Prevalence of Bacterial Vaginosis and *Candida* among Postmenopausal Women in the United States

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Objectives: To describe the prevalence of bacterial vaginosis (BV) and *Candida* among community-dwelling postmenopausal women in the United States and determine their change with age, using estimates based on Waves 1 and 2 of the National Social Life, Health and Aging Project (NSHAP).

Method: Self-administered vaginal swabs were collected in-home from women aged 57–85 ($n = 1,016$) in Wave 1 and again 5 years later in Wave 2 ($n = 883$). Gram-stained specimens were evaluated for BV using the Nugent score as well as presence of *Candida*.

Results: BV was prevalent in 23% and 38% of postmenopausal women in Waves 1 and 2 and increased with age. Women initially categorized with BV in Wave 1 were more than 10 times as likely to be categorized with BV in Wave 2, relative risk ratio (RRR) = 10.5; 95% confidence interval (CI) (4.45–24.7); $p < .001$, whereas women initially categorized as intermediate in Wave 1 were five times more likely to have a BV categorization, RRR = 5.0; 95% CI (2.56–9.75); $p < .001$. Although the presence of *Candida* was similar in both waves (6% and 5%), its relationship with age only became evident in Wave 2, with odds of detecting *Candida* decreasing by 7% with each year of age, OR = 0.93, 95% CI (0.88, 0.98); $p = .010$.

Discussion: In Wave 2, the prevalence of BV was higher and increased with age while the prevalence of *Candida* was low and declined with age. A 5-year age increase contributed to the prevalence change across waves. Methods refinements in Wave 2 improved the detection of BV and *Candida* and clarified their relationship with age.

Key Words: Bacterial vaginosis—*Candida*—Population—Postmenopause—Self-collection—Vaginal swab.

BACKGROUND

Postmenopause is a non-reproductive state marked by hormonal changes, more specifically a decrease in the levels of the ovarian hormones, estrogen, and progesterone. Without these hormones, the vaginal mucosa thins and begins to atrophy, becoming smaller and less elastic (Farage, Miller, & Sobel, 2010; Lobo, 2012). Functionally, the vagina produces fewer secretions, less lubrication and is more vulnerable to small tears during intercourse or masturbation which can contribute to dyspareunia (Lindau, Schumm, et al., 2007; Mac Bride, Rhodes, & Shuster, 2010). Vaginal atrophy affects up to 47% of postmenopausal women, but symptoms are often underreported by patients due to embarrassment discussing “personal” symptoms or a belief that it is normal part of aging (Johnston et al., 2004; Mac Bride et al., 2010). Thus, only about one-quarter of women that experience symptoms of vaginal atrophy, such as burning, itching, abnormal discharge, and urinary discomfort seek medical care (Cardozo, Bachmann, McClish, Fonda, & Birgerson, 1998). The postmenopausal vaginal environment, including vaginal atrophy may also contribute to a shift in the colonization of microbes that may cause vaginal infections in the older woman.

Bacterial vaginosis (BV) is the most common vaginal infection in reproductive-aged women and is categorized by a shift in vaginal flora from aerobic to predominately pathogenic anaerobic bacteria (Sobel, 1997). In the typical reproductive-aged vaginal environment, estradiol promotes glycogen production from the vaginal epithelium. The glycogen is hydrolyzed into glucose, which gets metabolized into lactic acid by beneficial lactobacilli, producing hydrogen peroxide. This process maintains an acidic environment and controls pathogenic overgrowth (Larsen, 1982, Larsen 1993; Mac Bride et al., 2010). This biological sequence is altered with the thinning of the vaginal wall in postmenopause, resulting in reduced vaginal epithelial exfoliation and increased pH, a prime environment for anaerobic bacterial overgrowth (Eschenbach et al., 1989; Hill, 1993; Murray, 1999). The colonization of anaerobic bacteria in the vagina, or BV, is usually found in an alkaline environment (pH greater than 4.5) more frequently observed in postmenopausal women (García-Closas et al., 1999; Plourd, 1997; Spinillo et al., 1997).

