaginal infections rather than a cause, and this controversy is particularly evident with regard to bacterial vaginosis.

1.2.5.2 Douching and the risk of BV

Douching agents are primarily composed of water, acidifying agents (vinegar, benzoic acid, sodium citrate, diazolidinyl urea), antimicrobials (cetylpyridinium chloride, edentate disodium), and/or surfactants (octoxynol-9) (3). Nearly all douching agents impact the vaginal flora, although different solutions differentially alter vaginal flora. In vitro studies have shown

that antiseptic and antimicrobial douches inhibit the growth of all vaginal organisms, including lactobacilli (77, 78). However, douches containing primarily acidifying agent and water, inhibited BV-associated organisms, but not lactobacilli (77). The length of vaginal disturbance after douching is also variable. Monif et al. (79) found that vaginal flora was reestablished within 120 minutes after douching with povidone-iodine solutions. However, while vaginal flora may quickly rebound after a single douching episode, repeated douching may diminish a woman's ability to reestablish H_2O_2+ lactobacilli predominant flora (3, 76). Additionally, other behaviors coinciding with douching, such as sexual activity, may inhibit the reestablishment of normal vaginal flora following a douching episode (3), thus providing an environment suitable for the growth of BV-associated organisms.

Numerous studies have examined the relationship between douching and bacterial vaginosis (Tables 3 and 4). Several cross-sectional studies have shown consistent and strong associations between douching frequency, recentness of douching, and bacterial vaginosis (1, 76, 81, 83, 85-88, 90). Chaiffarino et al. (83) showed a dose-dependent increase in risk with increased frequency of douching (OR associated with >1/week: 2.0; 95% CI: 1.0-3.9). Ness et al. (76) found that women who douched one or more times per month had a 1.4-fold increase in risk (95% CI: 1.1-1.9) and women who douched within the last week had a 2.1-fold increase in risk (95% CI: 1.3-3.1) compared to women who did not douche. Beigi et al. (1) found that douching greater than two times per month was significantly associated with an increased risk for lacking hydrogen peroxide-producing lactobacilli (OR=2.5; 95% CI: 1.1-6.0). They also found that douching during the past week was significantly associated with lacking H_2O_2+ lactobacilli (OR=2.6; 95% CI: 1.2-5.5). Additionally, Schwebke et al. (81) showed that among adolescent women who douched, recent douching within the last week was significantly

associated with the presence of bacterial vaginosis (p=0.04). However, douching frequency and douching for symptoms were not associated with bacterial vaginosis. A few cross-sectional studies have shown no relationship between douching and bacterial vaginosis; however, many of them did not determine frequency of douching or recentness of douching (28, 71, 80, 82).

While many cross-sectional studies exhibit strong associations between BV and douching, the question still remains as to whether douching causes BV or is a result of vaginal symptoms indicative of BV (3). Ness et al. (76) found significant associations with douching; however, a large proportion of the women douched because of symptoms, and it was not determined what effect this may have had on the relationship between douching frequency and BV. On the other hand, Holzman et al. (86) did not find any difference in risk based upon reason for douching. Women douching for symptoms had similar risk for BV compared to women who did not douche for symptoms, suggesting that douching may both precede and follow the development of BV (86).

Additionally, only two studies have been conducted prospectively and the long-term effect of douching on the maintenance of vaginal flora has not been assessed (72, 90). Hawes et al. (90) found that douching for cleanliness in a cohort of women recruited from an STD clinic was significantly associated with the acquisition of bacterial vaginosis (HR=2.1; 95% CI:1.0-4.3). However, Ness et al. (72) did not find that douching 2 or more times per month was associated with an increased risk of acquisition of bacterial vaginosis (HR=1.52; 95% CI: 0.67-1.90), which questions whether or not douching has a causal association with BV. Further research is warranted to not only understand the association between douching and the acquisition of BV, but also the long-term risk of BV associated with repeated douching.

Table 3: Literature review of published studies that report an association between douching and BV, Part A - Population, sample size, and study design.

Study	Population	N	Study Design
1. Ness, 2006 (72)	Women at high risk for STDs, recruited from clinics, student health services, and health departments	374	Longitudinal - discrete time survival analysis
2. Bradshaw, 2005 (71)	Women presenting with symptoms of abnormal discharge in Australia	342	Cross-sectional
3. Beigi, 2005 (1)	Four studies from Magee-Womens' Hospital: all women with BV	947	Cross-sectional
4. Demba, 2005 (80)	Women attending a Medical Research Clinic with self-reported vaginal discharge/itching in Gambia	230	Cross-sectional
5. Schwebke, 2004 (81)	Recruited women who douched from general adolescent clinics, primary care clinics and local universities in Alabama	250	Cross-sectional
6. Bailey, 2004 (82)	Women from sexual health clinics for lesbian and bisexuals	708	Cross-sectional
7. Chiaffarino, 2004 (83)	Recruited from outpatient gynecology clinics	926	Cross-sectional
8. Ness, 2003 (33)	Women at high risk for STDs, recruited from clinics, student health services, and health departments	1200	Longitudinal - cross-sectional at baseline
9. Marrazzo, 2002 (28)	Self-selected women from the community who reported having sex with women	326	Cross-sectional
10. Vermund, 2001 (84)	Cohort of HIV infected and high-risk HIV unifected adolescents	342	Cross-sectional
11. Fonck, 2001 (85)	RCT of antibiotic treatment of STDs to reduce the incidence of HIV/STDs in Kenya	543	Cross-sectional
12. Holzman, 2001 (86)	Non-pregnant women attending health care clinics	496	Cross-sectional
13. Newton, 2001 (87)	RCT of behavioral intervention for the prevention of STDs	617	Cross-sectional
14. Rajamanoharan, 1999 (88)	Women attending an STD clinic in London	200	Cross-sectional (case-control)
15. La Ruche, 1999 (89)	Pregnant women recruited from antenatal community clinic for STD screening	552	Cross-sectional
16. Hawes, 1996 (90)	Women from Seattle STD clinic	182	Cox PH survival analysis
17.Wolner-Hanssen, 1990 (99)	Recruited from STD clinics, Women's clinics, or emergency rooms in Seattle with suspected PID	981	Cross-section (case-control)

Table 4: Literature review of published studies that report an association between douching and BV, Part B – Outcome measure, exposure, risk estimate, and 95% confidence intervals.

Study	Outcome	Exposure	Risk Estimate	95% CI
1. Ness, 2006 (72)	BV (Gram-stain)	Douche: >2 times/month	HR = 1.52	(0.67-1.90)
2. Bradshaw, 2005 (71)	BV (Gram stain)	Vaginal douching: Yes	OR = 1.0	(0.6-1.5)
3. Beigi, 2005 (1)	Lack of H2O2+ lactobacilli	Douching during past week Douched > 2 times past month	**OR = 2.6**** **OR = 2.5**	**(1.2-5.5)** **(1.1-6.0)**
4. Demba, 2005 (80)	BV (Gram-stain)	Douching: Yes	OR = 0.73*	(0.30-1.75)
5. Schwebke, 2004 (81)	BV (Gram stain)	Douche <1 week ago*** Douche after period***	**OR = 1.84** **OR = 5.11**	**(1.07-3.18)** **(1.99-13.2)**
6. Bailey, 2004 (82)	BV (Amsel)	Douching: Yes	OR = 1.29	(0.69-2.42)
7. Chiaffarino, 2004 (83)	BV (PIP activity test)	Douching Occasional >1 / week	 **OR = 1.3** **OR = 2.0**	 **(1.0-1.7)** **(1.0-3.9)**
8. Ness, 2003 (33)	BV (Gram-stain)	Frequency: >1/month Reason: abnormal symptoms Reason: hygiene	**OR = 1.4** **OR = 1.7** **OR = 1.3**	**(1.1-1.9)** **(1.1-2.6)** **(1.0-1.9)**
9. Marrazzo, 2002 (28)	BV (Gram-stain)	Ever douched	OR = 1.5**	(0.9-2.5)
10. Vermund, 2001 (84)	BV (gram stain)	Douching: <1/month Douching: 1/month	OR = 1.03* OR = 1.03*	(0.49-2.18) (0.63-1.67)
11. Fonck, 2001 (85)	BV (gram stain)	Douching: any product Douching: water only Douching: ever with soap	**OR = 1.53*** OR = 1.42* **OR = 1.63***	**(1.0-2.35)** (0.6-3.32) **(1.04-2.54)**
12. Holzman, 2001 (86)	BV (gram stain)	Use of douche in past 2 mo. Douched because of sympt.	**OR = 2.9** **OR = 3.1**	**(1.5-5.6)** **(1.5-6.8)**
13. Newton, 2001 (87)	G. vaginalis	Douching >1/month	**OR = 2.4**	**p=0.05**
14. Rajamanoharan, 1999 (88)	BV (Gram-stain)	Douche Douche, bubble bath, antiseptic	**OR = 6.1** **OR = 2.7**	**(1.3-28.1)** **(1.2-6.0)**
15. La Ruche, 1999 (89)	M. hominis	Douche on day of exam Douche as common practice Use of intravaginal agents	RR = 0.9 RR = 1.8 RR = 1.0	p=0.76 p=0.25 p=0.88
16. Hawes, 1996 (90)	BV (Amsel)	Douches for cleanliness	**HR = 2.1**	**(1.0-4.3)**
17. Wolner-Hanssen, 1990 (99)	Not specified	Current douching	OR = 1.6**	(1.2-2.2)

1.2.6 Transmission of bacterial vaginosis

In addition to the lack of understanding regarding time-dependent variations in vaginal flora, methods of acquisition for bacterial vaginosis remain equally elusive. Both exogenous and endogenous sources have been shown to play significant roles in the development of BV; yet it is not known why or how BV develops. Organisms associated with BV are naturally found in the rectum, mouth, and oropharynx, and it has been postulated that BV associated organisms migrate from the intestinal tract (91). Additionally, studies involving partner cultivation of microbial organisms have generally not supported the concept that BV is sexually transmitted and there is no male equivalent of the disease (6, 31). Transient changes during the menstrual cycle also indicates that changes in vaginal flora may be common with changes in hormonal status or hormonal status may predispose women to BV (41).

Despite the evidence that suggests BV results from endogenous factors, exogenous factors clearly impact the development of BV. A number of factors related to sexual behavior are associated with bacterial vaginosis, causing some to speculate that BV is sexually transmitted (31, 92), and BV exhibits many, but not all, of the characteristics of the traditional STD (Table 5). Increasing number of sexual partners, sex during menses, new sexual partners, and a history of STDs have all been consistently identified risk factors for BV (24, 31, 83). While these risk factors mimic those of classic STDs, such as Chlamydia and Gonorrhea, other factors are in direct contradiction, such as the presence of BV in adolescent virgins (2, 25, 29). Additionally, studies among lesbians have been both positive and negative regarding the transmission of bacterial vaginosis (27, 110). BV may also be more common among women who do not use condoms; however, sperm may alter the pH of the vagina and cause an imbalance in vaginal flora (25).

Table 5: Is BV an STD? Comparison of risk factors for BV and Chlamydia/Gonorrhea.*

Risk Factor	Chlamydia/ Gonorrhea	BV
Increasing # sexual partners	Yes	Yes
Lower age first intercourse	Yes	Inconsistent
Found in virgins	No	Yes
Prevalence in lesbians	Decrease	Increase
Partner treatment/male counterpart	Yes	No
Age	<25	>25
Smoking	Yes	Yes
Black ethnicity	Yes	Yes
IUD use	Yes	Yes
Oral contraceptives (44)	Increase	Decrease

* Modified from Morris et al. (6) and Morris et al. (31).

The conflicting evidence has caused some to speculate that BV should not be considered as a traditional STD; however, the disease may still have a sexually transmitted component and still result from transmissible organisms (92). While partner treatment has generally proven to be an ineffective means for preventing BV in women (92); additional potential preventative measures, such as condom use, have not been well characterized and remain poorly understood. Discerning whether condoms protect against BV may help to clarify whether BV results from sexual transmission.

1.2.6.1 Condoms and sexually transmitted diseases

The use of condoms for protection against sexually transmitted diseases has been a key part of public health programs for many years (111, 112). Condoms are generally recognized as a practical tool for STD prevention and are known to provide an effective mechanical barrier against pathogens. Laboratory tests clearly indicate the effectiveness of condoms in the laboratory setting to provide an impermeable barrier against both viral and bacterial pathogens (93). However, the epidemiologic evidence is controversial regarding the overall role of

condoms in the reduction of STDs (94). A recent NIH workshop summary report concluded that condoms are effective for the prevention of HIV, but evidence was inconclusive for the prevention and risk reduction afforded for Chlamydia, gonorrhea (male to female), syphilis, trichomoniasis, herpes, and human papillomavirus (95).

The report also concluded, however, that the results were based upon scant data and very few prospective study designs, and the results did not constitute evidence for or against the effectiveness of condoms (94). Additionally, condom use is an inherently difficult exposure to study. Numerous factors, including infectivity of the STD, consistency of condom use, incorrect use, number of sex acts with an infected partner, and whether the STD can be transmitted by skin contact, all impact the effectiveness of condoms for the prevention of STDs (96). Due to the difficultly in measuring all factors that may impact the effectiveness of condoms, studies on condom effectiveness are fraught with potential bias (97, 98, 100-105). Additionally, reliance on self-report of condom use introduces recall bias, with a tendency to over-report condom use and potentially bias estimates towards the null (98).

Recently, the impact of condom use on STD acquisition was reexamined with a review of 18 prospective designs published since June 2000 (94). These studies indicated that condoms afford protection against bacterial STDs (94). However, Holmes et al. (94) does note that numerous methodological issues still existed with many of the studies, and potential bias from over-reporting of condom use, lack of adjustment for improper condom use, and lack of adjustment for confounding variables not easily measured may have been present. In order to address biases in studies evaluating condom use effectiveness, Warner et al (100) recently compared a general cohort repeated measures design with a case-crossover design to assess condom use and the risk of incident gonorrhea and chlamydia. By design, a case-crossover study

uses cases as their own controls and thus adjusts for time-independent factors that may not be easily measured (100); thus, bias caused by unmeasured confounding is reduced. Using the case-crossover analysis, Warner et al. (100) found a protective effect for consistent condom use without breakage (OR=0.49; 95% CI: 0.26-0.92) that was not apparent using the cohort design (OR=0.79; 95% CI: 0.53-1.17), suggesting that unmeasured confounding masks potential associations in traditional cohort designs using repeated measures analysis. Further application of this method may help to clarify the relationship between condom use and STDs, including the potential relationship between the condom use and bacterial vaginosis.

1.2.6.2 Condom use and BV

A number of studies have also addressed the relationship between condom use and bacterial vaginosis, yet no clear pattern has emerged regarding whether condoms may be protective against BV (Tables 6 and 7). The relationship with barrier methods of contraception has been inconsistent and has also been difficult to characterize due to the lack of knowledge about the sexual transmissibility of BV. Most studies addressing condom use and BV have been cross-sectional and have shown mixed results (30, 34, 35, 47, 71, 76, 81, 83, 85, 88, 108). Smart et al. (30) showed a significant protective effect for 100% condom use (OR=0.5; 95% CI: 0.30-0.71) in a cohort of women attending a sexual health center. Two European studies have also found protective associations for BV and condom use (35, 47). Similarly, Moi et al. (34) found a significant decrease in risk for barrier methods of contraception (OR=0.58; 95% CI: 0.45-0.75), although the authors did not adjust for confounding variables. However, eight cross-sectional studies have found no association between BV and condom use (71, 76, 81, 83, 85, 88, 106, 108). The largest of these was conducted by Ahmed et al. (106) who followed a cohort of 17,264 women from a randomized controlled trial of mass STD treatment in rural Uganda for

approximately 4 years at 10-month intervals. Consistent condom use during the last year was evaluated cross-sectionally using a repeated measures design (generalized estimating equations) and was not associated with bacterial vaginosis diagnosed by Gram-stain (OR=0.89; 95% CI: 0.74-1.07) when adjusting for baseline characteristics, multiple sex partners, and sex outside of marriage. This same study did, however, show protection against Chlamydia, gonorrhea, HIV, and syphilis.

Two prospective studies have also been conducted which examine the relationship between condom use and BV, and only one has shown a protective effect (107). Hawes et al. (90) followed a cohort of 182 women recruited from an STD clinic in Seattle, WA for 2-years and ascertained changes in vaginal flora, including the presence of Lactobacilli, bacterial vaginosis, yeast vaginitis, and trichomoniasis. Use of barriers methods was assessed throughout follow-up and did not show an association with the acquisition of BV (HR=0.8; 95% CI: 0.3-1.7) when adjusted for age, race, and time-dependent factors, such as new partners, antibiotic use, and douching. However, due to small sample size (n=182), this study may have lacked the power to observe an association. Baeten et al. (107) followed a cohort of 948 female sex workers in Kenya for a median of 421 days. Mean condom use during the follow-up was calculated and 100% condom use was associated with a significant, although slight, decreased risk of bacterial vaginosis (HR=0.9; 95% CI: 0.7-1.0) when adjusting for baseline characteristics, number of sexual partners per week, and number of sexual encounters.

From the current literature it is difficult to determine if condom use may provide a protective effect against bacterial vaginosis. Few prospective studies have addressed condom use and the acquisition of BV, and while the study by Baeten et al. (107) did show an association between condom use and the acquisition of BV, the effect size was small. In cross-sectional

studies it is difficult to determine timing with respect to disease acquisition. Ahmed et al. (106) did not show an association between condom use and BV, but did find an association between condom use and other STDs. However, the timing of condom use was broad (during past year) and the variability in BV over shorter periods of time may have made an association difficult to detect. Additionally, differences in results across studies may be due to differences in study designs, sample size, study populations, diagnostic criteria for BV, categorization and ascertainment of condom use, and adjustment for confounding factors. Several studies also failed to address biases associated with condom use (i.e. recall bias, self-report, other sexual behaviors, and partner characteristics). Application of study methods which can address biases inherent in condom use, such as the case-crossover study, may help to elucidate the relationship between condom use and bacterial vaginosis. While these methods cannot address all biases, they can help to further understand the transmission of BV and the role of condoms in the prevention of bacterial vaginosis.

Table 6: Literature review of published studies that report an association between condom use and bacterial vaginosis , Part A - Population, sample size, and study design.

Study	Population	N	Study Design
1. Saifuddin, 2001 (106)	RCT of STD control (mass treatment) in rural Uganda	17178	Longitudinal GEE
2. Hawes, 1996 (90)	Women from Seattle STD clinic	182	Cox PH survival analysis
3. Baeten, 2001 (107)	Cohort of female sex workers (HIV negative) in Kenya	948	Cox PH survival analysis
4. Bradshaw, 2005 (71)	Women presenting with symptoms of abnormal discharge in Australia	342	Cross-sectional
5. Chiaffarino, 2004 (83)	Recruited from outpatient gynecology clinics	926	Cross-sectional (case-control study)
6. Schwebke, 2004 (81)	Recruited women who douched from adolescent clinics, primary care clinics and universities.	250	Cross-sectional, no adjustment
7. Smart, 2003 (30)	Women attending Sydney Sexual Health Center	1780	Cross-sectional
8. Ness, 2003 (33)	Women at high risk for STDs, recruited from clinics, student health services, and health departments	1200	Longitudinal - reported cross-sectional at baseline
9. Fonck, 2001 (85)	RCT trial of antiobiotic treatment of STDs to reduce the incidence of HIV/STDs in Kenya	543	Cross-sectional
10. Calzolari, 2000 (35)	Women attending periodical preventive examinations at gynecology clinic in Rome	1314	Cross-sectional
11. Rajamanoharan, 1999 (88)	Women attending an STD clinic in London	200	Cross-sectional (case-control)
12. Shoubnikova, 1997 (47)	Swedish Women's Health Study recruited from family planning clinics	956	Cross - sectional
13. Rosenberg, 1992 (108)	Recruited from the Denver Metro Health Clinic for STDs	5353	Cross-sectional
14. Moi, 1990 (34)	STD Clinic	443 2259	Cross-sectional, no adjustment

Table 7: Literature review of published studies that report an association between condom use and bacterial vaginosis, Part B - Outcome measurement, definition of condom use (exposure assessment), risk estimate, and 95% confidence interval.

Study	Outcome	Exposure	Risk estimate	95% CI
1. Saifuddin, 2001 (106)	BV (Gram stain)	Condom Use Irregular Consistent	 OR = 1.11 OR = 0.89	 (0.99-1.25) (0.74-1.07)
2. Hawes, 1996 (90)	BV (Amsel)	Barrier methods	HR = 0.8	(0.3-1.7)
3. Baeten, 2001 (107)	BV (Gram stain)	100% Condom Use	HR = 0.9	(0.7-1.0)
4. Bradshaw, 2005 (71)	BV (Gram stain)	100% Condom Use	OR = 1.2	(0.7-1.9)
5. Chiaffarino, 2004 (83)	BV (PIP activity test)	Barrier method	OR = 1.0	(0.7-1.4)
6. Schwebke, 2004 (81)	BV (Gram stain)	Condom Use Condom use (Every time) Condom use last 5 times	OR = 1.08* OR = 1.09* OR = 0.78*	(0.65-1.78) (0.60-1.98) (0.44-1.37)
7. Smart, 2003 (30)	BV (Gram stain)	Condom Use last 3 mos Sometimes (<50%) Usually (>50%) Always (100%)	 OR = 0.80 **OR = 0.60** **OR = 0.50**	 (0.59-1.14) **(0.43-0.83)** **(0.50-0.71)**
8. Ness, 2003 (33)	BV (Gram stain)	Condom Use consistency <5/10 times 6-9/10 times 10/10 times	 OR = 1.4 OR = 1.2 OR = 0.9	 (0.9-2.2) (0.8-2.0) (0.6-1.4)
9. Fonck, 2001 (85)	BV (Gram stain)	Condom Use (Always as referent) Never Sometimes	 OR = 0.9 OR = 0.9	 (0.5-1.5) (0.5-1.5)
10. Calzolari, 2000 (35)	BV (Amsel)	Condom Use	**OR = 0.56**	**(0.33-0.96)**
11. Rajamanoharan, 1999 (88)	BV (Gram stain)	Barrier methods	OR = 0.5	(0.2-1.5)
12. Shoubnikova, 1997 (47)	BV (Amsel)	Condom Use	**OR = 0.3**	**(0.1-0.9)**
13. Rosenberg, 1992 (108)	BV (Amsel)	Condom Use	OR = 1.21	(0.97-1.53)
14. Moi, 1990 (34)	BV (Amsel)	Barrier methods	**OR = 0.58***	**(0.45-0.75)**

* Odds ratio computed from data.

1.2.7 Conclusions

Bacterial vaginosis is one of the least understood infections in reproductive age women. Despite the knowledge regarding the physiology of BV infection, relatively little is known about the underlying causes of changes in vaginal flora and the acquisition of BV. BV is an extremely complex disease that is marked by extensive variability among women. Half of women with BV are asymptomatic (15), and women with BV will experience extreme variability in the length of infection (2). Very little is understood about changes in vaginal flora or conditions that result in persistent infection. Douching significantly alters the vaginal flora (3) and despite the increasing evidence for harmful effects (4), douching is a common practice among women in the US (5). Numerous studies have shown an association between douching and BV; however, the timing of the relationship between douching and BV is not well characterized and it is unclear what effect douching has on the long-term maintenance of healthy vaginal flora. Additionally, very little is known about factors leading to the acquisition of BV and evidence linking BV to sexual transmission remains controversial (6). Studies investigating the association between male colonization of BV-associated organisms have not supported sexual transmission of BV (6); however, whether or not condoms may prevent BV has not been well studied. Thus, we propose to further characterize time-dependent variations in vaginal flora by assessing factors that influence the persistence and acquisition of BV.