However, the cause of BV remains unclear with behavioral factors such as frequency of intercourse, douching, pessaries, and smoking increasing the risk for infection (Sobel, 1997; Alnaif & Drutz, 2000; Allsworth & Peipert, 2007;

Brotman, et al., 2008; Verstraelen, Verhelst, Vaneechoutte, & Temmerman, 2010). For 50% of women, BV infection is asymptomatic, but for others it is not (Sobel, 2005). Symptoms may present as thin, homogeneous, off-white colored discharge with a fishy smelling odor along with pruritus, and burning during urination (Anderson, Klink, & Cochrane, 2004). While there are no complications from BV, there are risks as infection can compromise the function of the vulvovaginal barrier increasing susceptibility to and transmissibility of sexually transmitted infections (STIs), including HIV (Koumans & Kendrick, 2001). BV is considered a sexually enhanced disease (SED) because it affects both sexually active and inactive women (Verstraelen & Verhelst, 2009).

While large scale population estimates are available for younger women (Allsworth & Peipert, 2007), postmenopausal women have been understudied and reported clinical BV prevalence varies dramatically from 6% to 50% using the Gram stain Nugent scoring method (Burton & Reid, 2002; Cauci et al., 2002; Heinemann & Reid, 2005; Ocviyanti, Rosana, Olivia, & Darmawan, 2010). In addition, population estimates for BV remain unclear as an estimated 50%–75% of BV infections are asymptomatic and symptoms are nonspecific (Amsel et al., 1983; Klebanoff et al., 2004; Nyirjesy, 2008; Schaaf, Perez-Stable, & Borchardt, 1990). As a result, many women who have BV do not seek clinical care and thus do not contribute to clinical population estimates. The National Social Life Health and Aging Project (NSHAP) Waves 1 and 2 has filled this gap by collecting vaginal swab samples from a large representative population of United States women aged 57–90 (Lindau et al., 2009).

The presence of *Candida*, more commonly known as yeast, is considered typical fungal colonization in the vagina (Achkar & Fries, 2010). *Candida* species have been identified in 20–30% of reproductive-aged women and 6–7% of menopausal women (Goldacre et al., 1979; Tibaldi et al., 2009) but remain understudied in postmenopausal women. The presence of *Candida* spp. does not necessarily indicate a vaginal infection, however, its presence in combination with inflammation and vulvovaginal symptoms such as itching, burning and discharge indicates a clinical diagnosis of vulvovaginal candidiasis (VVC) (Achkar & Fries, 2010). Some risk factors for VVC include estrogen hormone replacement therapy, antibiotics, steroids, diabetes, and immunosuppression (Achkar & Fries, 2010; Bauters, Dhont, Temmerman, & Nelis, 2002; Beigi, Meyn, Moore, Krohn, & Hillier, 2004; Sobel et al., 1998).

Prevalence of VVC in postmenopausal women remains understudied but is reportedly low in non-HRT users, ranging from 3% to 7% (Fischer & Bradford, 2011; Ross, 1978). VVC more commonly occur at lower vaginal pH levels (less than 4.5), typical of estrogenized reproductive-aged women (Beigi et al., 2004; Plourd, 1997). However, hypoestrogenism in menopause results in higher vaginal pH levels (5.0

or above) and so a lower prevalence of VVC is expected in postmenopausal women. Reduced glycogen presence in the vaginal environment as well as lower levels of circulating ovarian hormones in postmenopausal women correlate with both vaginal atrophy and the lowered risk of VVC (Spinillo et al., 1997).

With the older United States population expected to double in the next 40 years, there is a need to continue to research this age group. Furthermore, with women living approximately a third of their life in postmenopause, it is important to understand transitions in the vaginal flora of older postmenopausal women, their attendant risk for vaginitis as well as related health concerns. This includes describing both changes typical of postmenopausal women, as well as those that may increase risk for infections, discomfort and urinary problems. The NSHAP survey sample was designed specifically to be representative of older adults residing in the United States. Thus, it is a unique longitudinal data set of older US adults, first studied in Wave 1 (aged 57–85) in 2005–2006 and again in Wave 2 (aged 62–90) in 2010–2011, aimed to re-interview original respondents surveyed in Wave 1 as well as their and co-residential romantic partners (married or not) and non-interviewed Wave 1 respondents. In this manuscript, we report the first large survey describing the prevalence of BV and *Candida* in a non-clinical, representative sample of postmenopausal women living in the United States.

METHOD

Study Population

Wave 1.—In NSHAP Wave 1 (fielded in 2005–2006) all women aged 57–85 (median = 67 years old) were eligible for vaginal biospecimen collection. Two-thirds (67.5%) of female respondents provided a vaginal biospecimen ($N = 1,028$). A total of 99.9% ($n = 1,016$) samples were processed and of these 89.3% ($n = 907$) samples were determined to be adequate for Nugent score evaluation and 94.2% ($n = 957$) for the presence of *Candida* (Lindau et al., 2009).

Wave 2.—In NSHAP Wave 2 (fielded in 2010–2011), Wave 1 respondents were re-interviewed. Individuals who declined participation in Wave 1 were recruited again for Wave 2. In addition, spouses or romantic partners living with the Wave 1 respondents were recruited for participation. The vaginal swab biomeasure was administered to a randomly selected two-thirds of female respondents selected prior to the interview ($N = 1,198$) (Jaszczak et al., 2014). The consent rate was 74.3% ($n = 889$). All collected samples were processed, and of these, 98.5% ($n = 870$) were determined to be adequate for analysis of Nugent score and *Candida* determination by a trained microbiologist. For these analyses, we focus on those women who met the Wave 1 age-eligibility requirement (born between 1920 and 1947) ($n = 790$,

median = 70 years old], excluding a small number of female spouses or partners who were outside the age range ($n = 80$).

Data Availability

Waves 1 and 2 data are publically available (NSHAP Wave 1: Waite, Linda J., Edward O. Laumann, Wendy Levinson, Stacy Tessler Lindau, and Colm A. O'Muircheartaigh. National Social Life, Health, and Aging Project (NSHAP): Wave 1. ICPSR20541-v6. Ann Arbor, MI: Inter-university Consortium for Political and Social Research (distributor), 2014-04-30. doi:10.3886/ICPSR20541.v6. NSHAP Wave 2: Waite, Linda J., Kathleen Cagney, William Dale, Elbert Huang, Edward O. Laumann, Martha K. McClintock, Colm A. O'Muircheartaigh, L. Phillip Schumm, and Benjamin Cornwell. National Social Life, Health, and Aging Project (NSHAP): Wave 2 and Partner Data Collection. ICPSR34921-v1. Ann Arbor, MI: Inter-university Consortium for Political and Social Research (distributor), 2014-04-29. doi:10.3886/ICPSR34921.v1).

Sample Collection, Storage and Shipping

Wave 1.—Self-administered vaginal swabs (BD BBL CultureSwab Plus Amies Gel without Charcoal, Double Swab, Catalog No. 220117; Becton, Dickinson and Company, Franklin Lakes, NJ) were collected in home by women using the procedure detailed in a eight-picture diagram (Lindau et al., 2009). The respondent was given oral and written instructions by the field interviewer before proceeding to the bathroom for sample collection. The respondent was instructed to insert a double-tipped swab with the tip up into her vagina. While counting to 10, the respondent gently rotated the swab in the vagina, and placed it directly into a white paper bag. The respondent returned the bag to the field interviewer, who removed the swab from the white bag, placed it into the transport tube and then into an insulated bag with a cold compress. The swab was shipped daily overnight to the Jordan clinical microbiology laboratory at Magee-Womens Hospital of UPMC, Pittsburgh, PA.

Wave 2.—In Wave 2, the vaginal swab collection protocol was refined to maximize sample collection. This included specific instructions in the eight-picture diagram to angle the swab towards the small of the back and to move the swab around within the vaginal lumen, making direct contact with the vaginal walls. Another refinement in Wave 2 was the use of an assay-specific swab. In Wave 1, a double swab was used for sample collection of two biomeasures, microbiology, and cytology. For Wave 2, a single swab with specimen specific media and transport tube (BD BBL CultureSwab Plus Amies Gel without Charcoal, Catalog No. 220116; Becton, Dickinson and Company, Franklin Lakes, NJ) was dedicated for BV and *Candida* specimen collection enabling singular use and direct shipment to the microbiology lab.

An additional Wave 2 protocol refinement instructed the respondent to immediately place the swab into its transport tube after specimen collection rather than first into a paper bag with later placement into a transport tube, a process that may have degraded the sample in Wave 1. The final refinement was the use of continuously replaced frozen (-20°C) refrigerant bricks to keep samples cold immediately after collection until arrival at the Jordan microbiology laboratory at George Washington University (GWU), Washington, DC. This was an improvement from the single (4°C) cold compress used in Wave 1 which quickly rose to room temperature upon shipment (O'Doherty et al., 2014).