1.3 LITERATURE CITED

1. Beigi R, Wiesenfeld H, Hillier S, Straw T, Krohn M. (2005) Factors Associated with Absence of H_2O_2 – Producing Lactobacillus among Women with Bacterial Vaginosis. *J Inf Dis*. 191:924-9.
2. McGregor J, French J. (2000) Bacterial Vaginosis in Pregnancy. *Obstet Gynecol Surv*. 55:1-19.
3. Martino J, Vermund S. (2002) Vaginal Douching: Evidence for Risks and Benefits to Women's Health. *Epidemiol Rev*. 24:109-124.
4. Zhang J, Thomas G, Leybovich E. (1997) Vaginal Douching and Adverse Health Effects: A Meta-Analysis. *Am J Public Health*. 87:1207-11.
5. Abma J, Chandra A, Mosher W, Peterson L, Piccinino L. Fertility, family planning, and women's health: New data from the 1995 National Survey of Family Growth. *Vital Health Stat*. 23:1-114.
6. Morris M, Nicoll A, Simms I, Wilson J, Catchpole M. (2001) Bacterial vaginosis: a public health review. *BJOG*. 108:439-50.
7. Wilson J. (2004) Managing recurrent bacterial vaginosis. *Sex Transm Infect*. 80:8-11.
8. Hay P. (2004) Life in the littoral zone: lactobacilli losing the plot. *Sex Transm Infect*. 81:100-2.
9. Sweet R. (2000) Gynecologic conditions and bacterial vaginosis: Implications for the Non-Pregnant Patient. *Inf Dis Obstet Gynecol*. 8:184-90.
10. Koumans E, Markowitz L, Hogan V. (2002) Indications for Therapy and Treatment Recommendations for Bacterial Vaginosis in Nonpregnant and Pregnant Women: A Synthesis of Data. *Clin Infect Dis*. 35(Suppl 2):S152-72.
11. Sobel J. (1997) Vaginitis. *NEJM*. 337:1896-1903.
12. Gutman R, Peipert J, Weitzen S, Blume J. (2005) Evaluation of Clinical Methods for Diagnosing Bacterial Vaginosis. *Obstet Gynecol*. 105:551-6.
13. Schwebke J, Hillier S, Sobel J, McGregor J, Sweet R. (1996) Validity of the Vaginal Gram Stain for the Diagnosis of Bacterial Vaginosis. *Obstet Gynecol*. 88:573-6.
14. Keane F, Maw, Pritchard C, Ison. (2004) Methods employed by genitourinary medicine clinics in the United Kingdom to diagnose bacterial vaginosis. *Sex Transm Infect*. 81:155-57.
15. Forsum U, Hallen A, Larsson P (2005) Bacterial vaginosis – a laboratory and clinical diagnostics enigma. *APMIS*. 113:153-61.
16. Nugent R, Krohn M, Hillier S. (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 29:297-301.
17. Sobel J. (2000) Bacterial Vaginosis. *Annu Rev Med*. 51:349-56.
18. Burstein G, Workowski K. (2003) Sexually transmitted diseases treatment guidelines. *Curr Opin Pediatr*. 15:391-7.
19. Koumans E, Kendrick J. (2000) Preventing Adverse Sequelae of Bacterial Vaginosis. *Sexually Trans Dis*. 28:292-7.

20. Potter B, Jhorden L. (2004) Should we screen for bacterial vaginosis in those at risk for preterm labor? *J Fam Pract*. 53:827-31.
21. Forsum U, Holst E, Larsson P, Vasquex A, Jakobsson T, Mattsby-Baltzer I. (2005) Bacterial vaginosis – a microbiological and immunological enigma. *APMIS*. 113:81-90.
22. Boon M, Claasen H, Kok L. (2002) Urbanization and baseline prevalence of genital infections including *Candida, Trichomonas*, and human papillomavirus and of a disturbed vaginal ecology as established in the Dutch Cervical Screening Program. *Am J Obstet Gynecol*. 187:365-9.
23. Eschenbach D. (1993) History and Review of Bacterial Vaginosis. *Am J Obstet Gynecol*. 169:441-45.
24. Kent H. (1991) Epidemiology of vaginitis. *Am J Obstet Gynecol*. 165:1168-76.
25. Priestly C, Kinghorn G. (1996) Bacterial Vaginosis. *Br J Clin Pract*. 50:331-4.
26. Mead P. (1993) Epidemiology of Bacterial Vaginosis. *Am J Obstet Gynecol*. 446-9.
27. Berger B, Kolton S, Zenilman J, Cummings M, Feldman J, McCormack. (1995) Bacterial Vaginosis in Lesbians: A Sexually Transmitted Disease. *Clin Inf Dis*. 21:1402-5.
28. Marrazzo J, Koutsky L, Eschenbach D, Agnew K, Stine K, Hillier S. (2002) Characterization of Vaginal Flora and Bacterial Vaginosis in Women Who Have Sex with Women. *J Inf Dis*. 185:1307-13.
29. Bump R, Buesching W. (1988) Bacterial vaginosis in virginal and sexually active adolescent females: Evidence against exclusive sexual transmission. *Obstet Gynecol*. 158:935-9.
30. Smart S, Singal A, Mindel A. (2004) Social and sexual risk factors for bacterial vaginosis. *Sex Trans Infect.* 80:58-62.
31. Morris M, Rogers P, Kinghorn G. (2001) Is bacterial vaginosis a sexually transmitted infection? *Sex Trans Inf*. 77:63-68.
32. Nelson D, Macones G. (2002) Bacterial Vaginosis in Pregnancy: Current Findings and Future Directions. *Epidem Rev*. 24:102-8.
33. Ness R, Hillier S, Richter H, Soper D, Stamm C, Bass D, Sweet R, Rice P. (2003) Can Known Risk Factors Explain Racial Differences in the Occurrence of Bacterial Vaginosis? *J Natl Med Assoc*. 95:201-212.
34. Moi H. (1990) Prevalence of bacterial vaginosis and its association with genital infections, inflammation, and contraceptive methods in women attending sexually transmitted disease and primary health clinics. *Int J STD & AIDS*. 1:86-94.
35. Calzolari E, Masciangelo R, Milite V, Verteramo R. (2000) Bacterial vaginosis and contraceptive methods. *Int J Gynecol Obstet*. 70:341-346.
36. Schwebke J. (2003) Gynecologic consequences of bacterial vaginosis. *Obstet Gynecol Clin N Am*. 30:685-94.
37. Flynn C, Helwig A, Meurer L. (1999) Bacterial Vaginosis in Pregnancy and the Risk of Prematurity: A Meta-Analysis. *J Family Prac*. 48:885-92.
38. Simms I, Stephenson J. (2000) Pelvic inflammatory disease epidemiology: what do we know and what do we need to know? *Sex Transm Infect.* 76:80-7.
39. Haggerty C, Hillier S, Bass D, Ness R. (2004) Bacterial Vaginosis and Anaerobic Bacteria are Associated with Endometritis. *Clin Infect Dis*. 39:990-5.
40. Pybus V, Onderdonk A. (1999) Microbial interactions in the vaginal ecosystem, with emphasis on the pathogenesis of bacterial vaginosis. *Microb Inf*. 1:285-92.

41. Keane F, Ison C, Taylor-Robinson D. (1997) A longitudinal study of the vaginal flora over a menstrual cycle. *Int J STD & AIDS*. 8:489-94.
42. Hillier S, Krohn M, Rabe L. (1993) Normal vaginal flora, H_2O_2-producing lactobacilli, and bacterial vaginosis in pregnant women. *Clin Infect Dis*. 16:S273-81.
43. Nagy E, Petterson M, Mardh P. (1991) Antibiosis between bacteria isolated from the vagina of women with and without signs of bacterial vaginosis. *APMIS*. 99:739-44.
44. Hillier S, Krohn M, Klebanoff S, Eschenbach D. (1992) The Relationship of Hydrogen Peroxide-Producing Lactobacilli to Bacterial Vaginosis and Genital Microflora in Pregnant Women. *Obstet Gynecol*. 79:369-73.
45. Taylor-Robinson D, Hay P. (1997) The pathogenesis of the clinical signs of bacterial vaginosis and possible reasons for its occurrence. *Int J STD & AIDS*. 8:13-6.
46. Iwasaka T, Wada T, Kidera Y, Sugimori H. (1986) Hormonal Status and Mycoplasma Colonization in the Female Genital Tract. *Obstet Gynecol*. 68:263-6.
47. Shoubnikova M, Hellberg D, Nilsson S, Per-Anders M. (1997) Contraceptive Use in Women with Bacterial Vaginosis. *Contraception*. 55:355-58.
48. Mardh P. (1991) The vaginal ecosystem. *Am J Obstet Gynecol*. 165:1163-8.
49. Brown D. (2005) Bacterial Vaginosis, in Benign Diseases of the Vulva and Vagina. Elsevier Mosby, Philadelphia PA. Fourth Edition.
50. Schwebke J. (2000) Asymptomatic bacterial vaginosis: Response to therapy. *Am J Obstet Gynecol*. 183:1434-9.
51. Cauci S. (2004) Vaginal Immunity in Bacterial Vaginosis. *Curr Infect Dis Reports*. 6:450-56.
52. Horowitz B, Mardh P, editors. (1991) Vaginitis and Vaginosis. Wiley-Liss, New York.
53. Kuby J (1997) Immunology. WH Freeman and Company, New York. Third Edition.
54. Cauci S, Scrimin F, Driussi S, Ceccone S, Monte R., Fant L, Quadrifoglio F. (1996) Gynecology: Specific immune response against *Gardnerella vaginalis* hemolysin in patients with bacterial vaginosis. *Am J Obstet Gynecol*. 175:1601-05.
55. Apgar B, Greenberg G, Yen G. (2005) Prevention of Group B Streptococcal Disease in the Newborn. *Am Fam Physician*. 71:903-10.
56. Moore K, Day R, Albers M. (2002) Pathogenesis of urinary tract infections: a review. *J Clin Nurs*. 11:568-73.
57. Hooton T, Hillier S, Johnson C, Roberts P, Stamm W. (1991) *Escherichia coli* Bacteriuria and Contraceptive Method. *JAMA*. 265:64-9.
58. Hill G. (1993) The microbiology of bacterial vaginosis. *Am J Obstet Gynecol*. 169:450-4.
59. Koch A, Bilina A, Teodorowicz L, Stary A. (1997) *Mycoplasma hominis* and *Ureaplasma urealyticum* in patients with sexually transmitted disease. *Wien Klin Woch*. 109:584-9.
60. Arya O, Tong C, Hart C, Pratt B, Hughes S, Roberts P, Kirby P, Howel J, McCormack A, Goddard A. (2001) *Sex Trans Inf*. 77:58-62.
61. Paavonen J, Miettinen A, Stevens C, Chen K, Holmes K. (1983) *Mycoplasma hominis* in Nonspecific Vaginitis. *Sex Trans Dis*. 10:271-5.
62. Hallen A, Pahlson C, Forsum U. (1988) Rectal occurrence of *Mobiluncus* species. *Genitourin Med*. 64:273-5.
63. Hallen A, Pahlson C, Forsum U. (1987) Bacterial vaginosis in women attending STD clinic: diagnostic criteria and prevalence of *Mobiluncus* spp. *Genitourin Med*. 63:386-9.
64. Fredricks D, Fiedler T, Marrazzo J. (2005) Molecular Identification of Bacteria Associated with Bacterial Vaginosis. *NEJM*. 353:1899-1911.

65. Taylor-Robinson D, Furr P. (1998) Update on sexually transmitted mycoplasmas. *Lancet*. 351:12-15.
66. Thorsen P, Jensen I, Jeune B, Ebbesen N, Magnus A, Bremmelgaard A, Moller B. (1998) Few microorganisms associated with bacterial vaginosis may constitute the pathologic core: A population-based microbiologic study among 3596 pregnant women. *Am J Obstet Gynecol*. 178:580-87.
67. Ness R, Kip K, Hillier S, Soper D, Stamm C, Sweet R, Rice P, Richter H. (2005) A Cluster Analysis of Bacterial Vaginosis-associated Microflora and Pelvic Inflammatory Disease. *Am J Epi*. 162:1-6.
68. Ness R, Hillier S, Kip K, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P, Richter H. (2004) Bacterial Vaginosis and Risk of Pelvic Inflammatory Disease. *Obstet Gynecol*. 104:761-9.
69. Boris J, Pahlson C, Larsson P. (1997) Six-year follow-up after successful treatment of bacterial vaginosis. *Int J STD AIDS*. 8(Suppl 1):41.
70. Cook R, Reedondo-Lopez V, Schmitt C, Meriwether C, Sobel J. (1992) Clinical, Microbiological, and Biochemical Factors in Recurrent Bacterial Vaginosis. *J Clin Microbio*. 30:870-7.
71. Bradshaw C, Morton A, Garland S, Morris M, Moss L, Fairley C. (2005) Higher-Risk Behavorial Practices Associated With Bacterial Vaginosis Compared With Vaginal Candidiasis. *Obstet Gynecol*. 106:105-14.
72. Ness R, Kip K, Soper D, Stamm C, Rice P, Richter H. (2006) Variability of Bacterial Vaginosis over 6-12 month intervals. *Sex Trans Dis*.
73. Schwebke J, Richey C, Weiss H. (1999) Correlation of Behaviors with Microbiologic Changes in Vaginal Flora. *J Inf Dis*. 180:1632-6.
74. Rosenberg M, Phillips R, Holmes M. (1991) Vaginal Douching: Who and Why? *J Repro Med*. 36:753-8.
75. Ness R, Hillier S, Richter H, Soper D, Stamm C, Bass D, Sweet R, Rice P, Downs J, Aral S. (2000) Why Women Douche and Why They May or May Not Stop. *Sex Trans Dis*. 30:71-4.
76. Ness R, Hillier S, Richter H, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P. (2002). Douching in Relation to Bacterial Vaginosis, Lactobacilli, and Facultative Bacteria in the Vagina. *Obstet Gynecol*. 100:765-72.
77. Pavlova S, Tao L. (2000) In vitro inhibition of commercial douche products against vaginal microflora. *Infect Dis Obstet Gynecol*. 8:99-104.
78. Juliano C, Piu L, Gavini E, Zanetti S, Fadda G. (1992) In vitro antibacterial activity of antiseptics against vaginal lactobacilli. *Eur J Clin Microbiol Infect Dis*. 11:1166-9.
79. Monif G, Thompson J, Stephen H, Baer H. (1980) Quantitative and qualitative effects of povidone-iodine liquid and gel on the aerobic and anaerobic flora of the female genital tract. *Am J Obstet Gynecol*. 137:432-8.
80. Demba E, Morison L, van der Loeff M, Awasana A, Gooding E, Bailey R, Mayaud P, West B. (2005) Bacterial vaginosis, vaginal flora patterns and vaginal hygiene practices in patients presenting with vaginal discharge syndrome in The Gambia, West Africa. *BMC Inf Dis*. 5:12.
81. Schwebke J, Desmond R, Oh M. (2004) Predictors of Bacterial Vaginosis in Adolescent Women who Douche. *Sex Trans Dis*. 31:433-36.

82. Bailey J, Farquhar C, Owen C. (2004) Bacterial Vaginosis in Lesbians and Bisexual Women. *Sex Trans Dis*. 31:691-94.
83. Chiaffarino F, Parazzini F, Besi P, Lavezzari M. (2004) Risk factors for bacterial vaginosis. *Eur J Obstet Gynecol Reprod Biol*. 117:222-6.
84. Vermund S, Sarr M, Murphy D, Levin L, Abdalian S, Ma Y, Crowley-Nowick P, Wilson C. (2001) Douching Practices Among HIV Infected and Uninfected Adolescents in the United States. *J Adolescent Health*. 29S:80-6.
85. Fonck K, Kaul R, Keli F, Bwayo J, Ngugi E, Moses S, Temmerman M. (2001) Sexually transmitted infections and vaginal douching in a population of female sex workers in Nairobi, Kenya. *Sex Trans Inf*. 77:271-75.
86. Holzman C, Leventhal J, Qiu H, Jones N, Wang J. (2001) Factors Linked to Bacterial Vaginosis in Nonpregnant Women. *Am J Public Health*. 91:1664-70.
87. Newton E, Piper J, Shain R, Perdue S, Paeirs W. (2001) Predictors of vaginal microflora. *Am J Obstet Gynecol*. 184:845-55.
88. Rajamanoharan S, Low N, Jones S, Pozniak A. (1999) Bacterial Vaginosis, Ethnicity, and the Use of Genital Cleaning Agents: A Case Control Study. *Sex Trans Dis*. 26:404-9.
89. La Ruche G, Messou N, Ali-Napo L, Noba V, Faye-Kette H, Combe P, Bonard D, Syllakoko F, Dheha D, Welffensekra C, Dosso M, Msellati P. (1999) Vaginal Douching: Association with Lower Genital Tract Infections in African Pregnant Women. *Sex Trans Dis*. 26:191-96.
90. Hawes S, Hillier S, Benedietti J, Stevens C, Koutsky L, Wolner-Hanssen, Holmes K. (1996) Hydrogen Peroxide-Producing Lactobacilli and Acquisition of Vaginal Infections. *J Infect Dis*. 174:1058-63.
91. Holst E. (1990) Reservoir of Four Organisms Associated with Bacterial Vaginosis Suggests Lack of Sexual Transmission. *J Clin Microbiol*. 28:2035-9.
92. Larsson P, Bergstrom M, Forsum U, Jacobsson b, Strand A, Wolner-Hanssen P. (2005) Bacterial vaginosis: Transmission, role in genital tract infection and Pregnancy outcome: an enigma. *APMIS*. 113:233-45.
93. Shlay J, McClung M, Patnaik J, Douglas J. (2004) Comparison of Sexually Transmitted Disease Prevalence by Reported Level of Condom Use Among Patients Attending an Urban Sexually Transmitted Disease Clinic. *Sex Trans Dis*. 31:154-60.
94. Holmes K, Levine R, Weaver M. (2004) Effectiveness of condoms in preventing sexually transmitted infections. *Bull World Health Org*. 82:454-461.
95. United States Department of Health and Human Services. (2001) Workshop summary: scientific evidence on condom effectiveness for sexually transmitted disease (STD) prevention. http://www.niaid.nih.gov/dmid/stds/condomreport.pdf. Accessed November 25, 2005
96. Fitch J. (2001) Are Condoms Effective in Reducing the Risk of Sexually Transmitted Disease? *Ann Pharmacother*. 35:1136-8.
97. Ellish N, Weisman C, Celentano D, Zenilman J. (1996) Reliability of Partner Reports of Sexual History in a Heterosexual Population at a Sexually Transmitted Disease Clinic. *Sex Trans Dis*. 23:446-52.
98. Crosby R, DiClemente R, Holtgrave D, Wingood G. (2002) Design, measurement, and analytical considerations for testing hypotheses relative to condom effectiveness against non-viral STIs. *Sex Trans Infect*. 78:228-31.

99. Wolner-Hanssen P, Eschenbach D, Paavonen J, Stevens C, Kiviat N, Critchlow C, DeRouen T, Koutsky L, Holmes K. (1990) Association Between Vaginal Douching and Acute Pelvic Inflammatory Disease. *JAMA*. 263:1936-41.
100. Warner L, Macaluso M, Austin H, Kleinbaum D, Artz L, Fleenor M, Brill I, Newman D, Hook E. (2005) Application of the Case-Crossover Design to Reduce Unmeasured Confounding in Studies of Condom Effectiveness. *Am J Epi*. 161:765-73.
101. Warner L, Newman D, Austin H, Kamb M, Douglas J, Malotte C, Zenilman J, Rogers J, Bolan G, Fishbein M, Kleinbaum D, Macaluso M, Peterman T. (2004) Condom Effectiveness for Reducing Transmission of Gonorrhea and Chlamydia: The Importance of Assessing Partner Infection Status. *Am J Epi*. 159:242-51.
102. Warner L, Clay-Warner J, Boles J, Williamson J. (1998) Assessing Condom Use Practices: Implications for Evaluating Method and User Effectiveness. *Sex Trans Dis.* 25:273-77.
103. Devine O, Aral S. (2004) The Impact of Inaccurate Reporting of Condom Use and Imperfect Diagnosis of Sexually Transmitted Disease Infection in Studies of Condom Effectiveness. *Sex Trans Dis*. 31:588-95.
104. Zenilman J, Weisman C, Rompalo A, Ellish N, Upchurch D, Hook E, Celentano D. (1995) Condom Use to Prevent Incident STDs: The Validity of Self-Reported Condom Use. *Sex Trans Dis.* 22:15-21.
105. Galavotti C. Cabral R, Beeker C. (1996) Condom Use to Prevent Incident STDs. *Sex Trans Dis.* 23:77-79.
106. Saifuddin A, Lutalo T, Wawer M, Serwadda D, Sewankambo N, Nalugoda F, Makumbi F, Wabwire-Mangen F, Kiwanuka N, Kigozi F, Kiddugavu M, Gray R. (2001) HIV incidence and sexually transmitted disease prevalence associated with condom use: a population study in Rakai, Uganda. *AIDS*. 15:2171-79.
107. Baeten J, Nyange P, Richardson B, Lavreys L, Cohan B, Martine H, Mandaliya K, Ndinya-Achola J, Bwayo J, Dreiss J. (2001) Hormonal contraception and risk sexually transmitted disease acquisition: Results from a prospective study. *Am J Obstet Gynecol*. 185:380-385.
108. Rosenberg M, Davidson A, Chen J, Judson F, Douglas J. (1992) Barrier Contraceptives and Sexually Transmitted Diseases in Women: A Comparison of Female-Dependent Methods and Condoms. *Am J Public Health*. 82:669-674.
109. Haukkamma M, Stranden P, Jousimies-Somer H, Siitonen A. (1986) Bacterial flora of the cervix in women using different methods of contraception. *Am J Obstet Gynecol*. 154:520-4.
110. McCaffrey M, Varney P, Evans B, Taylor-Robinson D. (1999) Bacterial vaginosis in lesbian: evidence for lack of sexual transmission. *Int J STD & AIDS*. 10:305-8.
111. Centers for Disease Control and Prevention. (1993) Update. Barrier protection against HIV infection and other sexually transmitted diseases. *Morb Mortal Wkly Report*. 42:589-91.
112. Centers for Disease Control and Prevention (2002). Sexually Transmitted Disease Guidelines, 2002. *Morb Mortal Wkly Report*. 51:1-80.
113. Verstraelen H, Verhelst R, Claeys G, Temmerman M, Vaneechoutte M. (2004) Culture-independent analysis of vaginal microflora: The unrecognized association of Atopobium vaginae with bacterial vaginosis. *Am J Obstet Gynecol.* 191:1130-2.

2.0 MANUSCRIPT 1: PERSISTENT BACTERIAL VAGINOSIS (BV): WHO HAS IT AND WHAT ARE THE RISK FACTORS?

Manuscript in preparation

Katherine H. Berger*, Kevin Kip*, Roberta B. Ness*;
for the Gynecologic Infections Follow-through (GIFT) Investigators

* Department of Epidemiology, University of Pittsburgh
Pittsburgh, Pennsylvania, 15261

2.1 ABSTRACT

Objectives: To examine characteristics of women with persistence of bacterial vaginosis (BV) and BV-associated organisms over 3-4 years of follow-up.

Methods: A total of 1032 women were followed for a median of 3 years. Vaginal swabs were obtained for Gram-stain diagnosis of BV, culture of microflora, and *Neisseria gonorrhoeae* and *Chlamydia trachomatis* at baseline and every 6 to 12 months thereafter. Baseline risk factors were evaluated for their association with the persistence of BV (BV at more than 50% of visits) and BV-associated organisms throughout follow-up.

Results: Thirty percent of women had BV diagnosed at more than half of visits. One-quarter to one-third of women persistently lacked hydrogen peroxide-producing lactobacilli and had persistently heavy growth of *G. vaginalis* and anaerobic Gram-negative pigmented rods. After adjusting for confounding factors, only black race (adjusted RR 1.47, 95% CI 1.09, 1.98) and a baseline Gram-stain of BV (adjusted RR 6.60, 95% CI 4.41, 9.87) significantly increased the risk of persistent BV. Other factors, associated with BV in univariate analyses (such as education, sexual activity, use of hormonal contraception, and a history of vaginal infections) were not significantly associated with persistent BV after adjustment for baseline BV status.

Conclusions: A large proportion of women had persistent BV or persistently high growth of BV-associated organisms. Baseline flora status and black race were the only significant predictors of persistent BV.

2.2 INTRODUCTION

Bacterial vaginosis (BV) is one of the most prevalent vaginal infections in women with prevalence estimates ranging from less than 5% to over 40% in various subgroups (1). BV results from a shift in vaginal flora in which anaerobic and facultative aerobic bacteria dominate at the expense of the normally dominant hydrogen peroxide-producing *Lactobacillus* species (2).

The natural history of BV is poorly understood (3, 4). While BV occurs in acute, chronic, and recurrent forms, little is understood about the causes of microflora heterogeneity among women or about variation over time in a given woman. Transient changes have been shown to occur during the menstrual cycle, and factors such as the number of sexual partners, spermicide use, frequency of vaginal intercourse and use of condoms have been associated with day-to-day changes in vaginal flora (4, 5). Nonetheless, recurrence (6) is common and studies following women after treatment for BV have shown recurrence rates of up to 30% after 3 months (2). Women who maintained abnormal vaginal symptoms following treatment, despite resolution of clinical BV, were particularly likely to have a recurrent infection, suggesting that some women treated for BV are unable to reestablish and maintain normal vaginal flora (7).

We sought to characterize women who experienced persistent infections with BV and high levels of BV-associated flora. We examined changes in vaginal flora over three years of follow-up in a cohort of women at high risk for STDs and assessed factors associated with persistent BV and high levels of BV-associated vaginal organisms.

2.3 MATERIALS AND METHODS

2.3.1 Patient selection

The methods used for subject enrollment, data collection and follow-up have been reported in detail elsewhere (8, 9). Briefly, women 13-36 years of age were recruited into the GYN Infections Follow-Through (GIFT) Study from family planning clinics, university health clinics, gynecology clinics, and sexually transmitted disease units at each of five US sites between May 1999 and June 2001. Human subjects approval was obtained at each participating institution, and all women signed informed consent. Women were eligible for the study if they were not specifically seeking care for a sexually transmitted disease, yet, based upon a previous risk stratification paradigm for chlamydial cervicitis (10), were considered at high risk for acquiring a bacterial sexually transmitted infection. Specifically, to be enrolled, a women had to have a score of 3 or more points on a algorithm in which points were derived as follows: age 24 or less = 1; black race = 2; never pregnant =1; 2 or more sexual partners = 1; douches at least once per month = 2; and any prior sexually transmitted infection, including *N. gonorrhoeae*, *C. trachomatis*, and *Trichomonas vaginalis* = 2. Of the 2740 women screened for study entry, 853 (31.1%) did not meet these inclusion criteria. An additional 259 (9.5%) women were excluded on the basis of priori criteria such as being pregnant, married, or virginal, or being on antibiotics at baseline. Among the 1628 women who were eligible for the study, 1199 (73.6%) completed a baseline questionnaire.

2.3.2 Microbiologic methods for evaluation and categorization of vaginal flora

At baseline and every 6-12 months thereafter, each subject obtained her own vaginal specimens with a cotton swab (11). Smears from these swabs were gram stained and a microscopy score of 0-10 was assigned by laboratory staff using the standardized method described by Nugent et al. (12). A score of 0-3 was interpreted as consistent with normal vaginal flora; a score of 4-6, corresponding to disturbed flora, was designated as intermediate; and a score of 7-10 was considered to be BV.

Two swabs, placed in an anaerobic transport vial, were also shipped to the microbiology laboratory for characterization of the following: *Lactobaccillus* species, anaerobic Gram-negative rods, *Gardnerella vaginalis*, group B streptococcus, *Enterococcus* species, *Escherichia coli*, *Candida* species, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. Lactobacilli were identified to the genus level on the basis of Gram's-stain morphology and production of lactic acid. The amount of growth for each of these microorganisms was recorded on a semiquantative scale from 0 to 4.

Consistency of vaginal flora scores was determined for women with two or more vaginal microbiology samples. Consistency of BV was categorized according to the proportion of visits in which women had BV (0, 1-50%, >50%). Consistency of other vaginal flora, including *G. vaginalis*, *M. hominis*, H_2O_2-producing lactobacilli, and anaerobic Gram-negative rods (pigmented and nonpigmented), were categorized based on a set of *a priori* categorizations as follows: 1) consistently high growth (score of 3 or more on at least 2 exams and no score less than 2, or 75% or more of visits with score of 3 or 4), 2) consistently low growth (score of 0 on at least 2 exams and no score greater than 1, or 75% or more of visits with score of 0), and 3) variable flora (all other combinations).

2.3.3 DNA amplification for *N. gonorrhoeae* and *C. trachomatis*

DNA amplification for *N. gonorrhoeae* and *C. trachomatis* was performed using a strand displacement DNA Amplification (SDA) Assay (Becton Dickinson, Sparks, MD) from self-obtained vaginal swabs. All positive test results for gonococcal or chlamydial infection were reported to the clinical sites within 1 week of enrollment where infected subjects were treated.

2.3.4 Other data collection

At baseline, women were asked about demographic factors, including age, race, education, income, pregnancy history, smoking, alcohol use, and drug use. They also reported relevant lifestyle behaviors such as number of sexual partners in the past 2 months, acquisition of a new partner in the past 2 months, contraception use, sex during menses, and douching practices. Douching practices were categorized according to frequency, reason for douching, and recentness of douching. Women were further requested to recall past episodes of sexually transmitted infections, including PID and gonococcal and/or chlamydial genital infections. Questions about pregnancy history, sexual activity, STDs, and douching were repeated during follow-up.

Consistency of behaviors for women with two or more follow-up visits were determined for douching, having a new partner, having more than 1 partner, use of hormonal contraception, and use of condoms according to the proportion of visits in which the behavior was recorded. Women were classified as never (no visits with behavior), 1 – 49% of visits with behavior, and 50% or more of visits with behavior.

2.3.5 Follow-up

Of the 1199 subjects, 31 (2.8%) had a baseline visit only and 6 (0.5%) did not obtain any vaginal swabs. Of the 1199 women, 1032 had at least two microbiology visits and were the focus of the analysis. Among the 1032 study participants, the median length of follow-up was 3.0 years (interquartile range: 2.4 – 3.4 years). The median number of follow-up visits was 6 (interquartile range: 5-7), and the median number of vaginal swab samples was 4 (interquartile range 3-4). Ninety-four percent of the women had four or more visits, and 85.8% of the women had 3 or more vaginal swab samples.

2.3.6 Statistical analyses

The proportion of visits in which BV and other vaginal flora were diagnosed was determined for each follow-up visit, and the consistency of vaginal flora throughout follow-up was described according to the proportion of visits in which vaginal flora was identified during the entire follow-up period. Descriptive baseline characteristics were compared for women according to the proportion of visits in which a woman was diagnosed with BV (0, 1-50%, >50%) using the chi-square test for trend. For behaviors that were recorded at each follow-up visit (douching, sexual activity, and contraceptive use), differences in the proportion of visits in which the behavior was recorded were determined for consistency of BV across visits. Differences between baseline status of vaginal Gram-stain and flora scores were also determined for consistency of BV throughout follow-up.

To assess the association between various baseline factors and persistent BV during the course of follow-up, risk ratios were estimated using log-binomial regression (13). When

models failed to converge, modified Poisson estimates with robust variance estimation were used (13, 14). The outcome was coded as 0 (0 – 49% of visits with BV) or 1 (>50% of visits with BV). Independent variables were identified based upon biologic plausibility and univariate analyses of possible predictors (Table 3). Final models included: black race (vs. white/other), less than high school education (vs. high school or more than high school education), current tobacco smoker (vs. previous/nonsmoker), use of hormonal contraception, history of BV, history of chlamydia/gonorrhea, only 1 sexual partner in the past 2 months (vs. 2 or more), new partner in past to 2 months, condom use in 10/10 sexual encounters (vs. <10/10 or no use), current douching, presence of chlamydia and/or gonorrhea, and vaginal flora growth score. Separate models were then run with each of the following outcomes based on consistently high flora growth for *G. vaginalis*, *M. hominis*, and anaerobic Gram-negative rods, and consistently low growth for hydrogen peroxide-producing lactobacilli. For all analyses, a *p*-value of <0.05 was considered statistically significant. All analyses were conducted using the SAS System for Window, version 8.02, Cary, NC.

2.4 RESULTS

Participants were predominantly aged 19-24 (66%), black (75%), and had an annual household income of less than $20,000 (74.0%). Almost half of participants reported a history of chlamydia and/or gonorrhea (46.1%) and upon study entry, 101 (9.8%) had a chlamydial infection and 47 (3.8%) had a gonococcal infection. Thirty-seven percent of women reported a history of BV and 405 (39.6%) women entered the study with a Gram-stain finding of BV.

Point-prevalence estimates for BV and H_2O_2-producing lactobacilli remained in a narrow range over time (Table 8). BV was present among 35% to 42% over the five follow-up visits and hydrogen peroxide producing lactobacilli were isolated from 45-54% of women over the visits. More than 80% of women harbored Gram-negative non-pigmented anaerobic bacteria over the course of visits. Some microflora showed a trend towards a decrease in prevalence, however, the proportion of missing microbiology samples significantly increased over time for *M. hominis* and *U. urealyticum*. Similarly, while there was a trend towards a decrease in the prevalence of *G. vaginalis*, the prevalence remained high (47-59%). In contrast, substantial declines in the prevalence of *C. trachomatis* and *N. gonorrhoeae* presumably resulted from study screening and treatment for these pathogens.

Thirty percent of women had BV at more than half of visits (categorized as consistently high), 32% were not diagnosed with BV at any point during follow-up (defined as consistently low) and 39% of women had BV at least once with at least one visit without BV (defined as variable) (Table 9). Nearly 26% of women had consistently heavy growth for H_2O_2-producing lactobacilli and 35% of women had consistently low growth. Over half of women showed significant variability for *M. hominis* and anaerobic Gram-negative rods (pigmented and nonpigmented). Relatively few women had consistently heavy growth for *M. hominis*, H_2O_2-negative lactobacilli, and anaerobic Gram-negative nonpigmented rods. Having consistently heavy growth was particularly common for anaerobic Gram-negative pigmented rods (33.5%) and *G. vaginalis* (31%).

Univariate analyses showed that women with persistent BV (>50% of visits) were significantly more likely than those without any BV or with BV at 50% or less of visits to have baseline characteristics of black race, less than high school education, and an income less than

$10,000 (Table 10). Furthermore, women who were current smokers, had ever been pregnant, had douched for more than 2 years, and had douched for reasons associated with hygiene were more likely to have persistent BV. Women who had a history of pelvic inflammatory disease, BV, chlamydia, gonorrhea, or trichomonas, and were young at first intercourse were more likely to have BV at any time during follow-up. Women who used hormonal contraception at 50% or more of visits had a lower likelihood of persistent BV (Table 11). Consistent use of douching throughout follow-up (at 50% or more of visits) was also associated with persistent BV. Other factors related to sexual activity, including number of partners, new partner, and use of condoms were not associated with persistent BV (Table 11). Sixty-one percent of women with BV at baseline had persistent BV (Table 12). Lacking H_2O_2-producing lactobacilli, having a high growth score (3 out of 4) for other vaginal organisms, and having chlamydia and/or gonorrhea cervicitis at baseline were all strongly associated with persistent BV (Table 12).

The strongest independent predictors for persistent BV were black race (adjusted RR = 1.47, 95% CI = 1.09, 1.98) and a baseline Gram-stain of BV (adjusted RR = 6.60, 95% CI = 4.41, 9.87) (Table 13). Growth of vaginal organisms was significantly associated with a dose-dependent increase in risk for persistent BV (Table 14). Heavy growth of *G. vaginalis*, anaerobic Gram-negative pigmented rods, and *M. hominis* were associated with a 2.9 to 4-fold increased risk of persistent BV (Table 14). Lacking H_2O_2+ lactobacilli was also strongly associated with persistent BV (adjusted RR = 3.79, 95% CI = 2.64, 5.44). Correspondingly, black race and baseline flora scores for each outcome (continuous 0-4) were also consistently associated with persistent heavy growth for *G. vaginalis*, *M. hominis*, anaerobic Gram-negative pigmented rods, and lacking hydrogen peroxide producing lactobacilli (Table 13). Smoking was also associated with persistently heavy growth of *G. vaginalis* (adjusted RR = 1.26, 95% CI =

1.06, 1.49) and anaerobic Gram-negative rods (adjusted RR = 1.30, 95% CI = 1.09, 1.55) (Table 13). Other factors commonly associated with BV in cross-sectional analyses, such as education, use of hormonal contraception, and a history of vaginal infections were not associated with persistent infection with BV, after adjusting for baseline flora score.

2.5 DISCUSSION

Among women at high risk for sexually transmitted infections, a significant proportion of women had persistent BV and persistently high scores of other flora throughout the 3-4 years of follow-up. Nearly one-third of women had BV at the majority of visits. Similar persistence of infection was seen with other vaginal flora. The strongest associations with persistent flora were seen for women with high vaginal Gram-stain scores or dense growth of BV-associated organisms at baseline. We found also found that black race was consistently associated with persistently high vaginal flora levels after controlling for confounding factors. Smoking was associated with the persistence of *G. vaginalis* and anaerobic Gram-negative rods, and condom use was associated with persistently heavy growth of *G. vaginalis*.

Longitudinal studies evaluating the occurrence of BV have found high rates of persistent or recurrent BV infections, however, few studies have examined the risk factors associated with recurrent or persistent BV. In studies on vaginal lactobacilli, little variation was observed and women predominantly maintained their vaginal status, irrespective of whether they had colonization with lactobacilli (15, 16). High levels of recurrence or persistence have also been observed in longitudinal studies and studies evaluating treatment efficacy. Schwebke et al. (6) found that among the 96 enrolled women, 67 women developed BV, and of those women, nearly

80% had either a persistent or recurrent episode during the study period. Studies evaluating recurrence of BV following treatment have also found high rates of recurrence. Women treated for BV with oral metronidazole have a recurrence rate of up to 30% after 3 months following successful treatment (3). In one study, during 6 years of follow-up, approximately 52% had at least one recurrent episode of BV (17). Despite the high rate of recurrence, it is unknown whether renewed episodes of BV result from reinfection, failure to completely treat an infection despite apparent resolution of BV symptoms, or failure to treat an unidentified pathogen. A study by Cook et al. (7) suggests that many women following treatment still have one or more abnormal symptoms (such as continued vaginal discharge, abnormal vaginal Gram-stain, and increased pH) despite clinical resolution of BV. These women were also more likely to have early recurrence of BV.

The continued persistence of BV among a high proportion of women may either be due to behavioral or intrinsic factors. Schwebke et al. (4) found that a history of bacterial vaginosis, as well as a few factors associated with sexual activity (number of lifetime sex partners, number of partners in the past 2 months, and episodes of receptive oral sex) were associated in univariate analyses with an overall cumulative characterization of unstable flora over a 6 week period (<85% of days with normal flora). However, only receptive oral sex remained significant when adjusting for potential confounders, suggesting that the impact of known behavioral factors on persistent unstable flora is limited and intrinsic factors may predominantly regulate persistence of BV. In the current study, we similarly found few factors were associated with persistent BV, after adjusting for confounding variables. Although characteristic inflammatory signs are absent in BV, a number of proinflammatory cytokines have been associated with BV and may mediate a women's ability to control and eradicate infection (18). Few studies have assessed genetic

polymorphisms in proinflammatory cytokines and direct association with BV. However, recent research has found a significant gene-environment interaction among BV and a TNF-α allele and the outcome of preterm birth, suggesting genetic polymorphisms in cytokines influence host response to BV (19). Additionally, recent research has shown significant variations in genetic polymorphisms for proinflammatory cytokines between black and white women (20). Black women were significantly more likely to have polymorphisms associated with increased expression of proinflammatory cytokines (IL1A, IL1B, IL6, IL18). Black race is a well known risk factor for BV, and black race is associated with BV independent of other risk factors (21). Similarly, in our analyses, black race was one of the few consistently significant factors associated with persistently high levels vaginal flora.

We also found that baseline flora status was a strong and consistent predictor of persistent BV. Similar to our work, a number of studies have shown that a history of BV significantly increased the likelihood of current BV (22, 23), and in a case-control study conducted in inner-city London which evaluated factors associated with BV, women with a history of BV had nearly a 13-fold increased likelihood of having BV (23). Thus, women with persistent BV may represent a subset of women that either cannot clear the infection, are unable to reestablish dominant levels of lactobacilli, or are more prone to reinfection following the initial acquisition of BV.

Nonetheless, it should be noted that in our study women who currently smoked were more likely to have persistently high levels of *G. vaginalis* and anaerobic Gram-negative rods. Additionally, prior studies on recurrent BV have shown that a history of vaginal infections and number of partners in the past year were associated with recurrent BV (24). Thus, environmental factors play some role in persistence or recurrence of BV and BV-associated flora.

Understanding how factors related influence persistent BV may also help us to understand whether women are reinfected, have an increased likelihood of reinfection, or are prone to recurrence following increased stress on the vaginal environment following the initial infection with BV.

The strengths of this study include the large number of women studied, enrollment of a high-risk population, which enhanced study power, use of consistent and standardized enrollment and data collection protocols, collection of biomarkers of effect, and the relatively long-term and complete longitudinal data collection. Women were assessed bi-annually, and annually over a 3-4 year period; thus, microbiology samples provide a fairly long-term evaluation of variability in vaginal flora.

There were a number of limitations to our study. One of the main limitations with this study was that microbiological assessments were taken over relatively long intervals and vaginal flora can vary on a day-to-day basis (6) and BV is more common at certain time points during the menstrual cycle (25). We did not evaluate the timing of the menstrual cycle in relation to vaginal swab collection. However, our repeated samples likely gave a general, albeit imprecise, estimate of variability in vaginal flora. Additionally, during follow-up we could not identify differences in persistent versus recurrent infection with BV. Factors leading to persistence of BV compared to those leading to a reinfection with may differ. We also did not obtain any information regarding treatment of BV infection, and were thus unable to assess recurrence/reinfection among women treated versus women who showed persistent infection or resolution of infection without treatment. However, BV status was not reported to participants; thus, any treatment would have been independent of this study.

The high level of persistence of BV among women in our study indicates that chronic BV is an important aspect in the natural history of BV. Given that BV is associated with increased acquisition of HIV and other adverse reproductive outcomes, it is necessary to increase awareness of BV in the clinical setting. Additionally, women should be counseled as to the high likelihood of recurrence. Finally, a search for host factors that re-set the vaginal microflora to preexisting conditions, is likely critical to long-term normalization of the vaginal ecosystem.

Table 8: Proportion of women with bacterial vaginosis, *N. gonorrheae*, *C. trachomatis*, lactobacilli (H_2O_2+ and H_2O_2-), *G. vaginalis*, *M. hominis*, and anaerobic bacteria and facultative aerobes.*

	Visit					
	Baseline (n=1185)	**6 Month (n=800)**	**12 month (n=784)**	**24 month (n=667)**	**36 month (n=397)**	***p***
Bacterial Vaginosis	39.6	34.8	38.2	34.6	42.1	0.84
Intermediate Flora	24.0	25.6	21.7	25.2	22.4	
Normal Flora	36.5	39.6	40.1	40.2	35.5	
N. gonorrheae	3.9	2.4	3.4	1.7	1.3	0.0006
C. trachomatis	10.0	6.2	5.7	7.3	5.3	0.001
Lactobacillus H_2O_2 +	49.6	50.9	51.2	54.3	45.2	0.72
Lactobacillus H_2O_2 -	19.9	21.8	16.2	12.9	9.6	<0.0001
G. vaginalis	58.9	54.0	52.9	47.9	49.1	<0.0001
M. hominis	45.1	44.2	42.0	37.2	43.3	0.01
Anaerobic GNR nonpigmented rods	81.8	83.0	83.7	83.3	82.8	0.69
Anaerobic GNR pigmented rods	50.0	45.4	49.0	46.3	55.5	0.58
U. urealyticum	66.7	63.9	64.2	61.3	61.0	0.01
Group B streptococcus	30.1	29.3	26.7	23.4	27.8	0.03
Enterococcus species	58.4	60.3	60.7	59.4	58.2	0.91
Escherichia coli	36.5	38.1	39.0	35.7	38.5	0.97
Yeast	17.0	16.0	15.8	10.9	16.9	0.14

* Not all subjects have complete microbiology data for all organisms assessed. Missing values range from 41 for Chlamydia to 200 for *M. hominis* at baseline. *M. hominis* and *U. urealyticum* are missing approximately 20% of samples across all visits. For other organisms, missing samples do not exceed 15 at any point during follow-up. Percentages are reported for proportions of available data for each organism.

Table 9: Consistency of bacterial vaginosis and other vaginal flora during follow-up for women who had 2 or more microbiology follow-up samples.

	Consistency of Vaginal Flora Across Follow-up					
	Low*	**%**	**Variable^**	**%**	**High****	**%**
Bacterial Vaginosis	326	31.6	397	38.5	309	29.9
H_2O_2 positive lactobacilli	356	34.5	409	39.7	266	25.8
H_2O_2 negative lactobacilli	773	75.0	247	24.0	11	1.1
G. vaginalis	280	27.2	427	41.4	324	31.4
M. hominis	347	38.9	451	50.5	95	10.6
Anaerobic Gram-negative black pigmented rods	68	6.6	618	59.9	345	33.5
Anaerobic Gram-negative nonpigmented rods	383	37.2	564	54.7	84	8.2

* Low score - BV: No diagnoses of BV, Other flora: score of 0 on at least 2 exams, no score greater than one or >75% of visits with score of 0

** High score - BV: >50% of visits with BV, Other flora: score of 3+ on at least 2 exams, no score less than 2, or >75% of visits with score of 3 or 4

^ Variable - BV: 1-49% of visits with BV, Other flora: All other combinations

Table 10: Descriptive baseline characteristics for women in the GIFT study by the proportion of visits in which a woman was diagnosed with BV throughout follow-up (n=1032).

	Proportion of visits with BV						
Baseline characteristic	**0**	**%**	**1 - 50%**	**%**	**> 50%**	**%**	***p*^**
	326	**31.6**	**397**	**38.5**	**309**	**29.9**	
Age							
13 - 18	17	5.2	28	7.1	18	5.8	0.20
19 - 24	227	69.6	258	65.0	197	63.8	
25 - 29	56	17.2	77	19.4	61	19.7	
30+	26	8.0	34	8.6	33	10.7	
Race							
White	111	34.1	62	15.6	34	11.0	<0.0001
Black	194	59.5	306	77.1	265	85.8	
Other	21	6.4	29	7.3	10	3.2	
Education							
< High school	40	12.3	81	20.4	75	24.3	<0.0001
High school	67	20.6	113	28.5	105	34.0	
> High school	219	67.2	203	51.1	129	41.8	
Income*							
< $10,000	108	36.4	177	48.8	155	56.2	<0.0001
$10,000 - $19,999	75	25.3	101	27.8	76	27.5	
≥ $20,000	114	38.4	85	23.4	45	16.3	
Smoking							
Current	88	27.0	140	35.4	143	46.3	<0.0001
Former	41	12.6	33	8.3	27	8.7	
Never	197	60.4	223	56.3	139	45.0	
Ever pregnant	129	39.6	245	61.7	204	66.0	<0.0001
Ever drink alcohol	241	73.9	290	73.1	235	76.1	0.55
History of vaginal infections							
PID	26	8.0	72	18.3	50	16.2	0.003
Bacterial Vaginosis	87	27.0	162	41.3	126	33.6	0.0001
Chlamydia	87	26.9	178	44.8	139	45.9	0.0001
Gonorrhea	47	14.5	102	25.7	82	27.2	0.0001
Trichomoniasis	49	15.0	111	28.0	83	27.4	0.0002
History of douching							
Never	213	65.3	228	57.4	150	48.5	<0.0001
< 2 years	30	9.2	50	12.6	42	13.6	
2 or more years	83	25.5	119	30.0	117	37.9	
Age at first Intercourse							
18+ years	75	23.1	66	16.7	47	15.2	0.003
16 - 17 years	129	39.7	131	33.1	117	37.9	
15 or less years	121	37.2	199	50.3	145	46.9	
Sex during menses	31	11.3	41	12.4	34	12.7	0.61
Use of antibiotics	72	22.1	92	23.2	58	18.8	0.33
Use of tampons	176	54.0	215	54.2	155	50.2	0.34

* 96 women missing information regarding income ^ *p* for trend

Table 11: Descriptive time-varying characteristics for women in the GIFT study by the proportion of visits in which a woman was diagnosed with BV throughout follow-up (n=1032).

	Proportion of visits with BV						
	0	**%**	**1 - 50%**	**%**	**> 50%**	**%**	***p*^**
	326	**31.6**	**397**	**38.5**	**309**	**29.9**	
Consistency of douching							
Never	184	56.4	172	38.1	101	22.7	<0.0001
1-49% of visits	67	20.6	123	40.9	95	34.8	
≥ 50% of visits	75	23	102	25.7	103	36.6	
New partner*							
Never	175	54.9	194	49.4	156	51.2	0.42
1-49% of visits	115	36.1	157	40	120	39.3	
≥ 50% of visits	29	9.1	42	10.7	29	9.5	
Number of partners >1*							
Never	224	70.2	243	61.8	179	58.7	0.02
1-49% of visits	69	21.6	112	28.5	99	32.5	
≥ 50% of visits	26	8.2	38	9.7	27	8.9	
Use of hormonal contraception							
Never	67	20.6	111	28	108	35	<0.0001
1-49% of visits	88	27	120	30.2	101	32.7	
≥ 50% of visits	171	52.5	166	41.8	100	32.4	
Condom Use*							
Never	42	13.2	43	10.9	46	15.1	0.86
1-49% of visits	81	25.4	105	26.7	69	22.6	
≥ 50% of visits	196	61.4	245	62.3	190	62.3	

* 15 women missing values throughout follow-up

^ *p* for trend

Table 12: Baseline vaginal flora by the proportion of visits in which a woman was diagnosed with BV throughout follow-up (n=1032).