Sample Processing

Upon receipt at the microbiology laboratory, each swab was removed from the transport tube, rolled onto a glass microscope slide, and allowed to air dry before being heat-fixed. Once fixed, the specimens were stable for storage at room temperature. All swabs were processed onto slides and Gram stained (Burke, 1922) on the same day that they were received.

Bacterial Vaginosis Determination

The Gram-stained microscope slide was scanned using a low power objective to locate epithelial cell clusters and bacterial flora. Once the cells and flora were located, the slide was determined to be adequate for analysis. The slide was then examined using 10–20 representative fields under oil immersion at 1000 $\times$ magnification. The quantity of the following bacterial morphotypes, *Lactobacilli*, *Gardereella/Bacteriodes* and *Mobiluncus*, were recorded and translated into a rank score (Table 1). Overgrowth of *Gardereella/Bacteriodes* and *Mobiluncus* is associated with BV and so higher counts of these bacteria are assigned higher rank scores. Conversely, higher counts of facultative bacteria, such as lactobacilli are given lower rank scores.

The rank scores from each type of bacteria were added together to determine an overall Nugent score, a standard measure of vaginal bacterial flora (Table 1). The Nugent scores were grouped into three diagnostic bacterial categories: normal (0–3), intermediate (4–6), and BV (7–10) (Nugent et al., 1991). This protocol was also used in Wave 1 (Lindau, Mendoza, Surawska, & Jordan, 2008).

Presence of *Candida*

The same heat-fixed, Gram-stained slide was evaluated under oil immersion at 1000 $\times$ magnification for the presence or absence of *Candida* cells, including budding, blastospore, and pseudohyphae forms. The samples were scored dichotomously as positive or negative for the presence of *Candida* in the genital tract. This protocol was also used in Wave 1 (Lindau, Mendoza, Surawska, & Jordan, 2007).

Statistical Analyses

To determine whether there is evidence of a difference between waves in the mean Nugent score, *Candida* prevalence

Table I. Determination of the Nugent Score using microscopic examination of Gram-stained vaginal smears

Morphotypes								
<i>Lactobacilli</i>			<i>Gardnerella,</i> <i>Bacteroides</i>			<i>Mobiluncus</i>		
Count	Score		Count	Score		Count	Score	Nugent Score
30 or >	0		0	0		0	0	Sum of 3 Scores (0–10)
5–30	1		<1	1		<1	1	
1–4	2	+	1–4	2	+	1–4	1	=
<1	3		5–30	3		5–30	2	
0	4		30 or >	4		30 or >	2	

and the likelihood of exceeding cut-points associated with diagnostic categories, we fit ordinary least squares, logistic, and ordinal logistic regression models to the combined data from both waves including age and wave (2 vs 1) as covariates. To account for possible non-linearities in the relationship between age and outcome, we also included age squared, dropping it if it was not statistically significant. The resulting coefficient for wave may thus be interpreted as the systematic change, if any, in the mean from Waves 1 to 2 holding constant age, and may be due either to changes in the population over time and/or changes in the measurement protocol.

We also tested for an interaction between age and wave, and in cases where this was significant, estimated the differences between waves for specific ages. These models were fit using the weights delivered with the Waves 1 and 2 data sets, and design-based variance estimates were obtained via the linearization method using information about strata and primary sampling units (PSUs). Note that because this permits observations within the same PSU to be correlated, this approach accommodates within-respondent correlation between waves.

Analyses presented here are based on the NSHAP Waves 1 and 2 in data release version 2.1. All analyses utilize the provided respondent sampling weights, which adjust for unequal probabilities of selection and non-response; results therefore apply to the United States population of community-dwelling women age 57–90. We used Stata version 12.1 (StataCorp, College Station, TX, 2011) for all data analysis.

RESULTS

Bacterial Vaginosis

Wave 1.—In Wave 1, 23.3% of older women (aged 57–85) in the United States population had a Gram stain consistent with BV, with women in the oldest age group (75–85) having the highest prevalence (28.2%). Concomitantly, the prevalence of women with normal flora decreased across age groups and was the lowest, 31.4%, at the oldest age group (ages 75–85). The prevalence of women with intermediate diagnosis did not differ across age groups (37.4%, 42.1% and 40.4%; Table 2).