	Proportion of Visits with BV						
Baseline Microbiology Status	**0**	**%**	**1-50%**	**%**	**>50%**	**%**	***p*^**
	326	**31.6**	**397**	**38.5**	**309**	**29.9**	
Gram-stain flora score							
Normal flora	220	59	127	34.1	26	7	n/a
Intermediate flora	103	42.0	110	44.9	32	13.1	
BV	--	--	158	39.0	247	61.0	
H_2O_2-producing lactobacilli							
0	75	14.5	207	40.1	234	45.4	<0.0001
1 - 2	24	22.4	49	45.8	34	31.8	
3 - 4	225	56.1	139	34.7	37	9.2	
Gardnerella vaginalis							
0	220	52.4	163	38.8	37	8.8	<0.0001
1 - 2	18	31.0	24	41.4	16	27.6	
3 - 4	86	15.8	208	38.1	252	46.2	
Anaerobic Gram-negative pigmented rods							
0	240	46.9	196	38.3	76	14.8	<0.0001
1 - 2	66	21.8	133	43.9	104	34.3	
3 - 4	17	8.2	66	31.7	125	60.1	
Anaerobic Gram-negative nonpigmented rods							
0	108	58.1	60	32.3	18	9.7	<0.0001
1 - 2	149	41.4	142	39.4	69	19.2	
3 - 4	66	13.8	193	40.5	218	45.7	
Mycoplasma hominis							
0	214	45.7	189	40.4	65	13.9	<0.0001
1 - 2	38	28.2	51	37.8	46	34.1	
3 - 4	17	6.8	89	35.6	144	57.6	
Chlamydia/gonorrhea							
No	292	33.3	345	39.3	241	27.5	<0.0001
Yes	25	19.4	43	33.3	61	47.3	

* N based on number of women with recorded baseline microbiology (9 women who had multiple follow-up visits did not have baseline microbiology)

^ *p* for trend

** 171 missing *M. hominis* at baseline

^^ 17 missing *C. trachomatis* and/or *N. gonorrhoeae* at baseline

Table 13: Adjusted risk ratios for baseline characteristics and persistent BV (50% or more of visits with BV) or high levels of microbiologic flora[#] over follow-up.

	BV		H_2O_2+ lactobacilli*		*G. vaginalis*^		*M. hominis*^		Anaerobic Gram-negative pigmented rods^	
Baseline characteristic	**Adj. RR**	**95% CI**	**Adj. RR**	**95% CI**	**Adj. RR**	**95% CI**	**Adj. RR**	**95% CI**	**Adj. RR**	**95% CI**
	n=1032		n=1031		n=1031		n=893		n=1031	
Black race (versus white)	1.47	(1.09, 1.98)	1.45	(1.15, 1.83)	1.85	(1.40, 2.45)	1.11	(0.60, 1.11)	1.63	(1.22, 2.18)
Less than high school education	1.04	(0.87, 1.24)	1.12	(0.95, 1.32)	1.10	(0.90, 1.34)	0.92	(0.62, 1.38)	1.20	(0.99, 1.45)
Currently smoking	1.17	(0.99, 1.39)	1.08	(0.94, 1.26)	1.26	(1.06, 1.49)	1.26	(0.87, 1.82)	1.30	(1.09, 1.55)
Use of hormonal contraception	1.06	(0.89, 1.25)	1.04	(0.90, 1.21)	1.07	(0.89, 1.26)	0.97	(0.63, 1.47)	0.88	(0.74, 1.05)
Monogamous	0.89	(0.72, 1.11)	1.08	(0.86, 1.36)	1.04	(0.82, 1.33)	0.85	(0.52, 1.37)	1.00	(0.79, 1.27)
New partner	0.93	(0.74, 1.17)	1.13	(0.91, 1.40)	1.15	(0.90, 1.46)	0.57	(0.30, 1.07)	1.07	(0.85, 1.36)
Condom use (10 out 10 sexual encounters)	0.86	(0.68, 1.09)	0.87	(0.71, 1.06)	0.75	(0.59, 0.96)	0.88	(0.55, 1.41	0.92	(0.74, 1.14)
Currently douching	1.11	(0.94, 1.31)	1.13	(0.97, 1.30)	0.99	(0.84, 1.17)	1.02	(0.71, 1.42)	1.18	(0.99, 1.41)
History of bacterial vaginosis	0.88	(0.73, 1.00)	0.99	(0.85, 1.16)	0.88	(0.74, 1.05)	0.66	(0.44, 1.00)	0.91	(0.76, 1.09)
History of chlamydia/ gonorrhea	1.04	(0.89, 1.25)	1.04	(0.89, 1.21)	1.02	(0.86, 1.21)	1.25	(0.83, 1.86)	1.02	(0.85, 1.22)
Chlamydia or gonorrhea infection	1.15	(0.93, 1.31)	0.98	(0.81, 1.18)	1.10	(0.90, 1.34)	1.12	(0.71, 1.74)	1.23	(1.00, 1.50)
Vaginal flora score (0-4)**	--	--	0.40	(0.33, 0.48)	1.75	(1.62, 1.90)	2.66	(2.28, 3.10)	1.43	(1.34, 1.51)
Gram stain score										
BV	6.6	(4.41, 9.87)	--	--	--	--	--	--	--	--
Intermediate	1.51	(0.90, 2.54)	--	--	--	--	--	--	--	--

persistent high growth: score of 3 or 4 in at least 2 visits with no score less than 2, or 75% of more of visits with score of 3 or 4.

* Outcome is consistently low score of H_2O_2+ lactobacilli

** Baseline vaginal flora score (continuous) for each outcome (eg. presence of *G. vaginalis* at baseline for outcome of persistent high growth of *G. vaginalis*)

^ Models failed to converge using log-binomial regression, estimates obtained with modified Poisson regression with robust variance estimation (Zou (14))

Table 14: Adjusted risk ratios for baseline flora and persistent BV.

	BV	
Baseline Flora	**Adjusted RR***	**95% CI**
	n=1032	
Gram-stain		
Normal	1.0	
Intermediate	1.51	(0.90, 2.54)
BV	6.60	(4.41, 9.87)
H_2O_2+ lactobacilli		
3 or 4	1.0	
1 or 2	2.89	(1.85, 4.52)
0	3.79	(2.64, 5.44)
G. vaginalis		
0	1.0	
1 or 2	2.68	(1.53, 4.70)
3 or 4	4.04	(2.85, 5.73)
Anaerobic Gram-negative rods (pigmented)		
0	1.0	
1 or 2	1.86	(1.39, 2.47)
3 or 4	2.88	(2.19, 3.47)
M. hominis		
0	1.0	
1 or 2	2.35	(1.64, 3.39)
3 or 4	3.48	(2.56, 4.73)
Chlamydia/Gonorrhea	1.15	(0.93, 1.31)

* Adjusted for race, education, smoking status, douching, history of BV, history of chlamydia/gonorrhea, use of hormonal contraceptives, use of condoms, number of sex partners, and new partners.

2.6 LITERATURE CITED

1. Priestly C, Kinghorn G. (1996) Bacterial Vaginosis. *Br J Clin Pract*. 50:331-4.
2. Wilson J. (2004) Managing recurrent bacterial vaginosis. *Sex Transm Infect*. 80:8-11.
3. McGregor J, French J. (2000) Bacterial Vaginosis in Pregnancy. *Obstet Gynecol Surv*. 55:1-19.
4. Schwebke J, Richey C, Weiss H. (1999) Correlation of Behaviors with Microbiologic Changes in Vaginal Flora. *J Inf Dis*. 180:1632-6.
5. Keane F, Ison C, Taylor-Robinson D. (1997) A longitudinal study of the vaginal flora over a menstrual cycle. *Int J STD & AIDS*. 8:489-94.
6. Schwebke J, Desmond R. (2005) Risk Factors for Bacterial Vaginosis in Women at High Risk for Sexually Transmitted Diseases. *Sexually Trans Dis*. 32:654-58.
7. Cook R, Reedondo-Lopez V, Schmitt C, Meriwether C, Sobel J. (1992) Clinical, Microbiological, and Biochemical Factors in Recurrent Bacterial Vaginosis. *J Clin Microbio*. 30:870-7.
8. Ness R, Hillier S, Richter H, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P. (2002). Douching in Relation to Bacterial Vaginosis, Lactobacilli, and Facultative Bacteria in the Vagina. *Obstet Gynecol*. 100:765-72.
9. Ness R, Hillier S, Kip K, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P, Richter H. (2004) Bacterial Vaginosis and Risk of Pelvic Inflammatory Disease. *Obstet Gynecol*. 104:761-9.
10. Stergachis A, Scholes D, Heidrich F. (1993) Selective screening for *Chlamydia trachomatis* infection in a primary care population of women. *Am J Epidem*. 138:143-53.
11. Schwebke J, Hillier S, Sobel J, McGregor J, Sweet R. (1996) Validity of the vaginal Gram stain for the diagnosis of bacterial vaginosis. *Obstet Gynecol*. 88:573-6.
12. Nugent R, Krohn M, Hillier S. (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 29:297-301.
13. Spiegelman D, Hertzmark E. (2005) Easy SAS Calculations for Risk or Prevalence Ratios and Differences. *Am J Epidemiol*. 162:199-200.
14. Zou G. (2004) A Modified Poisson Regression Approach to Prospective Studies with Binary Data. *Am J Epidemiol*. 159:702-6.
15. Vallor A, Antonio M, Hawes S, Hillier S. (2001) Factors associated with acquisition of, or persistent colonization by, vaginal lactobacilli: role of hydrogen peroxide production. *J Infect Dis*. 184:1431-6.
16. Hawes S, Hillier S, Benedietti J, Stevens C, Koutsky L, Wolner-Hanssen, Holmes K. (1996) Hydrogen Peroxide-Producing Lactobacilli and Acquisition of Vaginal Infections. *J Infect Dis*. 174:1058-63.
17. Boris J, Pahlson C, Larsson P. (1997) Six-year follow-up after successful treatment of bacterial vaginosis. *Int J STD AIDS*. 8(Suppl 1):41.
18. Cauci S. (2004) Vaginal Immunity in Bacterial Vaginosis. *Curr Infect Dis Reports*. 6:450-56.

19. Macones G, Parry S, Elkousy M, Clothier B, Ural S, Straus J. (2004) A polymorphism in the promotor region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. *Am J Obstet Gynecol*. 190:1504-8.
20. Ness R, Haggerty C, Harger G, Ferrel l R. (2004) Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. *Am J Epidemiol*. 160:1033-8.
21. Ness R, Hillier S, Richter H, Soper D, Stamm C, Bass D, Sweet R, Rice P. (2003) Can Known Risk Factors Explain Racial Differences in the Occurrence of Bacterial Vaginosis? *J Natl Med Assoc*. 95:201-212.
22. Chiaffarino F, Parazzini F, Besi P, Lavezzari M. (2004) Risk factors for bacterial vaginosis. *Eur J Obstet Gynecol Reprod Biol*. 117:222-6.
23. Rajamanoharan S, Low N, Jones S, Pozniak A. (1999) Bacterial Vaginosis, Ethnicity, and the Use of Genital Cleaning Agents: A Case Control Study. *Sex Trans Dis*. 26:404-9.
24. Morris M, Rogers P, Kinghorn G. (2001) Is bacterial vaginosis a sexually transmitted infection? *Sex Trans Inf*. 77:63-68.
25. Keane F, Ison C, Taylor-Robinson D. (1997) A longitudinal study of the vaginal flora over a menstrual cycle. *Int J STD & AIDS*. 8:489-94.

3.0 MANUSCRIPT 2: VAGINAL DOUCHING AND THE DEVELOPMENT OF BACTERIAL VAGINOSIS (BV) AMONG WOMEN WITH NORMAL AND ABNORMAL VAGINAL MICROFLORA

Manuscript in preparation

Katherine H. Berger*, Kevin Kip*, Roberta B. Ness*;
for the Gynecologic Infections Follow-through (GIFT) Investigators

* Department of Epidemiology, University of Pittsburgh
Pittsburgh, Pennsylvania, 15261

3.1 ABSTRACT

Objectives: To evaluate the relationship between douching and bacterial vaginosis (BV) among women with and without prior abnormal vaginal flora.

Methods: One thousand one hundred ninety-three women at high risk for sexually transmitted diseases were followed for a median of 3 years. Vaginal swabs were obtained for Gram-stain diagnosis of BV, culture of microflora, and *Neisseria gonorrhoeae* and *Chlamydia trachomatis* at baseline and every 6 to 12 months thereafter. The association between douching and BV was evaluated cross-sectionally and prospectively among subgroups of women defined by their vaginal flora status during the study.

Results: In cross-sectional analyses, douching at least once per month was significantly associated with BV among women who had BV at the immediately preceding visit (adj. OR 1.8, 95% CI 1.2, 2.6) and women who had a history of BV (adj. OR 1.6, 95% CI 1.2, 2.4), but not among women without prior experience of BV. In prospective analyses, douching increased the risk of acquisition for BV among women with intermediate flora at baseline (adj. HR 1.5, 95% CI 1.1-2.4); however women with normal flora at baseline did not have an increased risk of BV or BV-associated organisms.

Conclusions: Douching does not increase the risk of BV among women who previously lacked BV; however, douching does increase the risk of BV among women with already imbalanced flora or a history of BV.

3.2 INTRODUCTION

Vaginal douching is a common practice among women in the United States. Over one-quarter of reproductive aged women report douching regularly (1), and nearly three quarters of women report douching at some point in their life (2). Douching has been associated with a number of adverse outcomes including pelvic inflammatory disease (PID), bacterial vaginosis (BV), acquisition of sexually transmitted diseases, cervical cancer, preterm birth, ectopic pregnancy, and infertility (3, 4, 5); however controversy exists about the extent of risk and the likelihood that the association is causal.

Since douching and BV have been linked to the same adverse outcomes, the development of BV has been suggested as a possible mechanism or intermediate step towards the development of adverse sequelea following douching (2, 5). Cross-sectional studies consistently indicate that douching is associated with BV (6-11). However, it is difficult to determine the temporal and causal associations between douching and BV: Do women douche prior to developing BV, or do they douche as a result of symptoms associated with BV? Using longitudinal data, we previously reported that, among a group of women heterogeneous for pre-existing vaginal microflora, douching two or more times per month was not associated with the development of BV (12). However, we could not fully discern how pre-existing BV or already disturbed flora might impact any effect of douching. In addition, in previous cross-sectional studies, douching has been associated with several BV-associated organisms (5), however we did not explore this in our previous longitudinal analysis and it remains unclear how douching influences changes in specific vaginal flora.

In this analysis, we employed data from the Gyn Infections Follow-Through (GIFT) study to explore the effect of douching on the development of BV conditional upon previous

experience of BV; we also explored the relationship between douching and the acquisition of a variety of BV-associated organisms.

3.3 MATERIALS AND METHODS

3.3.1 Patient selection

The methods used for subject enrollment, data collection and follow-up have been reported in detail elsewhere (5, 13). Briefly, women 13-36 years of age were recruited into the GYN Infections Follow-Through (GIFT) Study from family planning clinics, university health clinics, gynecology clinics, and sexually transmitted disease units at each of five US sites between May 1999 and June 2001. Human subjects approval was obtained at each participating institution, and all women signed informed consent. Women were eligible for the study if they were not specifically seeking care for a sexually transmitted disease, yet, based upon a previous risk stratification paradigm for chlamydial cervicitis (14), were considered at high risk for acquiring a bacterial sexually transmitted infection. Specifically, to be enrolled, a women had to have a score of 3 or more points on a algorithm in which points were derived as follows: age 24 or less = 1; black race = 2; never pregnant =1; 2 or more sexual partners = 1; douches at least once per month = 2; and any prior sexually transmitted infection, including *N. gonorrhoeae*, *C. trachomatis*, and *Trichomonas vaginalis* = 2. Of the 2740 women screened for study entry, 853 (31.1%) did not meet these inclusion criteria. An additional 259 (9.5%) women were excluded on the basis of priori criteria such as being pregnant, married, or virginal, or being on antibiotics at baseline. Among the 1628 women who were eligible for the study, 1193 (73.3%) completed a baseline questionnaire and are the focus of these analyses.

3.3.2 Microbiologic methods for evaluation of vaginal flora

At baseline and every 6-12 months thereafter, each subject obtained her own vaginal specimens with a cotton swab (15). Smears from these swabs were gram stained and a microscopy score of 0-10 was assigned by laboratory staff using the standardized method described by Nugent et al. (16). A score of 0-3 was interpreted as consistent with normal vaginal flora; a score of 4-6, corresponding to disturbed flora, was designated as intermediate; and a score of 7-10 was considered to be BV.

Two swabs, placed in an anaerobic transport vial, were also shipped to the microbiology laboratory for characterization of the following: *Lactobaccillus* species, anaerobic Gram-negative rods, *Gardnerella vaginalis*, group B streptococcus, *Enterococcus* species, *Escherichia coli*, *Candida* species, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. Lactobacilli were identified to the genus level on the basis of Gram's-stain morphology and production of lactic acid. The amount of growth for each of these microorganisms was recorded on a semiquantative scale from 0 to 4.

3.3.3 DNA amplification for *N. gonorrhoeae* and *C. trachomatis*

DNA amplification for *N. gonorrhoeae* and *C. trachomatis* was performed using a strand displacement DNA Amplification (SDA) Assay (Becton Dickinson, Sparks, MD) from self-obtained vaginal swabs. All positive test results for gonococcal or chlamydial infection were reported to the clinical sites within 1 week of enrollment where infected subjects were treated.

3.3.4 Douching and other data collection

At baseline, women were asked about demographic factors, including age, race, education, income, pregnancy history, smoking, alcohol use, and drug use. They also reported relevant lifestyle behaviors such as number of sexual partners in the past 2 months, acquisition of a new partner in the past 2 months, contraception use, sex during menses, and douching practices. Women were further requested to recall past episodes of sexually transmitted infections, including PID and gonococcal and/or chlamydial genital infections. Questions about pregnancy history, sexual activity, STDs, and douching were repeated during follow-up.

For the purposes of analyses douching was categorized into frequency (never, less than once per month, at least once per month), and reason for douching according to previously published work (5, 9, 17). Reason for douching was created using hierarchically mutually exclusive categories of 1) Abnormal symptoms (including abnormal vaginal discharge, to reduce odor, and for bleeding between menses), 2) before or after sex, and 3) for hygiene (including general cleansing, after menses, because "it's normal to douche," and to prevent pregnancy).

3.3.5 Follow-up

Of the 1193 subjects, 27 (2.3%) had a baseline visit only. Among the remaining 1166 study participants, the median length of follow-up was 3.0 years (interquartile range: 2.4 – 3.4 years). The median number of follow-up visits was six (interquartile range: 5-7), and the median number of vaginal swab samples was 4 (interquartile range 2-4). Eighty-eight percent of the women had four or more visits, and 72% of the women had 3 or more vaginal swab samples.

3.3.6 Statistical analyses

To evaluate the association between douching and BV and other BV-associated organisms (*Gardnerella vaginalis*, *Mycoplasma hominis*, lacking H_2O_2 producing lactobacilli, and anaerobic Gram-negative pigmented and nonpigmented rods), we conducted both cross-sectional analyses and prospective analyses. Generalized estimating equations (GEEs) were used to estimate the adjusted odds ratios of BV and other microbes throughout follow-up in relation to frequency of reported douching and reason for douching. This method accounts for the correlation of multiple observations per subject. The final models were adjusted for: age, race (black vs. white or other), smoking status (current vs. prior or never), hormonal conception use (yes/no), ever pregnant, condom use (10/10 sexual encounters vs. <10/10 sexual encounters or never), number of sex partners (>1 vs. 1), and clinical site. Additionally, the impact of douching frequency on bacterial vaginosis was considered within the following subgroups: History of bacterial vaginosis (yes/no) and the presence of BV at the immediately preceding visit (yes/no).

To assess the relationship between douching and the development of BV prospectively, women who had at least one follow-up visit were considered. Subsets of women were separately assessed for the development of BV who at baseline had 1) normal flora, and 2) intermediate flora. Similarly, women were evaluated for the development of BV-associated organisms according the baseline status of lacking: 1) *G. vaginalis*, 2) *M. hominis*, 3) anaerobic Gram-negative pigmented rods, and 4) anaerobic Gram-negative pigmented rods. Loss of H_2O_2-producing lactobacilli was determined for women who had H_2O_2-producing lactobacilli at baseline. Since vaginal flora was routinely assessed at fixed semiannual and annual visits, discrete time hazard models were fit using the complementary log-log link. This model parallels the continuous-time proportional hazards model while accommodating interval-censored data in

which information about event occurrence is restricted to discrete time intervals (12, 18, 19). Time-varying and baseline status of douching were assessed in separate models. All models were adjusted for race, education, number of sex partners, baseline Gram-stain flora score, history of chlamydial/gonorrhea infection, history of BV, and current chlamydial or gonococcal cervical infection. Analyses were conducted using the SAS System for Windows, version 8.02, Cary, NC.

3.4 RESULTS

Participants were predominantly aged 19-24 (66.1%) and black (75%) (Table 15). At baseline, 425 (36.2%) reported a history of BV, 164 (13.8%) reported a history of pelvic inflammatory disease, and 464 (39.2%) reported a history of chlamydial infection. Four hundred seventy women (39.7%) entered the study with BV as determined by Gram-stain. Forty-four percent of the women reported douching, and of the women who douched, 464 (87.7%) douched at least once per month. Women who douched at least once per month were more likely to have BV, harbor BV-associated organisms (*Gardnerella vaginalis*, *Mycoplasma hominis*, and anaerobic Gram-negative black pigmented rods), and lack H_2O_2-producing lactobacilli (data not shown).

3.4.1 Cross-sectional analyses of douching and BV, stratified by history of BV

In cross-sectional analyses, which evaluated at each follow-up visit douching during the past 2 months, BV was more common among women who douched at least once per month (adjusted odds ratio 1.45, 95% CI = 1.20, 1.74) (Table 16). After stratification for factors

indicating a woman's prior history of BV, douching was only significantly associated with BV among women who either had a history of BV (adjusted OR 1.58, 95% CI = 1.17, 2.14) or had BV at the immediately preceding visit (adjusted OR 1.91, 95% CI = 1.29, 2.81). Conversely, douching was not associated with BV among women who did not have BV at the immediately preceding visit or among women who did not have a history of BV.

3.4.2 Douching and the development of BV among women who had normal flora and who had intermediate flora at baseline

Among women whose baseline Gram-stain score was normal (0-3), douching at least once per month at the immediately preceding visit was not associated with the development of BV (adjusted HR 1.01) (Table 17). For women who already had intermediate vaginal flora, frequency of douching at the preceding visit was strongly associated with the development of BV (adjusted HR 1.68, 95% CI = 1.17, 2.43). Douching for reasons associated with hygiene was also associated with a 1.9-fold significantly increased risk for developing BV, whereas douching for other reasons including hygiene and before or after sex were not significantly associated with douching (adjusted OR's 1.15 - 1.63). Douching at baseline was not associated with the development of BV among women with normal or intermediate flora. Additionally, douching was not significantly associated with the acquisition of any of the specific microflora studied including *G. vaginalis*, *M. hominis*, anaerobic Gram-negative nonpigmented rods, and lack of H_2O_2-producing lactobacilli.

3.5 DISCUSSION

The existing literature regarding the relationship between douching and BV is inconsistent. Cross-sectional studies generally link douching to BV (6-11) with risk estimates as high as 6-fold (11). Few prospective studies have been conducted, and of the three studies published (12, 20, 21), two suggest that douching is not related to the development of BV after adjusting for potential confounders (12, 20). Some suggest that the confusion arises because women douche following (rather than preceding) the development of BV in response to abnormal symptoms; however, in cross-sectional analyses, douching for reasons not associated with symptoms remains associated with BV (5, 9). Douching may also differentially affect vaginal flora depending on whether a women has normal or already disturbed flora, yet few studies have examined this association.

In the current study we found that the presence of already altered flora or a history of BV significantly impacted the association between douching and BV. Among women with a history of BV at baseline or a diagnosis of BV at an immediately preceding visit during follow-up, douching was significantly associated with a current diagnosis of BV. In contrast, douching was not associated with BV among women who lacked a history or previous diagnosis of BV. Similarly, we did not find douching to be associated with the development BV among women with normal flora at baseline, but douching was associated with BV among women with intermediate flora. Our results were also consistent across BV-associated organisms, that is, we did not find that douching significantly increased the risk of acquisition of these vaginal organisms among women who previously lacked them. These findings suggest that douching may disrupt imbalanced flora sufficiently to create BV; however, douching does not significantly impact women with normal flora.

To our knowledge, this is the first study to examine douching and the acquisition of vaginal microorganisms associated with BV. Irrespective of the time of measurement, our analyses showed that douching was not associated with the *de novo* acquisition of BV-associated organisms. The consistency of results across vaginal microorganisms is not surprising due to the high correlation among these organisms; however, these findings support the interpretation that douching is not associated with new acquisition of vaginal organisms among women who were previously free of them.

The strengths of this study include the large number of women studied, enrollment of a high-risk population, which enhanced study power, use of consistent and standardized enrollment and data collection protocols, collection of biomarkers of effect, and the relatively long-term and complete longitudinal data collection.

Our study also has a number of limitations. Relatively long intervals separated vaginal microbiologic assessments; interval assessment of BV and vaginal microbiology status was thus relatively gross. BV is variable over short periods of time and so infrequent assessment is necessarily incomplete. Prospective measurement of douching involved assessment at the immediately preceding visit, which was approximately 6 months prior; this, too, may also have been too distant to be optimally meaningful. However, we did observe a significantly harmful effect associated with douching among women with intermediate flora. Also, we cannot rule out that unmeasured confounding may have influenced our results. We did not assess factors such as partner sexual behavior or frequency of intercourse in these analyses. Additionally, treatment of BV was not ascertained in this study and treatment may influence or mask the effect of douching on vaginal flora. Finally, inclusion of high-risk women may limit the generalizability of our results.

In summary, among predominantly young black women followed longitudinally, we found that frequency of douching and reason for douching was not associated with progression to BV, the acquisition of *G. vaginalis*, *M. hominis*, and anaerobic Gram-negative rods, or the loss of H_2O_2-producing lactobacilli, among women with previously normal flora. However, douching was associated with the development of BV among women with already disturbed vaginal flora. In our own previous analyses of these data, this subset finding was washed-out by excluding women with intermediate flora from our analyses. As women are not screened for normality of vaginal flora prior to douching, the women at risk for BV (i.e. those with prior intermediate flora) are not identified as such. Thus, to avoid progression to BV in populations of unknown vaginal microflora status, douching is best avoided.

Table 15: Baseline descriptive characteristics of the 1,193 women in the GIFT study.

Variables	N	%
Age: 19-24	789	66.1
Race: Black	894	75.0
Education: greater than high school	624	52.3
Self reported history of*:		
Chlamydia	464	39.2
Gonorrhea	253	21.4
Bacterial Vaginosis	425	36.2
Pelvic Inflammatory Disease	164	13.8
Douching frequency		
Never	664	55.7
< 1 time per month	65	5.4
≥ 1 time per month	464	38.9
Reason for douching		
Never	664	55.7
Abnormal Symptoms	171	14.4
Before or after sex	93	7.8
Hygiene	263	22.1
2 or more sex partners in last 2 months**	190	18.7
New sex partner in past two months**	213	21.0
Vaginal Flora Gram Stain at study entry^		
Normal (score 0-3)	428	36.2
Intermediate (score 4-6)	283	23.9
Bacterial Vaginosis (score 7-10)	470	39.9
Chlamydia at study entry^	127	11.1
Gonorrhea at study entry^	48	4.2

* Missing values: chlamydia=10, history of gonorrhea=9, history of BV=18, history of PID=6

** among women reporting sexual activity in past two months (n=1016)

^ Missing vaginal microbiology: Gram-stain=12, chlamydia=50, gonorrhea=51

Table 16: Cross-sectional analyses - adjusted odds ratios for BV by frequency of douching during the past 2 months, and stratified by history of BV and BV at the immediately preceding visit.

Frequency of douching	Risk of BV	
	Adj. OR*	95% CI
Overall (n=3860)		
Never	1.0	
< 1 time/month	1.33	(0.91, 1.92)
≥ 1 time/month	1.45	(1.20, 1.74)
p for trend		0.0006
Bacterial vaginosis at previous visit (within 6 - 12 mo): Yes (n=868)		
Never	1.0	
< 1 time/month	2.00	(0.88, 4.56)
≥ 1 time/month	1.91	(1.29, 2.81)
p for trend		0.001
Bacterial vaginosis at previous visit (within 6 - 12 mo): No (n=1499)		
Never	1.0	
< 1 time/month	0.74	(0.36, 1.55)
≥ 1 time/month	1.10	(0.79, 1.56)
p for trend		0.66
History of bacterial vaginosis: yes (n=1410)		
Never	1.0	
< 1 time/month	1.52	(0.86, 2.67)
≥ 1 time/month	1.58	(1.17, 2.14)
p for trend		0.002
History of bacterial vaginosis: no (n=2389)		
Never	1.0	
< 1 time/month	1.15	(0.70, 1.89)
≥ 1 time/month	1.26	(0.99, 1.59)
p for trend		0.06

* Adjusted for age, race, smoking, use of hormonal contraception, pregnancy, condom use, number of partners, and clinical site.

Table 17: The risk of progression to BV or BV-associated microbologic outcomes by douching status at the preceding visit and douching at baseline.

	Bacterial Vaginosis				Lacking hydrogen peroxide producing lactobacilli*		*G. vaginalis***		Gram-negative nonpigmented rods**		Gram-negative black pigmented rods**		*M. hominis***	
	Normal flora to development of BV^		Intermediate flora to development of BV^^											
	n = 417		n = 277		n = 576		n = 474		n = 207		n = 578		n = 530	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Douching at Prior Visit														
Douching Frequency														
Never	1.0		1.0		1.0		1.0		1.0		1.0		1.0	
< 1 time/month	0.85	(0.37, 1.99)	0.66	(0.16, 2.70)	1.13	(0.57, 2.24)	0.52	(0.23, 1.17)	0.83	(0.35, 1.99)	0.91	(0.50, 1.66)	1.19	(0.62, 2.30)
≥ 1 time/month	1.01	(0.69, 1.48)	1.68	(1.17, 2.43)	1.08	(0.81, 1.46)	1.02	(0.76, 1.36	0.78	(0.52, 1.17)	1.19	(0.89, 1.59)	1.10	(0.79, 1.53)
p for trend		0.93		0.006		0.56		0.95		0.24		0.25		0.55
Reason for Douching														
Never	1.0		1.0		1.0		1.0		1.0		1.0		1.0	
Abn. Symptoms	1.05	(0.59, 1.89)	1.15	(0.64, 2.06)	0.87	(0.53, 1.45)	1.09	(0.71, 1.67)	0.45	(0.24, 0.87)	1.29	(0.86, 1.94)	1.23	(0.78, 1.93)
Before or after sex	1.14	(0.55, 2.33)	1.63	(0.78, 3.40)	0.98	(0.54, 1.79)	0.92	(0.51, 1.66)	1.18	(0.58, 2.40)	1.19	(0.63, 2.22)	1.14	(0.56, 2.31)
Hygiene	0.92	(0.59, 1.45)	1.86	(1.23, 2.83)	1.22	(0.88, 1.70)	0.90	(0.64, 1.28)	0.95	(0.59, 1.54)	1.05	(0.75, 1.48)	1.08	(0.73, 1.59)
Baseline Douching Status														
Douching Frequency														
Never	1.0		1.0		1.0		1.0		1.0		1.0		1.0	
< 1 time/month	0.75	(0.32, 1.79)	0.82	(0.37, 1.84)	1.03	(0.56, 1.88)	0.68	(0.34, 1.35)	1.35	(0.58, 3.14)	0.90	(0.52, 1.56)	0.90	(0.47, 1.72)
≥ 1 time/month	0.89	(0.62, 1.26)	0.84	(0.58, 1.22)	1.08	(0.82, 1.44)	1.00	(0.75, 1.34)	1.46	(0.98, 2.17)	0.82	(0.62, 1.08)	0.79	(0.58, 1.08)
p for trend		0.53		0.38		0.57		0.94		0.07		0.15		0.16
Reason for Douching														
Never	1.0		1.0		1.0		1.0		1.0		1.0		1.0	
Abnormal Symptoms	0.67	(0.35, 1.28)	0.91	(0.52, 1.58)	0.89	(0.53, 1.48)	0.91	(0.54, 1.54)	0.71	(0.34, 1.48)	1.10	(0.64, 1.87)	0.79	(0.41, 1.53)
Before or after sex	0.80	(0.43, 1.49)	0.59	(0.33, 1.06)	0.54	(0.33, 0.89)	0.85	(0.52, 1.37)	0.38	(0.20, 0.75)	0.92	(0.59, 1.45)	0.87	(0.54, 1.41)
Hygiene	0.76	(0.49, 1.19)	0.71	(0.46, 1.11)	0.86	(0.62, 1.21)	0.97	(0.67, 1.38)	0.99	(0.62, 1.58)	0.83	(0.60, 1.13)	0.74	(0.51, 1.07)

^ Includes women who had normal flora at baseline. ^^ Includes women who had intermediate flora at baseline.

* Includes women who had lactobacilli at baseline and were followed for the outcome of lacking hydrogen peroxide producing lactobacilli.

** Includes women who did not have the vaginal organism at baseline and were followed for subsequent development of the vaginal organism.

3.6 LITERATURE CITED

1. Abma J, Chandra A, Mosher W, Peterson L, Piccinino L. Fertility, family planning, and women's health: New data from the 1995 National Survey of Family Growth. *Vital Health Stat*. 23:1-114.
2. Rosenberg M, Phillips R, Holmes M. (1991) Vaginal Douching: Who and Why? *J Repro Med*. 36:753-8.
3. Martino J, Vermund S. (2002) Vaginal Douching: Evidence for Risks and Benefits to Women's Health. *Epidemiol Rev*. 24:109-124.
4. Zhang J, Thomas G, Leybovich E. (1997) Vaginal Douching and Adverse Health Effects: A Meta-Analysis. *Am J Public Health*. 87:1207-11.
5. Ness R, Hillier S, Richter H, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P. (2002). Douching in Relation to Bacterial Vaginosis, Lactobacilli, and Facultative Bacteria in theVagina. *Obstet Gynecol*. 100:765-72.
6. Schwebke J, Desmond R, Oh M. (2004) Predictors of Bacterial Vaginosis in Adolescent Women who Douche. *Sex Trans Dis*. 31:433-36.
7. Chiaffarino F, Parazzini F, Besi P, Lavezzari M. (2004) Risk factors for bacterial vaginosis. *Eur J Obstet Gynecol Reprod Biol*. 117:222-6.
8. Fonck K, Kaul R, Keli F, Bwayo J, Ngugi E, Moses S, Temmerman M. (2001) Sexually transmitted infections and vaginal douching in a population of female sex workers in Nairobi, Kenya. *Sex Trans Inf*. 77:271-75.
9. Holzman C, Leventhal J, Qiu H, Jones N, Wang J. (2001) Factors Linked to Bacterial Vaginosis in Nonpregnant Women. *Am J Public Health*. 91:1664-70.
10. Newton E, Piper J, Shain R, Perdue S, Paeirs W. (2001) Predictors of vaginal microflora. *Am J Obstet Gynecol*. 184:845-55.
11. Rajamanoharan S, Low N, Jones S, Pozniak A. (1999) Bacterial Vaginosis, Ethnicity, and the Use of Genital Cleaning Agents: A Case Control Study. *Sex Trans Dis*. 26:404-9.
12. Ness R, Kip K, Soper D, Stamm C, Rice P, Richter H. (2006) Variability of Bacterial Vaginosis over 6-12 month intervals. *Sex Trans Dis*. 33:1-5.
13. Ness R, Hillier S, Kip K, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P, Richter H. (2004) Bacterial Vaginosis and Risk of Pelvic Inflammatory Disease. *Obstet Gynecol*. 104:761-9.
14. Stergachis A, Scholes D, Heidrich F. (1993) Selective screening for *Chlamydia trachomatis* infection in a primary care population of women. *Am J Epidem*. 138:143-53.
15. Schwebke J, Hillier S, Sobel J, McGregor J, Sweet R. (1996) Validity of the vaginal Gram stain for the diagnosis of bacterial vaginosis. *Obstet Gynecol*. 88:573-6.
16. Nugent R, Krohn M, Hillier S. (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 29:297-301.
17. Scholes D, Stergachis A, Ichikawa L, Heidrich F, Holmes K, Stamm W. Vaginal douching as a risk factor for cervical *Chlamydia trachomatis* infection. *Obstet Gynecol*. 91:993-7.

18. Hosmer D, Lemeshow S. Applied Logistic Regression. 2nd ed. New York, NY: Wiley, 2000.
19. Allison P. (1995) Survival Analysis Using the SAS System: A Practical Guide. The SAS Institute. Cary, NC.
20. Schwebke J, Desmond R. (2005) Risk Factors for Bacterial Vaginosis in Women at High Risk for Sexually Transmitted Diseases. *Sexually Trans Dis*. 32:654-58.
21. Hawes S, Hillier S, Benedietti J, Stevens C, Koutsky L, Wolner-Hanssen, Holmes K. (1996) Hydrogen Peroxide-Producing Lactobacilli and Acquisition of Vaginal Infections. *J Infect Dis*. 174:1058-63.

4.0 MANUSCRIPT 3: CONDOM USE AND THE ASSOCIATION WITH BACTERIAL VAGINOSIS (BV) AND BV-ASSOCIATED VAGINAL FLORA

Manuscript in preparation

Katherine H. Berger*, Kevin Kip*, Roberta B. Ness*;
for the Gynecologic Infections Follow-through (GIFT) Investigators

* Department of Epidemiology, University of Pittsburgh
Pittsburgh, Pennsylvania, 15261

4.1 ABSTRACT

Objectives: To evaluate whether condom use is associated with bacterial vaginosis (BV) and BV-associated microflora.

Study Design: A total of 1143 women at high risk for sexually transmitted diseases were followed for a median of 3 years. At baseline and every 6 to 12 months thereafter, vaginal swabs were obtained for Gram-stain diagnosis of BV, culture of microflora, and *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Both cross-sectional analyses using generalized estimating equations and case-crossover analyses using conditional logistic regression were used to assess the association between condom use and vaginal flora.

Results: Consistent condom use (10 out 10 sexual encounters) was associated with a decreased frequency of bacterial vaginosis in case-crossover analyses (adjusted OR = 0.68, 95% CI = 0.49-0.94, p for trend = 0.047). Similar results were seen for carriage of *M. hominis* (adjusted OR=0.61, 95% CI: 0.41-0.93) and anaerobic Gram-negative pigmented rods (OR=0.65, 95% CI: 0.47-0.91). Analyses repeated with first incident case intervals showed a strong inverse association between condom use and the acquisition of BV (adjusted OR = 0.33, 95% CI = 0.15-0.76). The association between condom use and BV was not significant in cross-sectional analyses.

Conclusions: Consistent condom use was associated with a significant decrease in the risk for BV, suggesting that BV is sexually transmitted.

4.2 INTRODUCTION

Bacterial vaginosis (BV) is characterized by complex alterations of the normal vaginal flora (1). Healthy vaginal flora primarily consists of hydrogen peroxide (H_2O_2) producing Lactobacillus species, which help to maintain an acidic environment that is inhibitory to the growth of other endogenous organisms such as *Gardnerella vaginalis*, *Mycoplasma hominis*, and anaerobic Gram-negative rods (*Bacteroides* and *Prevotella* spp.) (2, 3). While the direct causes for changes in vaginal flora are poorly understood, BV results in a dramatic decrease in lactobacilli, an increase in pH, and an increase in other mixed flora in which anaerobic and facultative aerobic bacteria dominate (4). BV is one of the most common vaginal infections and prevalence estimates range from <5% to over 40% depending on the characteristics of the populations (5). Despite the lack of severe acute symptoms, research over the last two decades has indicated that BV is associated with a number of adverse sequelea, including pre-term delivery, intrapartum and postpartum infections, post-abortion endometritis, pelvic inflammatory disease, and the acquisition of chlamydia, gonorrhea, and HIV (6-9).

Numerous risk factors have been identified for bacterial vaginosis, however it is still unclear as to whether BV results from an endogenous infection, exogenous influences, or both (4). Smoking, black race, older age, douching, and the use of IUDs have been consistently associated with an increased risk of BV (10, 11). Additionally, several behaviors related to sexual activity have been associated with BV, including increasing number of sexual partners, new sexual partners, early age at first intercourse, sex during menses, and a history of sexually transmitted infections (12, 13). While these risk factors mimic those of classic STDs (including chlamydia and gonorrhea) and have raised the possibility that BV may be sexually transmitted, other factors, such as the presence of BV in adolescent virgins, are in direct contradiction to

models of sexual transmission (14). Additionally, partner treatment has generally proven to be an ineffective means for preventing BV in women (15); however, other preventative measures, particularly condom use, have not been well characterized for BV.

Condoms are a generally recognized tool for STD prevention and are known to provide an effective mechanical barrier against pathogens (16). However, the efficacy of condoms for reducing bacterial STD incidence in populations has been controversial (17, 18). Factors that have been shown to impact the apparent effectiveness of condoms, such as inconsistent condom use, incorrect use, and infection status of partner, are difficult variables to measure and control for in studies (19, 20). A recent study by Warner et al. (21) evaluated the use of a case-crossover design to reduce potential bias encountered in studies of condom effectiveness. The case-crossover design utilizes only case and corresponding control intervals from the same participant; thus, all time-independent variables, whether measured or unmeasured are automatically controlled for in the design. The authors found that condoms were significantly protective against bacterial STDs in a case crossover design, but not in corresponding cross-sectional analyses, which are often utilized in the current literature. Thus, the case-crossover design may prove to be a more useful tool when assessing the risk associated with condom use.

With respect to BV, evaluation of condom use will help to understand whether BV has a sexually transmitted component, and may provide a preventative measure when counseling women against recurrent BV. In order to assess the relationship between condom use and bacterial vaginosis, we conducted initial cross-sectional analyses using a cohort of women at high risk for sexually transmitted infection. Additionally, we repeated analyses using the case-crossover methodology to assess whether unmeasured confounding may be present in our

assessment of condom use and BV. We also examined whether condom use reduced the risk of carriage of several BV-associated organisms using both methodologies.

4.3 MATERIALS AND METHODS

4.3.1 Patient selection

The methods used for subject enrollment, data collection and follow-up have been reported in detail elsewhere (22, 23). Briefly, women 13-36 years of age were recruited into the GYN Infections Follow-Through (GIFT) Study from family planning clinics, university health clinics, gynecology clinics, and sexually transmitted disease units at each of five US sites between May 1999 and June 2001. Human subjects approval was obtained at each participating institution, and all women signed informed consent. Women were eligible for the study if they were not specifically seeking care for a sexually transmitted disease, yet, based upon a previous risk stratification paradigm for chlamydial cervicitis (24), were considered at high risk for acquiring a bacterial sexually transmitted infection. Specifically, to be enrolled, a women had to have a score of 3 or more points on a algorithm in which points were derived as follows: age 24 or less = 1; black race = 2; never pregnant =1; 2 or more sexual partners = 1; douches at least once per month = 2; and any prior sexually transmitted infection, including *N. gonorrhoeae*, *C. trachomatis*, and *Trichomonas vaginalis* = 2. Of the 2740 women screened for study entry, 853 (31.1%) did not meet these inclusion criteria. An additional 259 (9.5%) women were excluded on the basis of priori criteria such as being pregnant, married, or virginal, or being on antibiotics at baseline. Among the 1628 women who were eligible for the study, 1143 (70.2%) completed a baseline questionnaire and are the focus of these analyses.

4.3.2 Microbiologic methods for evaluation of vaginal flora

At baseline and every 6-12 months thereafter, each subject obtained her own vaginal specimens with a cotton swab (25). Smears from these swabs were gram stained and a microscopy score of 0-10 was assigned by laboratory staff using the standardized method described by Nugent et al. (26). A score of 0-3 was interpreted as consistent with normal vaginal flora; a score of 4-6, corresponding to disturbed flora, was designated as intermediate; and a score of 7-10 was considered to be BV.

Two swabs, placed in an anaerobic transport vial, were also shipped to the microbiology laboratory for characterization of the following: *Lactobaccillus* species, anaerobic Gram-negative rods, *Gardnerella vaginalis*, group B streptococcus, *Enterococcus* species, *Escherichia coli*, *Candida* species, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. Lactobacilli were identified to the genus level on the basis of Gram's-stain morphology and production of lactic acid. The amount of growth for each of these microorganisms was recorded on a semiquantative scale from 0 to 4.

4.3.3 DNA amplification for *N. gonorrhoeae* and *C. trachomatis*

DNA amplification for *N. gonorrhoeae* and *C. trachomatis* was performed using a strand displacement DNA Amplification (SDA) Assay (Becton Dickinson, Sparks, MD) from self-obtained vaginal swabs. All positive test results for gonococcal or chlamydial infection were reported to the clinical sites within 1 week of enrollment where infected subjects were treated.

4.3.4 Condom use and other data collection

At baseline, women were asked about demographic factors, including age, race, education, income, pregnancy history, smoking, alcohol use, and drug use. They also reported relevant lifestyle behaviors such as number of sexual partners in the past 2 months, acquisition of a new partner in the past 2 months, contraception use, sex during menses, and douching practices. Women were further requested to recall past episodes of sexually transmitted infections, including PID and gonococcal and/or chlamydial genital infections. Questions about pregnancy history, sexual activity, STDs, and douching were repeated during follow-up.

Among women who reported being sexually active during the 2 months prior to the interview, condom use (during the past 2 months) was categorized according to use (yes/no) and consistency of use (never, five or less times out of 10 acts of vaginal intercourse, 6 to 9 times out of ten acts of vaginal intercourse, and 10 out of 10 times of vaginal intercourse—100% use). For the cross-sectional analyses, condom use was also categorized according to the proportion of visits (up until the recorded visit) in which any level of condom use was reported (never used, use less than 49% of visits, use in 50% or more of visits).

4.3.5 Follow-up

Of the 1143 subjects, 22 (1.9%) had a baseline visit only. Among the remaining 1121 study participants, the median length of follow-up was 3.0 years. The median number of follow-up visits was six (interquartile range: 5-7), and the median number of vaginal swab samples was 4 (interquartile range 3-4). Eighty-nine percent of the women had four or more visits, and 75.6% of the women had 3 or more vaginal swab samples.

4.3.6 Statistical analyses

To determine the effect of condom use on the presence of bacterial vaginosis, both cross-sectional and case crossover analyses were conducted. For the cross-sectional analysis, all visits with a recorded microbiologic sample and information regarding condom use were included in the analysis. Because participants had multiple visits, adjusted odds ratios and 95% confidence intervals were estimated using generalized estimating equations (GEEs). This method accounts for the correlation of multiple observations per subject. Potential confounders were entered into the model based upon biologic plausibility and univariate analyses which indicated associations with the main outcome variable ($p<0.10$). Variables were eliminated if they were consistently insignificant ($p>0.10$) and did not alter the effect size of the primary independent variable (condom use). Factors associated with sexual activity (number of partners, new partners, and use of spermicide) that were not significant in the final model were retained as they were variables of interest. The final model included: age (25 or greater vs. less than 25), race (black vs. white or other), education (more than high school vs. high school or less), smoking status (current vs. prior or never), hormonal conception use (yes/no), history of pelvic inflammatory disease (yes/no), recent douching (yes/no), number of sex partners (>1 vs. 1), new sex partner (yes/no), and use of spermicide (yes/no). Analyses were repeated for each of the following microbiologic outcomes: Chlamydia and/or gonorrhea, presence of H_2O_2 producing lactobacilli, *Gardnerella vaginalis*, *Mycoplasma hominis*, and anaerobic Gram-negative rods (pigmented and nonpigmented). Additionally, the effect of condom use on bacterial vaginosis was considered within the following subgroups: bacterial vaginosis at previous visit (within 6-12 months) (yes/no), history of BV at baseline (yes/no), number of sexual partners in the past 2 months

(>1/1), new partner in the past 2 months (yes/no), use of hormonal contraception in the past 2 months (yes/no).

For the case-crossover analyses, exposure assessment intervals were designated to occur between each of the 6-month follow-up visits. Case-intervals corresponded to visits in which bacterial vaginosis was diagnosed at the end of the interval. Control-intervals corresponded to intervals in which the woman was diagnosed with normal/intermediate flora or solely normal flora. All intervals for women who experienced a change or "cross-over" in infection status were included in the primary analyses. Thus, women were allowed to have multiple case and control intervals, irrespective of the sequence in which the control and case intervals occurred. Control intervals or prior case intervals were considered etiologically irrelevant to infection status during a subsequent (or prior) case interval due to their remote temporal sequence. Given that BV is persistent in many women, the case-crossover analysis was repeated using only the first incident case interval and available controls intervals in order to capture only forward crossovers (lacking BV to obtaining BV). Thus, all intervals up until the first incident interval, for women who developed BV during follow-up were included in secondary incident analyses. Analyses which assessed only the first incident case interval and the immediate prior control interval (within 6 months) were similar to those that included all control intervals (i.e. multiple control intervals); thus, results are not reported. Adjusted odds ratios were estimated using conditional logistic regression. Given that time-independent factors (both measured and unmeasured) are controlled for with the case-crossover design, only time-dependent variables were considered for confounding adjustment. Final models included: number of sex partners (>1/1), new partner (yes/no), use of spermicide (yes/no), recent douching (yes/no), and use of hormonal contraception (yes/no). Cross-sectional analyses were conducted using the SAS System for

Windows, version 8.02, Cary, NC. Unconditional logistic regression analyses were conducted using STATA, version 8, College Station, TX.

4.4 RESULTS

Participants were predominantly aged 19-24 (66.4%), black (75%), and had a greater than high school education (52.1%). At baseline, 416 (37.0%) reported a history of BV, 160 (14.1%) reported a history of pelvic inflammatory disease, and 451 (39.8%) reported a history of chlamydial infection. One thousand twenty women were sexually active at baseline and 628 (61.5%) used condoms in the past 2 months. One hundred ninety women had two or more sexual partners in the past two months (18.7%) and 213 (21.0%) had a new sexual partner. Four hundred forty-nine women entered the study with a Gram-stain finding of bacterial vaginosis (39.6%), 123 (10.9%) had chlamydial infection, and 48 (4.2%) had a gonococcal infection.

Women were sexually active during 5732 (83.2%) of the visits. Condom use was reported 53.6% of the time during the 4712 follow-up visits. At baseline, women who smoked, had ever been pregnant, had sex during menses, had sex 2 or more times per week, and douched 1 or more times per month were more less likely use condoms during every sexual encounter (Table 18). Women who had two or more sex partners or had a new sex partner in the past 2 months were more likely to consistently use condoms than to not use condoms.

Over all follow-up visits, microbiology samples were obtained in 3358 of the 5732 visits (58.6%) where sexual activity was reported (Table 19). Women were diagnosed with BV in 1315 (39.2%) of the visits. The prevalence of BV ranged from 34.6% at the 6 month follow-up visit to 41.8% at the 3-year follow-up visit (results not shown). Chlamydial and/or gonococcal

infection was diagnosed in 330 (9.8%) of the visits (222 with chlamydia, 66 with gonorrhea, and 41 with both infections) (Table 19), and the prevalence ranged from 6.3% (3-year) to 8.3% (2-year) (results not shown).

4.4.1 Cross-sectional analyses of condom use and the risk of BV, other vaginal flora, and chlamydial/gonococcal infection

Overall women used condoms in 743 visits (56.5%) in which BV was diagnosed and women used condoms in 1167 (57.1%) visits in which they were diagnosed normal or intermediate flora (Table 19). Condom use was not associated with BV after adjusting for relevant confounders (OR = 0.95; 95% CI = 0.81- 1.11). When consistency of use was considered, there was evidence of protection for BV (condoms used in 10/10 sexual acts: adjusted OR=0.81, p=0.038), although the trend was not significant (p=0.07). Condom use at 50% or more of visits was not associated with BV (adjusted OR = 0.91, 95% CI = 0.75-1.11).

In relevant subgroups of women, including those with or without BV at the visit immediately prior, women with a history of BV, women with 1 or 2+ sexual partners in the past 2 months, women with/without a new sexual partner, and women who used hormonal contraception in the past 2 months, condom use was not significantly associated with BV (Table 21). The exception was seen among women who did not use oral contraceptives, wherein consistent condom use reduced BV risk (adjusted OR = 0.68, 95% CI: 0.53, 0.87, *p* for trend = 0.004).

Assessment of condom use in relation to other vaginal infections and microbiology outcomes (Table 20), showed a significant protective effect for both lack of H_2O_2-producing lactobacilli (condom use in 10/10 sex acts: adjusted OR = 0.73, p for trend = 0.001) and

Mycoplasma hominis (100% condom use: OR = 0.71, p for trend = 0.004). No association was observed between condoms and chlamydia/gonorrhea, anaerobic Gram-negative nonpigmented rods, *Gardnerella vaginalis*, and anaerobic Gram-negative pigmented rods.

4.4.2 Case-crossover analyses for the association between condom use and BV and other vaginal microorganisms

For the initial case-crossover analyses, we considered all prevalent case intervals to compare results with cross-sectional analyses. In prevalent analyses (Table 22), 606 women had a crossover in vaginal flora status from normal or intermediate flora to bacterial vaginosis. Among these women, condoms were used in 1077 (56.1%) of the intervals. Bacterial vaginosis was diagnosed in 47.3% of the intervals in which women reported condom use. When comparing BV to intermediate or normal flora, women who consistently used condoms had a 32% decreased risk of BV compared to women who did not use condoms (condom use in 10/10 sex acts: adjusted OR = 0.68, 95% CI = 0.49-0.94, p for trend = 0.047). Estimates were similar when comparing only normal flora to BV, suggesting a protective effect against development of BV for women with both intermediate and normal flora.

We repeated our analyses with only the first incident case-interval and found that condoms non-significantly decreased the risk of acquiring BV (adjusted OR = 0.57, 95% CI: 0.32-1.02) when comparing to intervals with both normal and intermediate flora control intervals (Table 22). However, the risk significantly decreased when only normal flora intervals were compared to BV intervals (condom use in 10/10 sex acts: adjusted OR = 0.41, 95% CI = 0.19-0.86, *p* for trend = 0.01).

Similar to the cross-sectional analysis, assessment of the relationship between condom use and microbiology outcomes showed a significant decrease in the risk for *M. hominis* with consistent condom use (adjusted OR=0.61, 95% CI: 0.41-0.93, p for trend=0.06) (Table 23). Consistent condom use was also significantly protective against anaerobic Gram-negative pigmented rods (OR=0.65, 95% CI: 0.47-0.91, p for trend=0.007). No association was observed between condoms and chlamydia/gonococcal infection, *G. vaginalis*, and anaerobic Gram-negative nonpigmented rods. These also remained non-significant when assessing only incident case intervals. In contrast to cross-sectional analyses, lacking H_2O_2-producing lactobacilli was non-significant when assessing both prevalent and incident case periods.

4.5 DISCUSSION

Consistent condom use significantly reduced the risk of bacterial vaginosis in this large cohort of women at high risk for sexually transmitted infections. We observed a significant decrease in the risk of bacterial vaginosis with increasing consistency of condom use using a case-crossover study design. The strength of the association was strongest when assessing only incident cases intervals of bacterial vaginosis. A protective effect was also observed in the cross-sectional analyses, although the results were non-significant.

The stronger results from the case-crossover analyses, compared to the cross-sectional analyses are of particular interest in this study. Sufficient control of confounding with respect to measuring condom effectiveness has proven to be difficult (27). Traditional study designs in which important factors cannot be controlled for because they are either unmeasured or unknown may result in biased conclusions. Thus, while the case-crossover design does not control for

unmeasured time-dependent confounders, the design does control for all measured or unmeasured time-independent confounders, which may significantly reduce the bias in estimated effects. The difference in our results suggests that unmeasured confounding was present in our assessment of condom use. Only one other study, to our knowledge, has used the case-crossover design to assess condom effectiveness for the prevention of STDs (21). This study found a significant protective effect for condoms against chlamydia and gonorrhea infection with the case-crossover design compared to the traditional cross-sectional method (21), which was attributed to the reduction in unmeasured confounding.

Our results were also strongest among women reporting consistent condom use. Whereas condom use, defined as a dichotomous variable (yes/no) was not protective against BV, 100% condom use was protective against BV, which is consistent with previous studies on condom effectiveness (21, 27). Given that consistency of use is a fairly crude measure of condom effectiveness, the true measure of condom effectiveness is likely greater than that observed in this study. We did not observe any significant protective effect when assessing persistent condom use across visits. This variable, however, was calculated based upon the dichotomous condom use variable, which may account for the null results.

We also found that condoms were protective against BV-associated organisms. The gold-standard of diagnosis for BV is Nugent's criteria based upon the Gram-stain, which assesses the proportion of *Lactobacilli* species, *G. vaginalis*, and curved Gram-variable rods or *Mobiluncus* species (26). Thus, our corresponding results for a protective effect against lacking H_2O_2 producing lactobacilli in the cross-sectional results support our findings for BV. Results were not as strong, although of similar magnitude, for *G. vaginalis*. We additionally saw a protective effect against *Mycoplasma hominis*; however, 20% of data are missing for this

microbiologic outcome, so results should be interpreted cautiously. The case-crossover analysis yielded similar results to cross-sectional analysis, although the magnitude of the effect was slightly greater, particularly when assessing incident case intervals. Of interest, was the pronounced decrease in risk seen for anaerobic Gram-negative pigmented rods. Anaerobic Gram-negative rods have often been associated with BV (28-30). A recent study by Ness et al. (31), also found that taking into account the presence of anaerobic Gram-negative rods when assessing BV-associated organisms increased the risk for PID compared to a traditionally diagnosed BV from Nugent's criteria.

One of the factors complicating our understanding of how BV is acquired is the natural history of the disease: BV occurs as an acute, chronic, and recurrent condition and very little is understood about the causes of variation among women. Women commonly have persistent BV, which would likely obscure any protective effect afforded by condom use. Among women with or without prior BV, we observed a non-significant trend towards a protective effect. This would indicate that condoms are protective whether or not the diagnosis of BV is an incident infection, recurrent infection, or reinfection. Of note, condom use was also most protective when assessing incident BV. Thus, condoms may prevent against the acquisition of BV in addition to potentially protecting the vaginal environment from further disruptions, infections, or recurrence of a prior infection.

Previous findings have been inconsistent in showing a relationship between condom and bacterial vaginosis. Several cross-sectional studies have shown protective effect for condoms. In a cohort of women attending a sexual health center, Smart et al. (32) found that 100% condom use decreased the risk of BV by 50%. Additionally, studies by Calzolari et al. (33) and Shoubnikova et al. (34) in European populations also found that condom use decreased the risk

of BV. Similarly, Moi et al. (35) found a significant decrease in the risk of BV for barrier methods of contraception ($p<0.05$), although the estimate was not adjusted for confounding factors. However, several studies have shown no association with condom use (36-42).

To date, only one longitudinal cross-sectional study and three cohort studies have assessed condom use in relation to bacterial vaginosis, and results have been inconsistent. Saifuddin et al. (36) followed a cohort of 17,264 women in rural Uganda for approximately 4 years at 10-month intervals. The risk of BV was not associated with consistent condom use (OR=0.89, 95% CI = 0.74-1.07). The authors did find an association with other sexually transmitted infections. However, the broad assessment of condom use (in the last year) and the variability of BV over shorter periods of time, may have made an association difficult to detect. Similarly, a small study by Hawes et al. (43), which followed a cohort of 182 women recruited from an STD clinic for 2 years, found that barrier methods of contraception did not decrease the risk for acquisition of BV (HR=0.8, 95% CI: 0.3-1.7). In contrast, Baeten et al. (44) followed a cohort of 948 female sex workers in Kenya for a median of 421 days and found a slight although significant decrease in the risk for bacterial vaginosis (HR=0.9, 95% CI = 0.7, 1.0). Similarly, Schwebke et al. (45) found that always using condoms was protective against the development of BV, but only in occasional partners (RR = 0.80, 95% CI = 0.67, 0.98).

Due to the wide variability between study designs, the different assessments of both BV (Gram-stain vs. Amsel's criteria) and condom use, and the inherent difficulty with accurately measuring condom effectiveness, the inconsistency in risk across studies is not surprising. Consistency of condom use or other factors associated with condom effectiveness are often not delineated in studies of BV. Further complicating the issue, BV involves multiple organisms, and one or more BV-associated organisms may also be transmissible, such as a subset of

anaerobic Gram-negative rods. Similarly, another, as yet unidentified organism, may be the transmissible agent. Recent studies have isolated numerous previously unidentified organisms associated with BV, and these studies may further our understanding of the components of BV and how factors may be transmitted (46, 47).

The strengths of this study include the large number of women studied, enrollment of a high-risk population, which enhanced study power, use of consistent and standardized enrollment and data collection protocols, collection of biomarkers of effect, and the relatively long-term and complete longitudinal data collection. Additionally, to our knowledge this is the first study to assess the association between condom use and bacterial vaginosis using a case crossover study design.

There are a number of limitations with our study. Condom use did not reduce chlamydial or gonococcal infection in either the cross-sectional or case-crossover analyses. The cross-sectional analyses indicated a harmful effect due to condom use, although this disappeared in the case-crossover analyses where the results showed a non-significant protective effect. These results may have been due to the imprecise nature of condom use assessment. We did not ascertain whether there was slippage or breakage of condoms, and condom use consistency is an imprecise assessment of condom effectiveness. Assessment of condom use according to the number of unprotected sex acts, may be a more precise measurement of condom effectiveness (21). Additionally, we only had 113 incident cases of chlamydia and gonorrhea which reduced our power for detecting an association, and this also likely reduced our power for assessing other microbiologic outcomes. Further limiting this study was the relatively long intervals between vaginal microbiologic assessments which only allowed for a somewhat gross assessment of BV infection status. BV is variable over short periods of time and incident versus prevalent or

recurrent infections cannot be accurately assessed with long intervals. Finally, women were not treated for BV or referred for treatment as part of the study, and information on treatment of BV was not ascertained for this study.

Given the complexity of bacterial vaginosis and the evidence against uniform sexual transmission (eg. BV diagnosed in adolescent virgins), it is highly unlikely that BV is solely sexually transmitted. However, research continues to suggest that sexual activity plays a role in the risk for BV. Factors such as number of sexual partners and new sexual partners are consistently linked with BV and are known risk factors for traditional STDs. Additionally, prevalence estimates are often highest among women attending STD clinics or among women who are sex workers (4, 5). In this study we demonstrated that consistent condom use was significantly associated with reduced risk of bacterial vaginosis, further lending support to the potential sexual transmissibility of bacterial vaginosis.

Table 18: Descriptive characteristics of the 1,020 women reporting sexual activity at baseline by consistency of condom use (Never, <5/10 times, 6-9/10 times, 10/10 times)*

	Condom use during the past 2 months								
	Never		≤ 5/10 times		6-9/10 times		10/10 times		
Baseline characteristic	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
	392	**38.4**	**213**	**20.9**	**166**	**16.3**	**249**	**24.4**	***p***
Age									
13-18	20	5.1	14	6.6	11	6.6	22	8.8	0.08
19-24	246	62.8	151	70.9	120	72.3	162	65.1	
25-29	91	23.2	37	17.4	28	16.9	44	17.7	
30+	35	8.9	11	5.2	7	4.2	21	8.4	
Race									
White	88	22.5	31	14.6	35	21.1	46	18.5	0.13
Black	276	70.4	172	80.8	125	75.3	188	75.5	
Other	28	7.1	10	4.7	6	3.6	15	6.0	
Education									
< High School	96	24.5	45	21.1	27	16.3	46	18.5	0.13
High School	106	27.0	58	27.2	54	32.5	60	24.1	
> High School	190	48.5	110	51.6	85	51.2	143	57.4	
Income									
< $10,000	178	49.2	99	51.3	68	44.7	110	48.3	0.31
$10,000 - $19,999	88	24.3	56	29.0	46	30.3	53	23.3	
≥ $20,000	96	26.5	38	19.7	38	25.0	65	28.5	
Smoking Status									
Current	169	43.1	68	32.1	65	39.2	70	28.1	0.007
Former	35	8.9	20	9.4	16	9.6	24	9.6	
Never	188	48.0	124	58.5	85	51.2	155	62.3	
Gravidity									
Ever	235	60.0	127	59.6	76	45.8	136	54.6	0.01
Self reported history of:									
Chlamydia	146	37.6	97	45.9	79	47.9	90	36.6	0.03
Gonorrhea	88	22.7	57	27.1	34	20.6	47	18.9	0.19
Trichmoniasis	91	23.3	54	25.6	40	24.5	56	22.6	0.88
Bacterial Vaginosis	145	37.5	76	36.2	69	42.1	81	33.3	0.35
Pelvic Inflammatory Disease	55	14.1	35	16.4	26	15.8	28	11.3	0.40
Yeast	253	65.0	148	69.8	110	67.1	160	64.3	0.58
Age at first intercourse									
≤ 15	177	45.3	104	48.8	79	47.6	114	46.2	0.14
16-17	153	39.1	80	37.6	59	35.5	77	31.2	
18+	61	15.6	29	13.6	28	16.9	56	22.7	
Sex during menses	47	12.1	39	18.3	19	11.5	17	6.8	0.002

Table continued

Table 18 continued: Descriptive characteristics of the 1,020 women reporting sexual activity at baseline by consistency of condom use (Never, <5/10 times, 6-9/10 times, 10/10 times)*

	Condom Use during the past 2 months								
	Never		≤ 5/10 times		6-9/10 times		10/10 times		
	N	%	N	%	N	%	N	%	*p*^
Average number of times have sex per week									
≤ 1	138	35.2	80	37.6	89	53.6	155	62.3	<0.0001
2 or more	254	64.8	133	62.4	77	46.4	94	37.8	
Number of sex partners in last 2 months									
≤ 1	350	89.3	162	76.1	118	71.1	200	80.3	<0.0001
2 or more	42	10.7	51	23.9	48	28.9	49	19.7	
New sex partner in past two months									
No	339	86.5	159	74.7	122	73.5	186	74.7	<0.0001
Yes	53	13.5	54	25.4	44	26.5	63	25.3	
Douching frequency									
Never	187	47.7	112	52.6	102	61.5	145	58.2	0.03
< 1 time per month	125	31.9	61	28.6	36	21.7	70	28.1	
≥ 1 time per month	80	20.4	40	18.8	28	16.9	34	13.7	
Use of hormonal contraceptives	181	46.2	102	47.9	65	39.2	109	43.8	0.34
Vaginal Flora Gram Stain at study entry									
Normal (score 0-3)	143	36.9	58	28.0	59	36.0	91	37.0	0.12
Intermediate (score 4-6)	88	22.7	56	27.1	37	22.6	71	28.9	
Bacterial Vaginosis (score 7-10)	157	40.5	93	44.9	68	41.5	84	34.2	
Chlamydia at study entry	40	10.5	22	10.8	24	14.8	25	10.3	0.47
Gonorrhea at study entry	19	5.0	9	4.4	8	4.9	9	3.7	0.89

* Missing cases: annual household income, n=85; smoking status, n=1; history of pelvic inflammatory disease, n=4; history of bacterial vaginosis, n=16; history of chlamydial infection, n=10, history of gonorrhea infection, n=9; history of trichomoniasis, n=7; history of yeast infection, n=6; age at first intercourse, n=3; sex during menses, n=4; vaginal flora, n=15; chlamydia and/or gonorrhea infection, n=31.
^ *p* for trend

Table 19: Cross-sectional analyses - condom use and the risk of BV and chlamydia/gonorrhea.

	Bacterial Vaginosis				Chlamydia and/or Gonorrhea			
Time dependent variables	**BV**	**Int./ Normal**	**Adj. OR***	**95% CI**	**C/G**	**None**	**Adj. OR****	**95% CI**
Condom Use								
Yes	743	1167	0.95	(0.81, 1.11)	205	1688	1.18	(0.92, 1.52)
No	572	876	1.0		125	1311	1.0	
Consistency of Use								
10/10	281	515	0.81	(0.66, 0.99)	72	715	1.02	(0.74, 1.40)
6-9/10	205	295	1.06	(0.85, 1.32)	58	436	1.25	(0.89, 1.77)
≤ 5/10	257	357	1.02	(0.84, 1.24)	75	537	1.34	(0.99, 1.81)
None	572	876	1.0		125	1331	1.0	
p for trend				0.07				0.81
Condom Use: Across visits								
≥ 50% of the time	836	1331	0.91	(0.75, 1.11)	225	1923	0.99	(0.74, 1.33)
1-49% of the time	133	233	0.92	(0.68, 1.24)	28	334	0.75	(0.48, 1.17)
Never	346	479	1.0		77	742	1.0	
p for trend				0.35				0.86

* Adjusted for age, race, education, baseline smoking status, number of sexual partners, new partner, use of spermicide, use of hormonal contraceptives, douching and history of PID

** Adjusted for age, race, education, use of hormonal contraceptives, number of sexual partners, new partner, and history of PID

Table 20: Cross-sectional analyses - condom use and the risk of microbial organisms, including H_2O_2-producing lactobacilli, *G. Vaginalis*, *M. hominis*, and anaerobic Gram-negative rods (pigmented and nonpigmented).

	Lacking H_2O_2 + lactobacilli		*G. vaginalis*		*M. hominis*		Anaerobic GN nonpigmented rods		Anaerobic GN pigmented rods	
Time dependent variables	**Adj. OR***	**95% CI**	**Adj. OR***	**95% CI**	**Adj. OR***	**95% CI**	**Adj. OR***	**95% CI**	**Adj. OR***	**95% CI**
Condom Use										
Yes	0.8	(0.69, 0.93)	1.04	(0.89, 1.21)	0.91	(0.77, 1.07)	1.10	(0.91, 1.35)	0.99	(0.84, 1.15)
No	1.0		1.0		1.0		1.0		1.0	
Consistency of Use										
10/10	0.73	(0.60, 0.89)	0.88	(0.72, 1.07)	0.71	(0.57, 0.88)	1.07	(0.83, 1.37)	0.87	(0.73, 1.06)
6-9/10	0.82	(0.66, 1.01)	1.17	(0.94, 1.47)	1.02	(0.81, 1.29)	1.02	(0.76, 1.35)	0.92	(0.75, 1.14)
≤ 5/10	0.86	(0.71, 1.03)	1.17	(0.96, 1.42)	1.08	(0.86, 1.36)	1.22	(0.94, 1.59)	1.08	(0.90, 1.30)
None	1.0		1.0		1.0		1.0		1.0	
p for trend		0.001		0.34		0.004		0.71		0.13
Condom Use: Across visits										
≥ 50% of the time	0.78	(0.64, 0.95)	0.97	(0.80, 1.19)	0.79	(0.64, 0.98)	1.19	(0.94, 1.52)	0.96	(0.80, 1.16)
1-49% of the time	0.90	(0.69, 1.18)	0.96	(0.73, 1.26)	0.86	(0.63, 1.16)	1.16	(0.82, 1.63)	0.92	(0.72, 1.18)
Never	1.0		1.0		1.0		1.0		1.0	
p for trend		0.01		0.83		0.03		0.17		0.72

* Adjusted for age, race, education, baseline smoking status, number of sexual partners, new partner, use of spermicide, use of hormonal contraceptives, douching and history of PID.

Table 21: Adjusted odds ratios for BV by condom use during the past 2 months and stratified by potentially modifying factors.

Condom use and potentially modifying factors	Obs.	Adjusted OR^	95% CI
Bacterial vaginosis at previous visit*: Yes	886		
None		1.0	
≤ 5/10		0.87	(0.59, 1.28)
6-9/10		1.03	(0.68, 1.56)
10/10		0.70	(0.49, 1.00)
p for trend			0.09
Bacterial vaginosis at previous visit*: No	1456		
None		1.0	
≤ 5/10		0.97	(0.69, 1.37)
6-9/10		0.95	(0.66, 1.36)
10/10		0.88	(0.64, 1.22)
p for trend			0.44
History of bacterial vaginosis: yes	1283		
None		1.0	
≤ 5/10		1.36	(1.00, 1.85)
6-9/10		1.10	(0.76, 1.58)
10/10		0.91	(0.65, 1.26)
p for trend			0.60
History of bacterial vaginosis: no	2028		
None		1.0	
≤ 5/10		0.87	(0.67, 1.13)
6-9/10		1.09	(0.84, 1.43)
10/10		0.76	(0.59, 0.98)
p for trend			0.10
Sexual partners in the past 2 months: ≥ 2	484		
None		1.0	
≤ 5/10		0.96	(0.56, 1.65)
6-9/10		0.70	(0.41, 1.18)
10/10		0.76	(0.43, 1.33)
p for trend			0.21
Sexual partners in the past 2 months: < 2	2874		
None		1.0	
≤ 5/10		1.03	(0.83, 1.28)
6-9/10		1.16	(0.91, 1.49)
10/10		0.82	(0.66, 1.02)
p for trend			0.19
New partner in the past 2 months: Yes	553		
None		1.0	
≤ 5/10		1.36	(0.81, 2.30)
6-9/10		0.88	(0.50, 1.55)
10/10		0.90	(0.55, 1.46)
p for trend			0.34

Table continues.

Table 21 continued: Adjusted odds ratios for BV by condom use during the past 2 months and stratified by potentially modifying factors.

Condom use and potentially modifying factors	Obs.	Adjusted OR^	95% CI
New partner in the past 2 months: No	2805		
None		1.0	
≤ 5/10		0.96	(0.78, 1.19)
6-9/10		1.12	(0.88, 1.42)
10/10		0.81	(0.65, 1.02)
p for trend			0.18
Use of hormonal contraception in past 2 months: Yes	1454		
None		1.0	
≤ 5/10		1.08	(0.79, 1.48)
6-9/10		1.28	(0.89, 1.85)
10/10		1.08	(0.77, 1.52)
p for trend			0.48
Use of hormonal contraception in past 2 months: No	1904		
None		1.0	
≤ 5/10		0.99	(0.77, 1.27)
6-9/10		0.95	(0.73, 1.26)
10/10		0.68	(0.53, 0.87)
p for trend			0.003

* With in the past 6-12 months

^ Adjusted for age, race, education, smoking, new partner, use of spermicide, douching, and history of PID.

Table 22: Case-crossover analyses - the association between condom use and BV using both prevalent and incident case intervals.

	Case crossover: Prevalent							
	BV vs. Int/Normal				BV vs. Normal			
Measures of condom use	**Case intervals**	**Control intervals**	**Adj. OR***	**95% CI**	**Case intervals**	**Control intervals**	**Adj. OR***	**95% CI**
	n=606				n=388			
Condom use during past 2 mo.								
Yes	510	567	0.87	(0.67, 1.11)	297	293	0.95	(0.67, 1.33)
No	411	432	1.0		250	231		
Consistency of use during past 2 mo.								
10/10	188	249	0.68	(0.49, 0.94)	112	148	0.68	(0.44, 1.04)
6-9/10	145	135	1.04	(0.73, 1.49)	81	66	1.26	(0.78, 2.03)
≤ 5/10	177	183	0.95	(0.69, 1.29)	104	79	1.1	(0.71, 1.71)
None	411	432	1.0		250	231	1.0	
p for trend				0.047				0.145
	Case crossover: Incident							
	n=256				n=133			
Condom use during past 2 mo.								
Yes	141	252	0.65	(0.40, 1.05)	71	115	0.55	(0.30, 1.00)
No	115	162	1.0		62	83	1.0	
Consistency of use during past 2 mo.								
10/10	55	107	0.57	(0.32, 1.02)	27	57	0.41	(0.19, 0.86)
6-9/10	40	63	0.68	(0.35, 1.32)	21	30	0.57	(0.25, 1.31)
≤ 5/10	46	82	0.71	(0.40, 1.26)	23	28	0.81	(0.36, 1.81)
None	115	162	1.0		62	83	1.0	
p for trend				0.07				0.01

* Adjusted for number of partners, new partner, use of spermicide, use of hormonal contraception, and douching

Table 23: Case-crossover analyses - the association between condom use, BV-associated organisms, and chlamydial/gonococcal infection using both prevalent and incident case intervals.

	Case-crossover: prevalent											
	Chlamydia/ gonorrhea		**Lacking H_2O_2 + lactobacilli**		***G. vaginalis***		***M. hominis***		**Anaerobic GN nonpigmented rods**		**Anaerobic GN pigmented rods**	
Measures of condom use	**Adj. OR**	**95% CI**	**Adj. OR**	**95% CI**	**Adj. OR**	**95% CI**	**Adj. OR**	**95% CI**	**Adj. OR**	**95% CI**	**Adj. OR**	**95% CI**
	n=255		n=483		n=534		n=394		n=497		n=345	
Condom use during past 2 mo.												
Yes	1.08	(0.75, 1.57)	0.80	(0.62, 1.03)	1.05	(0.82, 1.34)	0.98	(0.73, 1.32)	0.90	(0.66, 1.22)	0.82	(0.63, 1.06)
No	1.0		1.0		1.0		1.0		1.0		1.0	
Consistency of use during past 2 mo.												
10/10	0.81	(0.50, 1.32)	0.77	(0.56, 1.07)	0.81	(0.59, 1.10)	0.61	(0.41, 0.93)	0.93	(0.63, 1.38)	0.65	(0.47, 0.91)
6-9/10	1.03	(0.63, 1.70)	0.90	(0.63, 1.30)	1.33	(0.95, 1.87)	1.16	(0.77,1.75)	0.86	(0.56, 1.31)	0.79	(0.55, 1.12)
≤ 5/10	1.44	(0.91, 2.25)	0.77	(0.56, 1.05)	1.16	(0.85, 1.57)	1.17	(0.82, 1.66)	0.90	(0.61, 1.33)	1.00	(0.73, 1.37)
None	1.0				1.0		1.0		1.0		1.0	
p for trend		0.36		0.18		0.35		0.06		0.65		0.007
	Case-crossover: Incident											
	n=113		n=281		n=268		n=215		n=171		n=284	
Condom use during past 2 mo.												
Yes	0.84	(0.42, 1.68)	0.69	(0.42, 1.13)	0.82	(0.52, 1.28)	0.65	(0.38, 1.09)	0.81	(0.42, 1.55)	0.60	(0.37, 0.98)
No	1.0		1.0		1.0		1.0		1.0		1.0	
Consistency of use during past 2 mo.												
10/10	0.81	(0.33, 1.97)	0.69	(0.37, 1.28)	0.62	(0.35, 1.08)	0.55	(0.28, 1.10)	0.75	(0.22, 1.05)	0.54	(0.29, 1.00)
6-9/10	0.54	(0.20, 1.45)	0.81	(0.43, 1.54)	1.24	(0.64, 2.44)	0.57	(0.27, 1.21)	0.88	(0.40, 1.73)	0.63	(0.33, 1.21)
≤ 5/10	1.01	(0.46, 2.23)	0.63	(0.35, 1.19)	0.90	(0.49, 1.63)	0.76	(0.40, 1.46)	0.81	(0.42, 1.55)	0.64	(0.36, 1.16)
None	1.0		1.0		1.0		1.0		1.0		1.0	
p for trend		0.45		0.36		0.15		0.07		0.55		0.056

***** Adjusted for number of partners, new partner, use of spermicide, use of hormonal contraception, and douching

4.6 LITERATURE CITED

1. Koumans E, Markowitz L, Hogan V. (2002) Indications for Therapy and Treatment Recommendations for Bacterial Vaginosis in Nonpregnant and Pregnant Women: A Synthesis of Data. *Clin Infect Dis*. 35(Suppl 2):S152-72.
2. Pybus V, Onderdonk A. (1999) Microbial interactions in the vaginal ecosystem, with emphasis on the pathogenesis of bacterial vaginosis. *Microb Inf*. 1:285-92.
3. Nagy E, Petterson M, Mardh P. (1991) Antibiosis between bacteria isolated from the vagina of women with and without signs of bacterial vaginosis. *APMIS*. 99:739-44.
4. Forsum U, Holst E, Larsson P, Vasquex A, Jakobsson T, Mattsby-Baltzer I. (2005) Bacterial vaginosis – a microbiological and immunological enigma. *APMIS*. 113:81-90.
5. Priestly C, Kinghorn G. (1996) Bacterial Vaginosis. *Br J Clin Pract*. 50:331-4.
6. Hay P. (2004) Life in the littoral zone: lactobacilli losing the plot. *Sex Transm Infect*. 81:100-2.
7. Koumans E, Kendrick J. (2000) Preventing Adverse Sequelae of Bacterial Vaginosis. *Sexually Trans Dis*. 28:292-7.
8. Schwebke J. (2003) Gynecologic consequences of bacterial vaginosis. *Obstet Gynecol Clin N Am*. 30:685-94.
9. Flynn C, Helwig A, Meurer L. (1999) Bacterial Vaginosis in Pregnancy and the Risk of Prematurity: A Meta-Analysis. *J Family Prac*. 48:885-92.
10. Wilson J. (2004) Managing recurrent bacterial vaginosis. *Sex Transm Infect*. 80:8-11.
11. Smart S, Singal A, Mindel A. (2004) Social and sexual risk factors for bacterial vaginosis. *Sex Trans Infect.* 80:58-62.
12. Morris M, Nicoll A, Simms I, Wilson J, Catchpole M. (2001) Bacterial vaginosis: a public health review. *BJOG*. 108:439-50.
13. Nelson D, Macones G. (2002) Bacterial Vaginosis in Pregnancy: Current Findings and Future Directions. *Epidem Rev*. 24:102-8.
14. Bump R, Buesching W. (1988) Bacterial vaginosis in virginal and sexually active adolescent females: Evidence against exclusive sexual transmission. *Obstet Gynecol*. 158:935-9.
15. Larsson P, Bergstrom M, Forsum U, Jacobsson b, Strand A, Wolner-Hanssen P. (2005) Bacterial vaginosis: Transmission, role in genital tract infection and Pregnancy outcome: an enigma. *APMIS*. 113:233-45.
16. Shlay J, McClung M, Patnaik J, Douglas J. (2004) Comparison of Sexually Transmitted Disease Prevalence by Reported Level of Condom Use Among Patients Attending an Urban Sexually Transmitted Disease Clinic. *Sex Trans Dis*. 31:154-60.
17. Holmes K, Levine R, Weaver M. (2004) Effectiveness of condoms in preventing sexually transmitted infections. *Bull World Health Org*. 82:454-461.
18. United States Department of Health and Human Services. (2001) Workshop summary: scientific evidence on condom effectiveness for sexually transmitted disease (STD)

prevention. http://www.niaid.nih.gov/dmid/stds/condomreport.pdf. Accessed November 25, 2005
19. Crosby R, DiClemente R, Holtgrave D, Wingood G. (2002) Design, measurement, and analytical considerations for testing hypotheses relative to condom effectiveness against non-viral STIs. *Sex Trans Infect*. 78:228-31.
20. Devine O, Aral S (2004) The impact of inaccurate reporting of condom use and imperfect diagnosis of sexually transmitted disease infection in studies of condom effectiveness: a simulation-based assessment. *Sex Transm Dis*. 10:588-95.
21. Warner L, Macaluso M, Austin H, Kleinbaum D, Artz L, Fleenor M, Brill I, Newman D, Hook E. (2005) Application of the Case-Crossover Design to Reduce Unmeasured Confounding in Studies of Condom Effectiveness. *Am J Epi*. 161:765-73.
22. Ness R, Hillier S, Richter H, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P. (2002). Douching in Relation to Bacterial Vaginosis, Lactobacilli, and Facultative Bacteria in the Vagina. *Obstet Gynecol*. 100:765-72.
23. Ness R, Hillier S, Kip K, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P, Richter H. (2004) Bacterial Vaginosis and Risk of Pelvic Inflammatory Disease. *Obstet Gynecol*. 104:761-9.
24. Stergachis A, Scholes D, Heidrich F. (1993) Selective screening for *Chlamydia trachomatis* infection in a primary care population of women. *Am J Epidem*. 138:143-53.
25. Schwebke J, Hillier S, Sobel J, McGregor J, Sweet R. (1996) Validity of the vaginal Gram stain for the diagnosis of bacterial vaginosis. *Obstet Gynecol*. 88:573-6.
26. Nugent R, Krohn M, Hillier S. (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 29:297-301.
27. Warner L, Stone K, Macaluso M, Buehler J, Austin H. (2006) Condom Use and Risk of Gonorrhea and Chlamydia: A Systematic Review of Design and Measurement Factors Assessed in Epidemiologic Studies. *Sex Trans Dis*. 33:36-51.
28. Hill G. (1993) The microbiology of bacterial vaginosis. *Am J Obstet Gynecol*. 169:450-4.
29. McGregor J, French J. (2000) Bacterial Vaginosis in Pregnancy. *Obstet Gynecol Surv*. 55:1-19.
30. Holst E, Wathne B, Hovelius B, Mardh P. (1987) Bacterial Vaginosis: Microbiological and Clinical Findings. *Eur J Clin Microbiol.* 6:536-41.
31. Ness R, Kip K, Hillier S, Soper D, Stamm C, Sweet R, Rice P, Richter H. (2005) A Cluster Analysis of Bacterial Vaginosis-associated Microflora and Pelvic Inflammatory Disease. *Am J Epi*. 162:1-6.
32. Smart S, Singal A, Mindel A. (2004) Social and sexual risk factors for bacterial vaginosis. *Sex Trans Infect.* 80:58-62.
33. Calzolari E, Masciangelo R, Milite V, Verteramo R. (2000) Bacterial vaginosis and contraceptive methods. *Int J Gynecol Obstet*. 70:341-346.
34. Shoubnikova M, Hellberg D, Nilsson S, Per-Anders M. (1997) Contraceptive Use in Women with Bacterial Vaginosis. *Contraception*. 55:355-58.
35. Moi H. (1990) Prevalence of bacterial vaginosis and its association with genital infections, inflammation, and contraceptive methods in women attending sexually transmitted disease and primary health clinics. *Int J STD & AIDS*. 1:86-94.
36. Saifuddin A, Lutalo T, Wawer M, Serwadda D, Sewankambo N, Nalugoda F, Makumbi F, Wabwire-Mangen F, Kiwanuka N, Kigozi F, Kiddugavu M, Gray R. (2001) HIV incidence

and sexually transmitted disease prevalence associated with condom use: a population study in Rakai, Uganda. *AIDS*. 15:2171-79.
37. Bradshaw C, Morton A, Garland S, Morris M, Moss L, Fairley C. (2005) Higher-Risk Behavorial Practices Associated With Bacterial Vaginosis Compared With Vaginal Candidiasis. *Obstet Gynecol*. 106:105-14.
38. Chiaffarino F, Parazzini F, Besi P, Lavezzari M. (2004) Risk factors for bacterial vaginosis. *Eur J Obstet Gynecol Reprod Biol*. 117:222-6.
39. Schwebke J, Desmond R, Oh M. (2004) Predictors of Bacterial Vaginosis in Adolescent Women who Douche. *Sex Trans Dis*. 31:433-36.
40. Fonck K, Kaul R, Keli F, Bwayo J, Ngugi E, Moses S, Temmerman M. (2001) Sexually transmitted infections and vaginal douching in a population of female sex workers in Nairobi, Kenya. *Sex Trans Inf*. 77:271-75.
41. Rajamanoharan S, Low N, Jones S, Pozniak A. (1999) Bacterial Vaginosis, Ethnicity, and the Use of Genital Cleaning Agents: A Case Control Study. *Sex Trans Dis*. 26:404-9.
42. Rosenberg M, Davidson A, Chen J, Judson F, Douglas J. (1992) Barrier Contraceptives and Sexually Transmitted Diseases in Women: A Comparison of Female-Dependent Methods and Condoms. *Am J Public Health*. 82:669-674.
43. Hawes S, Hillier S, Benedietti J, Stevens C, Koutsky L, Wolner-Hanssen, Holmes K. (1996) Hydrogen Peroxide-Producing Lactobacilli and Acquisition of Vaginal Infections. *J Infect Dis*. 174:1058-63.
44. Baeten J, Nyange P, Richardson B, Lavreys L, Cohan B, Martine H, Mandaliya K, Ndinya-Achola J, Bwayo J, Dreiss J. (2001) Hormonal contraception and risk sexually transmitted disease acquisition: Results from a prospective study. *Am J Obstet Gynecol*. 185:380-385.
45. Schwebke J, Desmond R. (2005) Risk Factors for Bacterial Vaginosis in Women at High Risk for Sexually Transmitted Diseases. *Sexually Trans Dis*. 32:654-58.
46. Fredricks D, Fiedler T, Marrazzo J. (2005) Molecular Identification of Bacteria Associated with Bacterial Vaginosis. *NEJM*. 353:1899-1911.
47. Verstraelen H, Verhelst R, Claeys G, Temmerman M, Vaneechoutte M. (2004) Culture-independent analysis of vaginal microflora: The unrecognized association of Atopobium vaginae with bacterial vaginosis. *Am J Obstet Gynecol*. 191:1130-2.

5.0 DISCUSSION

In this study we utilized data from a cohort of women at high risk for sexually transmitted diseases followed for 3 to 4 years. The longitudinal dataset with multiple measurements of vaginal flora obtained through self-collected vaginal swabs taken every 6 to 12 months allowed us to evaluate the natural history of bacterial vaginosis and evaluate factors associated with the development and persistence of BV.

Our findings suggest that acquisition of BV often leads to persistent infections that are unable to completely resolve. Nearly one-third of women in our study had persistent infection with BV. Similarly, one-third of women had persistently high levels of vaginal flora associated with BV, and persistently low levels of protective H_2O_2-producing lactobacilli. Race and baseline vaginal flora score were strongly associated with persistent BV, and while currently smoking was associated with persistent high growth of *G. vaginalis* and anaerobic Gram-negative rods, other behavioral factors, including sexual behaviors, douching, and use of hormonal contraceptives, were not associated with persistent BV or persistently high growth of vaginal organisms. These results suggest that persistence of BV is predominantly dependent upon host factors (i.e. immune function, genetic polymorphisms) versus environmental factors (i.e. sexual behavior, use of vaginal products).

Prior infection with bacterial vaginosis or a history of abnormal flora was also found to play a significant role in the association between douching and acquisition bacterial vaginosis. In cross-sectional results we found that douching was only associated with bacterial vaginosis among women who had a history of bacterial vaginosis. We also found that douching was

associated with acquisition of BV during the study period among women who had abnormal flora (defined as intermediate flora) at baseline. Among women who had normal flora at baseline, douching was not associated with acquiring BV, suggesting that douching likely disrupts already unbalanced flora and further stresses an already unstable vaginal environment leading to the development of BV. These findings are of particular concern, because women are unlikely to be aware of their infection status, especially for women with intermediate flora or asymptomatic BV. Additionally, many women douche due to abnormal vaginal symptoms (1), which may then lead to persistence of infection.

While factors associated with BV are well characterized and variations in BV over time are becoming better understood, it is still not understood how BV is acquired; thus we evaluated the relationship between condom use and BV to assess whether BV may be sexually transmitted. In this study, we utilized a case-crossover design to decrease unmeasured confounding often associated with studies on condom use (2). Our findings showed that women who used condoms were significantly less likely to have BV, suggesting that sexual transmission plays a significant role in the development of BV. These findings increase the mounting evidence for a sexually transmitted etiology for BV. Currently, few, if any, options exist for preventing BV and increased awareness of BV as a sexually transmitted disease and the protection afforded through consistent condom use may help to reduce the prevalence of the disease.

BV is an extremely complex and not well understood disease. In this study, we were able to further characterize the natural history of the disease, as well as evaluate two very controversial issues: 1) Does douching cause BV?, and 2) Is BV a sexually transmitted disease? Our results emphasize the need for further research to understand the causes of BV.

5.1 FUTURE RESEARCH

5.1.1 The etiology of BV

Our analyses allowed for long-term evaluation of persistent infection; however we were limited in our ability to characterize more precise changes in vaginal flora after the initial diagnosis of BV. Persistent diagnoses of BV may have been due to reinfection or persistent infection, and differentiating between these is necessary for understanding the etiology of BV. Studies have yet to determine whether specific organisms persist in the vaginal flora leading to persistent infection or increased likelihood of recurrence, or whether organisms are reacquired. One longitudinal study evaluating changes in G. vaginalis found a trend towards acquisition of new biotypes in women who had persistent infection (3). Further research in this area is warranted, and recent utilizations of PCR analysis to identify the myriad of organisms involved with BV (4) will help to identify whether specific bacteria persistent.

Additional prospective studies with evaluation of vaginal flora at closer intervals are also warranted. Our data collection was limited in that we were unable to adjust for natural variations that occur in flora on a daily basis. Changes in vaginal flora have been shown to occur daily basis (5), and vaginal flora varies significantly with the menstrual cycle (6). Adjustments for natural variations are necessary to capture the differences between women who have persistently high levels of BV-associated flora and women who have more variable changes in vaginal flora.

Evaluating the consequences of persistent vaginal infection are also necessary. BV has been associated with numerous adverse outcomes (7), and evaluating whether women with persistent and recurrent BV have an increased risk of BV over women who are able to resolve

infection with BV. Recent research has also indicated strong gene-environmental interactions between genetic polymorphisms in cytokines and BV which increases the likelihood of preterm birth (8). Further research in this area and the role of cytokines in persistent BV are warranted.

5.1.2 Black race and the increased risk of BV

Race is consistently associated with BV across studies, and was one of the strongest risk factors for persistent BV infection in our study. These results suggest that intrinsic host factors are predominantly responsible for persistent infections. Other studies have found that race is associated with BV independent of other factors (9), which may be partly explained by variations in genetic polymorphisms associated with proinflammatory cytokines (10). Few studies have evaluated cytokines and regulation of BV; however, studies do show significant variations in cytokine levels among women with BV (8), and additional research in this area may help to understand the racial disparity associated with BV.

5.1.3 Douching and the acquisition of BV

Few prospective studies have been conducted on the relationship between douching and BV. Our study found different causal results for the association between douching and BV depending on whether a woman had normal or intermediate flora at baseline, which suggests douching stresses already disturbed flora. This is of particular concern has it may promote persistent infection. Additional prospective studies which are able to delineate between variations in vaginal flora are needed to confirm these results.

One of our main limitations with this study was relatively long intervals between follow-up visits. We were only able to assessing douching practices every six months, and douching has been shown to have strong short term effects (1); thus, evaluation of douching six months prior to assessment of microbiology may have been too far distant to be meaningful. Prospective studies which are able to obtain accurate assessment of the timing of douching in relation to acquisition of BV may help to better understand the effect of douching on both short and long-term changes in vaginal flora.

5.1.4 BV as a sexually transmitted disease

One of the primary controversies surrounding BV is whether BV has a sexually transmitted component. Our results suggest a strong and consistent protective effect for condom use against BV and BV-associated organisms, which supports a role for sexual transmission and the acquisition of BV. Further studies are warranted to confirm our results. Additionally, our study was not specifically designed to evaluate condom use and studies which capture other factors related to condom use (such as the number of unprotected sex acts (2) will be able to further improve the assessment of condom effectiveness in relation to bacterial vaginosis. Partner infection status has also been shown to significantly impact risk estimates associated with condom use and other STDs; however, a male equivalent has yet to be identified for BV. Most studies evaluating partner infection status have been relatively small, and additional studies which are able to capture a broad range of potential organisms among larger populations are needed.

Studies evaluating BV consistently suggests both sexual and nonsexual etiologies. Studies consistently find that number of sexual partners, new sexual partners, and history of

vaginal infections are associated with BV (11-13), all of which are markers of traditional STDs. However, BV has been found in adolescent virgins (14) and treatment of males has not proven to be effective (15), although that may be related to the general lack of understanding of which organism causes BV. Given the extreme differences, it is very likely that BV results from multiple sources. The next step will be to delineate between sexual acquisition of BV and innate development of BV.

5.2 APPLICATION TO PUBLIC HEALTH

Bacterial vaginosis is a highly prevalent but not well understood disease. However, research continues to indicate that BV is a large public health problem. BV is associated with a number of adverse sequelae, including preterm delivery, pelvic inflammatory disease, infertility, cervical cancer, increased acquisition of STDs, pre-term birth, and intrapartum and postpartum infections (7, 16, 17), which underscores the need for further research regarding the etiology of bacterial vaginosis. In this study we were able to provide further insight into the natural history of BV, and demonstrate high levels of persistent infection. This adds to the work which shows significant variability in vaginal flora between women and identifies distinct groups of women which are prone to persistent infections. Additionally, this work underscores the need to identify effective treatment methods, particularly for women known to have a history of BV. The high levels of persistent infection also suggest low recognition and treatment of BV in the clinical setting, and further education is necessary to increase awareness of BV among women and clinicians.

Our study also provides increased understanding of the association between douching and BV. Douching is consistently associated with BV in the literature; however the question still remains about whether or not douching causes BV. In this study we demonstrated that douching adversely effects women with already disturbed flora and promotes infection among women with intermediate flora. It may also promote persistent infection among women with a history of BV. Thus, while douching does not likely cause BV in women with normal flora, it does aggravate vaginal flora among women with already stressed vaginal ecosystems. This has important implications, particularly for women with a history of BV and the large number of women with asymptomatic BV or intermediate flora. The prevalence of douching and BV are also high among African Americans, and reducing douching among these women may help to reduce the prevalence of BV.

Additionally, this study provides important evidence for the role of sexual transmission in the etiology of BV. This work also helps to increase our understanding of the etiology of BV and further work is warranted to understand the protection afforded through condom use. Currently there are no methods for preventing BV and identification of BV as a sexually transmitted disease could potentially increase the ability to effectively treat and prevent the disease. Women with a history of BV may also be a subset of women at increased risk for reinfection and may be an effective targeted intervention group.

5.3 LITERATURE CITED

1. Ness R, Hillier S, Richter H, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P. (2002). Douching in Relation to Bacterial Vaginosis, Lactobacilli, and Facultative Bacteria in the Vagina. *Obstet Gynecol*. 100:765-72.
2. Warner L, Macaluso M, Austin H, Kleinbaum D, Artz L, Fleenor M, Brill I, Newman D, Hook E. (2005) Application of the Case-Crossover Design to Reduce Unmeasured Confounding in Studies of Condom Effectiveness. *Am J Epi*. 161:765-73.
3. Briselden A, Hillier S. (1990) Longitudinal Study of the Biotypes of Gardnerella vaginalis. *J Clin Microbio*. 28:2761-4.
4. Fredricks D, Fiedler T, Marrazzo J. (2005) Molecular Identification of Bacteria Associated with Bacterial Vaginosis. *NEJM*. 353:1899-1911.
5. Schwebke J, Richey C, Weiss H. (1999) Correlation of Behaviors with Microbiologic Changes in Vaginal Flora. *J Inf Dis*. 180:1632-6.
6. Keane F, Ison C, Taylor-Robinson D. (1997) A longitudinal study of the vaginal flora over a menstrual cycle. *Int J STD & AIDS*. 8:489-94.
7. Morris M, Nicoll A, Simms I, Wilson J, Catchpole M. (2001) Bacterial vaginosis: a public health review. *BJOG*. 108:439-50.
8. Cauci S. (2004) Vaginal Immunity in Bacterial Vaginosis. *Curr Infect Dis Reports*. 6:450-56.
9. Ness R, Hillier S, Richter H, Soper D, Stamm C, Bass D, Sweet R, Rice P. (2003) Can Known Risk Factors Explain Racial Differences in the Occurrence of Bacterial Vaginosis? *J Natl Med Assoc*. 95:201-212.
10. Ness R, Haggerty C, Harger G, Ferrel l R. (2004) Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. *Am J Epidemiol*. 160:1033-8.
11. Kent H. (1991) Epidemiology of vaginitis. *Am J Obstet Gynecol*. 165:1168-76.
12. Morris M, Rogers P, Kinghorn G. (2001) Is bacterial vaginosis a sexually transmitted infection? *Sex Trans Inf*. 77:63-68.
13. Chiaffarino F, Parazzini F, Besi P, Lavezzari M. (2004) Risk factors for bacterial vaginosis. *Eur J Obstet Gynecol Reprod Biol*. 117:222-6.
14. Bump R, Buesching W. (1988) Bacterial vaginosis in virginal and sexually active adolescent females: Evidence against exclusive sexual transmission. *Obstet Gynecol*. 158:935-9.
15. Larsson P, Bergstrom M, Forsum U, Jacobsson b, Strand A, Wolner-Hanssen P. (2005) Bacterial vaginosis: Transmission, role in genital tract infection and Pregnancy outcome: an enigma. *APMIS*. 113:233-45.
16. McGregor J, French J. (2000) Bacterial Vaginosis in Pregnancy. *Obstet Gynecol Surv*. 55:1-19.
17. Sweet R. (2000) Gynecologic conditions and bacterial vaginosis: Implications for the Non-Pregnant Patient. *Inf Dis Obstet Gynecol*. 8:184-90.

BIBLIOGRAPHY

Abma J, Chandra A, Mosher W, Peterson L, Piccinino L. Fertility, family planning, and women's health: New data from the 1995 National Survey of Family Growth. *Vital Health Stat*. 23:1-114.

Allison P. (1995) Survival Analysis Using the SAS System: A Practical Guide. The SAS Institute. Cary, NC.

Apgar B, Greenberg G, Yen G. (2005) Prevention of Group B Streptococcal Disease in the Newborn. *Am Fam Physician*. 71:903-10.

Arya O, Tong C, Hart C, Pratt B, Hughes S, Roberts P, Kirby P, Howel J, McCormack A, Goddard A. (2001) *Sex Trans Inf*. 77:58-62.

Baeten J, Nyange P, Richardson B, Lavreys L, Cohan B, Martine H, Mandaliya K, Ndinya-Achola J, Bwayo J, Dreiss J. (2001) Hormonal contraception and risk sexually transmitted disease acquisition: Results from a prospective study. *Am J Obstet Gynecol*. 185:380-5.

Bailey J, Farquhar C, Owen C. (2004) Bacterial Vaginosis in Lesbians and Bisexual Women. *Sex Trans Dis*. 31:691-4.

Beigi R, Wiesenfeld H, Hillier S, Straw T, Krohn M. (2005) Factors Associated with Absence of H_2O_2 – Producing Lactobacillus among Women with Bacterial Vaginosis. *J Inf Dis*. 191:924-9.

Berger B, Kolton S, Zenilman J, Cummings M, Feldman J, McCormack. (1995) Bacterial Vaginosis in Lesbians: A Sexually Transmitted Disease. *Clin Inf Dis*. 21:1402-5.

Boon M, Claasen H, Kok L. (2002) Urbanization and baseline prevalence of genital infections including *Candida, Trichomonas*, and human papillomavirus and of a disturbed vaginal ecology as established in the Dutch Cervical Screening Program. *Am J Obstet Gynecol*. 187:365-9.

Boris J, Pahlson C, Larsson P. (1997) Six-year follow-up after successful treatment of bacterial vaginosis. *Int J STD AIDS*. 8(Suppl 1):41.

Bradshaw C, Morton A, Garland S, Morris M, Moss L, Fairley C. (2005) Higher-Risk Behavorial Practices Associated With Bacterial Vaginosis Compared With Vaginal Candidiasis. *Obstet Gynecol*. 106:105-14.

Briselden A, Hillier S. (1990) Longitudinal Study of the Biotypes of Gardnerella vaginalis. *J Clin Microbio*. 28:2761-4.

Brown D. (2005) Bacterial Vaginosis, in Benign Diseases of the Vulva and Vagina. Elsevier Mosby, Philadelphia PA. Fourth Edition.

Bump R, Buesching W. (1988) Bacterial vaginosis in virginal and sexually active adolescent females: Evidence against exclusive sexual transmission. *Obstet Gynecol*. 158:935-9.

Burstein G, Workowski K. (2003) Sexually transmitted diseases treatment guidelines. *Curr Opin Pediatr*. 15:391-7.

Calzolari E, Masciangelo R, Milite V, Verteramo R. (2000) Bacterial vaginosis and contraceptive methods. *Int J Gynecol Obstet*. 70:341-6.

Cauci S, Scrimin F, Driussi S, Ceccone S, Monte R., Fant L, Quadrifoglio F. (1996) Gynecology: Specific immune response against *Gardnerella vaginalis* hemolysin in patients with bacterial vaginosis. *Am J Obstet Gynecol*. 175:1601-5.

Cauci S. (2004) Vaginal Immunity in Bacterial Vaginosis. *Curr Infect Dis Reports*. 6:450-6.

Centers for Disease Control and Prevention. (1993) Update. Barrier protection against HIV infection and other sexually transmitted diseases. *Morb Mortal Wkly Report*. 42:589-91.

Centers for Disease Control and Prevention (2002). Sexually Transmitted Disease Guidelines, 2002. *Morb Mortal Wkly Report*. 51:1-80.

Chiaffarino F, Parazzini F, Besi P, Lavezzari M. (2004) Risk factors for bacterial vaginosis. *Eur J Obstet Gynecol Reprod Biol*. 117:222-6.

Cook R, Reedondo-Lopez V, Schmitt C, Meriwether C, Sobel J. (1992) Clinical, Microbiological, and Biochemical Factors in Recurrent Bacterial Vaginosis. *J Clin Microbio*. 30:870-7.

Crosby R, DiClemente R, Holtgrave D, Wingood G. (2002) Design, measurement, and analytical considerations for testing hypotheses relative to condom effectiveness against non-viral STIs. *Sex Trans Infect*. 78:228-31.

Demba E, Morison L, van der Loeff M, Awasana A, Gooding E, Bailey R, Mayaud P, West B. (2005) Bacterial vaginosis, vaginal flora patterns and vaginal hygiene practices in patients presenting with vaginal discharge syndrome in The Gambia, West Africa. *BMC Inf Dis*. 5:12.

Devine O, Aral S. (2004) The Impact of Inaccurate Reporting of Condom Use and Imperfect Diagnosis of Sexually Transmitted Disease Infection in Studies of Condom Effectiveness. *Sex Trans Dis*. 31:588-95.

Diggle P, Heagerty P, Liang K, Zeger S. (2002) Analysis of Longitudinal Data. Oxford University Press Inc., New York, Second Edition. Horowitz B, Mardh P, editors. (1991) Vaginitis and Vaginosis. Wiley-Liss, New York.

Ellish N, Weisman C, Celentano D, Zenilman J. (1996) Reliability of Partner Reports of Sexual History in a Heterosexual Population at a Sexually Transmitted Disease Clinic. *Sex Trans Dis*. 23:446-52.

Eschenbach D. (1993) History and Review of Bacterial Vaginosis. *Am J Obstet Gynecol*. 169:441-5.

Fitch J. (2001) Are Condoms Effective in Reducing the Risk of Sexually Transmitted Disease? *Ann Pharmacother*. 35:1136-8.

Flynn C, Helwig A, Meurer L. (1999) Bacterial Vaginosis in Pregnancy and the Risk of Prematurity: A Meta-Analysis. *J Family Prac*. 48:885-92.

Fonck K, Kaul R, Keli F, Bwayo J, Ngugi E, Moses S, Temmerman M. (2001) Sexually transmitted infections and vaginal douching in a population of female sex workers in Nairobi, Kenya. *Sex Trans Inf*. 77:271-5.

Forsum U, Hallen A, Larsson P (2005) Bacterial vaginosis – a laboratory and clinical diagnostics enigma. *APMIS*. 113:153-61.

Forsum U, Holst E, Larsson P, Vasquex A, Jakobsson T, Mattsby-Baltzer I. (2005) Bacterial vaginosis – a microbiological and immunological enigma. *APMIS*. 113:81-90.

Fredricks D, Fiedler T, Marrazzo J. (2005) Molecular Identification of Bacteria Associated with Bacterial Vaginosis. *NEJM*. 353:1899-1911.

Galavotti C. Cabral R, Beeker C. (1996) Condom Use to Prevent Incident STDs. *Sex Trans Dis.* 23:77-9.

Gutman R, Peipert J, Weitzen S, Blume J. (2005) Evaluation of Clinical Methods for Diagnosing Bacterial Vaginosis. *Obstet Gynecol*. 105:551-6.

Haggerty C, Hillier S, Bass D, Ness R. (2004) Bacterial Vaginosis and Anaerobic Bacteria are Associated with Endometritis. *Clin Infect Dis*. 39:990-5.

Hallen A, Pahlson C, Forsum U. (1988) Rectal occurrence of *Mobiluncus* species. *Genitourin Med*. 64:273-5.

Hallen A, Pahlson C, Forsum U. (1987) Bacterial vaginosis in women attending STD clinic: diagnostic criteria and prevalence of *Mobiluncus* spp. *Genitourin Med*. 63:386-9.

Haukkamma M, Stranden P, Jousimies-Somer H, Siitonen A. (1986) Bacterial flora of the cervix in women using different methods of contraception. *Am J Obstet Gynecol*. 154:520-4.

Hawes S, Hillier S, Benedietti J, Stevens C, Koutsky L, Wolner-Hanssen, Holmes K. (1996) Hydrogen Peroxide-Producing Lactobacilli and Acquisition of Vaginal Infections. *J Infect Dis*. 174:1058-63.

Hay P. (2004) Life in the littoral zone: lactobacilli losing the plot. *Sex Transm Infect*. 81:100-2.

Hill G. (1993) The microbiology of bacterial vaginosis. *Am J Obstet Gynecol*. 169:450-4.

Hillier S, Krohn M, Klebanoff S, Eschenbach D. (1992) The Relationship of Hydrogen Peroxide-Producing Lactobacilli to Bacterial Vaginosis and Genital Microflora in Pregnant Women. *Obstet Gynecol*. 79:369-73.

Hillier S, Krohn M, Rabe L. (1993) Normal vaginal flora, H_2O_2-producing lactobacilli, and bacterial vaginosis in pregnant women. *Clin Infect Dis*. 16:S273-81.

Holmes K, Levine R, Weaver M. (2004) Effectiveness of condoms in preventing sexually transmitted infections. *Bull World Health Org*. 82:454-461.

Holst E. (1990) Reservoir of Four Organisms Associated with Bacterial Vaginosis Suggests Lack of Sexual Transmission. *J Clin Microbiol*. 28:2035-9.

Holzman C, Leventhal J, Qiu H, Jones N, Wang J. (2001) Factors Linked to Bacterial Vaginosis in Nonpregnant Women. *Am J Public Health*. 91:1664-70.

Hooton T, Hillier S, Johnson C, Roberts P, Stamm W. (1991) *Escherichia coli* Bacteriuria and Contraceptive Method. *JAMA*. 265:64-9.

Hosmer D, Lemeshow S. Applied Logistic Regression. 2nd ed. New York, NY: Wiley, 2000.

Iwasaka T, Wada T, Kidera Y, Sugimori H. (1986) Hormonal Status and Mycoplasma Colonization in the Female Genital Tract. *Obstet Gynecol*. 68:263-6.

Juliano C, Piu L, Gavini E, Zanetti S, Fadda G. (1992) In vitro antibacterial activity of antiseptics against vaginal lactobacilli. *Eur J Clin Microbiol Infect Dis*. 11:1166-9.

Keane F, Maw, Pritchard C, Ison. (2004) Methods employed by genitourinary medicine clinics in the United Kingdom to diagnose bacterial vaginosis. *Sex Transm Infect*. 81:155-7.

Keane F, Ison C, Taylor-Robinson D. (1997) A longitudinal study of the vaginal flora over a menstrual cycle. *Int J STD & AIDS*. 8:489-94.

Kent H. (1991) Epidemiology of vaginitis. *Am J Obstet Gynecol*. 165:1168-76.

Klebanoff M, Hauth J, MacPherson C, Carey J, Heine R, Wapner R, Iams J, Moawad A, Miodovnik M, Sibai B, vanDorsten J, Dombrowski M. (2004) Time course of the

regression of asymptomatic bacterial vaginosis in pregnancy with and without treatment. *Am J Obstet Gynecol.* 190:363-70.

Koch A, Bilina A, Teodorowicz L, Stary A. (1997) *Mycoplasma hominis* and *Ureaplasma urealyticum* in patients with sexually transmitted disease. *Wien Klin Woch*. 109:584-9.

Koumans E, Kendrick J. (2000) Preventing Adverse Sequelae of Bacterial Vaginosis. *Sexually Trans Dis*. 28:292-7.

Koumans E, Markowitz L, Hogan V. (2002) Indications for Therapy and Treatment Recommendations for Bacterial Vaginosis in Nonpregnant and Pregnant Women: A Synthesis of Data. *Clin Infect Dis*. 35(Suppl 2):S152-72.

Kuby J (1997) Immunology. WH Freeman and Company, New York. Third Edition.

La Ruche G, Messou N, Ali-Napo L, Noba V, Faye-Kette H, Combe P, Bonard D, Syllakoko F, Dheha D, Welffensekra C, Dosso M, Msellati P. (1999) Vaginal Douching: Association with Lower Genital Tract Infections in African Pregnant Women. *Sex Trans Dis*. 26:191-6.

Larsson P, Bergstrom M, Forsum U, Jacobsson b, Strand A, Wolner-Hanssen P. (2005) Bacterial vaginosis: Transmission, role in genital tract infection and Pregnancy outcome: an enigma. *APMIS*. 113:233-45.

Macones G, Parry S, Elkousy M, Clothier B, Ural S, Straus J. (2004) A polymorphism in the promotor region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. *Am J Obstet Gynecol*. 190:1504-8.

Mardh P. (1991) The vaginal ecosystem. *Am J Obstet Gynecol*. 165:1163-8.

Marrazzo J, Koutsky L, Eschenbach D, Agnew K, Stine K, Hillier S. (2002) Characterization of Vaginal Flora and Bacterial Vaginosis in Women Who Have Sex with Women. *J Inf Dis*. 185:1307-13.

Martino J, Vermund S. (2002) Vaginal Douching: Evidence for Risks and Benefits to Women's Health. *Epidemiol Rev*. 24:109-124.

McCaffrey M, Varney P, Evans B, Taylor-Robinson D. (1999) Bacterial vaginosis in lesbian: evidence for lack of sexual transmission. *Int J STD & AIDS*. 10:305-8.

McGregor J, French J. (2000) Bacterial Vaginosis in Pregnancy. *Obstet Gynecol Surv*. 55:1-19.

Mead P. (1993) Epidemiology of Bacterial Vaginosis. *Am J Obstet Gynecol*. 446-9.

Moi H. (1990) Prevalence of bacterial vaginosis and its association with genital infections, inflammation, and contraceptive methods in women attending sexually transmitted disease and primary health clinics. *Int J STD & AIDS*. 1:86-94.

Monif G, Thompson J, Stephen H, Baer H. (1980) Quantitative and qualitative effects of povidone-iodine liquid and gel on the aerobic and anaerobic flora of the female genital tract. *Am J Obstet Gynecol*. 137:432-8.

Moore K, Day R, Albers M. (2002) Pathogenesis of urinary tract infections: a review. *J Clin Nurs*. 11:568-73.

Morris M, Nicoll A, Simms I, Wilson J, Catchpole M. (2001) Bacterial vaginosis: a public health review. *BJOG*. 108:439-50.

Morris M, Rogers P, Kinghorn G. (2001) Is bacterial vaginosis a sexually transmitted infection? *Sex Trans Inf*. 77:63-8.

Nagy E, Petterson M, Mardh P. (1991) Antibiosis between bacteria isolated from the vagina of women with and without signs of bacterial vaginosis. *APMIS*. 99:739-44.

Nelson D, Macones G. (2002) Bacterial Vaginosis in Pregnancy: Current Findings and Future Directions. *Epidem Rev*. 24:102-8.

Ness R, Hillier S, Richter H, Soper D, Stamm C, Bass D, Sweet R, Rice P, Downs J, Aral S. (2000) Why Women Douche and Why They May or May Not Stop. *Sex Trans Dis*. 30:71-4.

Ness R, Hillier S, Richter H, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P. (2002). Douching in Relation to Bacterial Vaginosis, Lactobacilli, and Facultative Bacteria in the Vagina. *Obstet Gynecol*. 100:765-72.

Ness R, Hillier S, Richter H, Soper D, Stamm C, Bass D, Sweet R, Rice P. (2003) Can Known Risk Factors Explain Racial Differences in the Occurrence of Bacterial Vaginosis? *J Natl Med Assoc*. 95:201-12.

Ness R, Hillier S, Kip K, Soper D, Stamm C, McGregor J, Bass D, Sweet R, Rice P, Richter H. (2004) Bacterial Vaginosis and Risk of Pelvic Inflammatory Disease. *Obstet Gynecol*. 104:761-9.

Ness R, Haggerty C, Harger G, Ferrel l R. (2004) Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. *Am J Epidemiol*. 160:1033-8.

Ness R, Kip K, Hillier S, Soper D, Stamm C, Sweet R, Rice P, Richter H. (2005) A Cluster Analysis of Bacterial Vaginosis-associated Microflora and Pelvic Inflammatory Disease. *Am J Epi*. 162:1-6.

Ness R, Kip K, Soper D, Stamm C, Rice P, Richter H. (2006) Variability of Bacterial Vaginosis over 6-12 month intervals. *Sex Trans Dis*. 33:1-5.

Newton E, Piper J, Shain R, Perdue S, Paeirs W. (2001) Predictors of vaginal microflora. *Am J Obstet Gynecol*. 184:845-55.

Nugent R, Krohn M, Hillier S. (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 29:297-301.

Paavonen J, Miettinen A, Stevens C, Chen K, Holmes K. (1983) *Mycoplasma hominis* in Nonspecific Vaginitis. *Sex Trans Dis*. 10:271-5.

Pavlova S, Tao L. (2000) In vitro inhibition of commercial douche products against vaginal microflora. *Infect Dis Obstet Gynecol*. 8:99-104.

Potter B, Jhorden L. (2004) Should we screen for bacterial vaginosis in those at risk for preterm labor? *J Fam Pract*. 53:827-31.

Priestly C, Kinghorn G. (1996) Bacterial Vaginosis. *Br J Clin Pract*. 50:331-4.

Pybus V, Onderdonk A. (1999) Microbial interactions in the vaginal ecosystem, with emphasis on the pathogenesis of bacterial vaginosis. *Microb Inf*. 1:285-92.

Rajamanoharan S, Low N, Jones S, Pozniak A. (1999) Bacterial Vaginosis, Ethnicity, and the Use of Genital Cleaning Agents: A Case Control Study. *Sex Trans Dis*. 26:404-9.

Rosenberg M, Phillips R, Holmes M. (1991) Vaginal Douching: Who and Why? *J Repro Med*. 36:753-8.

Rosenberg M, Davidson A, Chen J, Judson F, Douglas J. (1992) Barrier Contraceptives and Sexually Transmitted Diseases in Women: A Comparison of Female-Dependent Methods and Condoms. *Am J Public Health*. 82:669-74.

Saifuddin A, Lutalo T, Wawer M, Serwadda D, Sewankambo N, Nalugoda F, Makumbi F, Wabwire-Mangen F, Kiwanuka N, Kigozi F, Kiddugavu M, Gray R. (2001) HIV incidence and sexually transmitted disease prevalence associated with condom use: a population study in Rakai, Uganda. *AIDS*. 15:2171-9.

Scholes D, Stergachis A, Ichikawa L, Heidrich F, Holmes K, Stamm W. Vaginal douching as a risk factor for cervical *Chlamydia trachomatis* infection. *Obstet Gynecol*. 91:993-7.

Schwebke J, Hillier S, Sobel J, McGregor J, Sweet R. (1996) Validity of the Vaginal Gram Stain for the Diagnosis of Bacterial Vaginosis. *Obstet Gynecol*. 88:573-6.

Schwebke J, Richey C, Weiss H. (1999) Correlation of Behaviors with Microbiologic Changes in Vaginal Flora. *J Inf Dis*. 180:1632-6.

Schwebke J. (2000) Asymptomatic bacterial vaginosis: Response to therapy. *Am J Obstet Gynecol*. 183:1434-9.

Schwebke J. (2003) Gynecologic consequences of bacterial vaginosis. *Obstet Gynecol Clin N Am*. 30:685-94.

Schwebke J, Desmond R, Oh M. (2004) Predictors of Bacterial Vaginosis in Adolescent Women who Douche. *Sex Trans Dis*. 31:433-6.

Schwebke J, Desmond R. (2005) Risk Factors for Bacterial Vaginosis in Women at High Risk for Sexually Transmitted Diseases. *Sexually Trans Dis*. 32:654-8.

Shlay J, McClung M, Patnaik J, Douglas J. (2004) Comparison of Sexually Transmitted Disease Prevalence by Reported Level of Condom Use Among Patients Attending an Urban Sexually Transmitted Disease Clinic. *Sex Trans Dis*. 31:154-60.

Shoubnikova M, Hellberg D, Nilsson S, Per-Anders M. (1997) Contraceptive Use in Women with Bacterial Vaginosis. *Contraception*. 55:355-8.

Simms I, Stephenson J. (2000) Pelvic inflammatory disease epidemiology: what do we know and what do we need to know? *Sex Transm Infect.* 76:80-7.

Smart S, Singal A, Mindel A. (2004) Social and sexual risk factors for bacterial vaginosis. *Sex Trans Infect.* 80:58-62.

Sobel J. (2000) Bacterial Vaginosis. *Annu Rev Med*. 51:349-56.

Sobel J. (1997) Vaginitis. *NEJM*. 337:1896-1903.

Spiegelman D, Hertzmark E. (2005) Easy SAS Calculations for Risk or Prevalence Ratios and Differences. *Am J Epidemiol*. 162:199-200.

Stergachis A, Scholes D, Heidrich F. (1993) Selective screening for *Chlamydia trachomatis* infection in a primary care population of women. *Am J Epidem*. 138:143-53.

Sweet R. (2000) Gynecologic conditions and bacterial vaginosis: Implications for the Non-Pregnant Patient. *Inf Dis Obstet Gynecol*. 8:184-90.

Taylor-Robinson D, Hay P. (1997) The pathogenesis of the clinical signs of bacterial vaginosis and possible reasons for its occurrence. *Int J STD & AIDS*. 8:13-6.

Taylor-Robinson D, Furr P. (1998) Update on sexually transmitted mycoplasmas. *Lancet*. 351:12-5.

Thorsen P, Jensen I, Jeune B, Ebbesen N, Magnus A, Bremmelgaard A, Moller B. (1998) Few microorganisms associated with bacterial vaginosis may constitute the pathologic core: A population-based microbiologic study among 3596 pregnant women. *Am J Obstet Gynecol*. 178:580-7.

United States Department of Health and Human Services. (2001) Workshop summary: scientific evidence on condom effectiveness for sexually transmitted disease (STD) prevention. http://www.niaid.nih.gov/dmid/stds/condomreport.pdf. Accessed November 25, 2005

Vallor A, Antonio M, Hawes S, Hillier S. (2001) Factors associated with acquisition of, or persistent colonization by, vaginal lactobacilli: role of hydrogen peroxide production. *J Infect Dis*. 184:1431-6.

Vermund S, Sarr M, Murphy D, Levin L, Abdalian S, Ma Y, Crowley-Nowick P, Wilson C. (2001) Douching Practices Among HIV Infected and Uninfected Adolescents in the United States. *J Adolescent Health*. 29S:80-6.

Verstraelen H, Verhelst R, Claeys G, Temmerman M, Vaneechoutte M. (2004) Culture-independent analysis of vaginal microflora: The unrecognized association of Atopobium vaginae with bacterial vaginosis. *Am J Obstet Gynecol.* 191:1130-2.

Warner L, Clay-Warner J, Boles J, Williamson J. (1998) Assessing Condom Use Practices: Implications for Evaluating Method and User Effectiveness. *Sex Trans Dis.* 25:273-7.

Warner L, Newman D, Austin H, Kamb M, Douglas J, Malotte C, Zenilman J, Rogers J, Bolan G, Fishbein M, Kleinbaum D, Macaluso M, Peterman T. (2004) Condom Effectiveness for Reducing Transmission of Gonorrhea and Chlamydia: The Importance of Assessing Partner Infection Status. *Am J Epi*. 159:242-51.

Warner L, Macaluso M, Austin H, Kleinbaum D, Artz L, Fleenor M, Brill I, Newman D, Hook E. (2005) Application of the Case-Crossover Design to Reduce Unmeasured Confounding in Studies of Condom Effectiveness. *Am J Epi*. 161:765-73.

Wilson J. (2004) Managing recurrent bacterial vaginosis. *Sex Transm Infect*. 80:8-11.

Wolner-Hanssen P, Eschenbach D, Paavonen J, Stevens C, Kiviat N, Critchlow C, DeRouen T, Koutsky L, Holmes K. (1990) Association Between Vaginal Douching and Acute Pelvic Inflammatory Disease. *JAMA*. 263:1936-41.

Zhang J, Thomas G, Leybovich E. (1997) Vaginal Douching and Adverse Health Effects: A Meta-Analysis. *Am J Public Health*. 87:1207-11.

Zenilman J, Weisman C, Rompalo A, Ellish N, Upchurch D, Hook E, Celentano D. (1995) Condom Use to Prevent Incident STDs: The Validity of Self-Reported Condom Use. *Sex Trans Dis.* 22:15-21.

Zou G. (2004) A Modified Poisson Regression Approach to Prospective Studies with Binary Data. *Am J Epidemiol*. 159:702-6.