Table II. Estimated US prevalence of Nugent Score diagnostic categories based on Waves 1 and 2 separately (overall and split by age group)

Diagnostic categories				
	Sample size	Normal (0–3)	Intermediate (4–6)	Bacterial vaginosis (7–10)
Wave 1				
Total	907	36.9 (1.9)	39.8 (2.0)	23.3 (1.9)
Age groups				
57–64	301	40.5 (2.8)	37.4 (3.2)	22.1 (2.9)
65–74	341	36.2 (3.4)	42.1 (2.9)	21.8 (2.8)
75–85	265	31.4 (2.8)	40.4 (3.1)	28.2 (3.3)
Wave 2				
Total	790	20.6 (2.0)	41.4 (2.5)	38.0 (2.4)
Age groups				
62–69	316	27.6 (3.0)	43.7 (3.9)	28.7 (3.3)
70–79	325	16.9 (2.6)	38.0 (3.5)	45.1 (3.8)
80–90	149	9.12 (2.2)	43.2 (4.2)	47.7 (4.1)

The Nugent score increased linearly with age, when it was treated as a continuous variable (age coef = 0.05, $t = 4.01$, $p \leq .001$; Figure 1, Table 3). Likewise, using Nugent score diagnostic categories, the change was away from normal flora and towards BV with age (age coef = 0.02, $t = 2.75$, $p = .008$, ordinal logistic regression of diagnostic category on age, Figure 3).

Wave 2.—In Wave 2, 38.0% of older women (aged 62–90) in the United States had a Gram stain consistent with BV and prevalence increased within each of the three age groups (29%, 45%, and 48%; Table 2; Figure 2). Similar to Wave 1, the prevalence of women with normal flora decreased across age groups (28%, 17%, and 9% for each age group, respectively). Women in the oldest age group (80–90) had the highest prevalence of BV (48%) and lowest prevalence of normal flora (9%). There was little change in the prevalence of women with an intermediate diagnosis (41.4%; Table 2).

The estimated Nugent score increased linearly with chronological age (age coef = 0.08, $t = 6.28$, $p \leq .001$, linear regression; Table 3, Figure 1), with the predicted probability of BV diagnosis increasing until 90 years of age (Figure 3). Likewise, using Nugent score diagnostic categories, there was a significant change away from normal flora categorization and towards BV categorization with age (age

Figure 1. Effect of chronological age on the predicted Nugent score (95% CIs) in Waves 1 (ages 57–85) and 2 (ages 62–90).

Table III. Estimated coefficients from the regression of Nugent score on age, separately by wave

Predictor	Wave 1 (N = 907)		Wave 2 (N = 790)	
	Regression coefficient	SE	Regression coefficient	SE
Age	0.05***	0.01	0.08***	0.01
Intercept	0.98	0.86	−0.37	0.94

*** $p < .001$

coef = 0.06, $t = 6.05$, $p \leq .001$, ordinal logistic regression of diagnostic category on age, Figure 3).

Wave comparison.—Overall, Nugent scores in Wave 2 were significantly higher than in Wave 1, both for all women (quasi-independent $t = 5.77$, $p < .001$) and for a subset of women providing data in both waves ($n = 377$, Wave 2 = 5.1 ± 0.2 vs Wave 1 = 3.9 ± 0.2 , paired $t = 7.85$, $p < .001$). Among these 377 women, the average change in Nugent scores from Waves 1 to 2 was 1.24 ± 0.2 .

We used linear regression models to examine the mean difference of the Nugent score between waves for respondents of similar ages by fitting a model of Nugent score based on age, wave, and wave moderated by age. We then used linear combinations to estimate the effect of wave at different 5-year age points and the corresponding standard errors. We found that there was a statistically significant age by wave interaction (Table 3). We also found that between wave differences were statistically significant, with differences increasing at each age point (65, 70, 75, 80, and 85 years) (Table 4).

Finally, we asked whether a participant's Wave 1 diagnostic category (normal, intermediate, and BV) predicted her status 5 years later in Wave 2. Using normal flora as a baseline, a multinomial logistic regression model revealed that women originally categorized as intermediate in Wave 1 were three times more likely to be categorized again as intermediate in Wave 2, RRR = 3.1, 95% CI (1.66–5.89); $p < .001$. Furthermore, women categorized as intermediate in

Figure 2. Wave 2 differences between age groups in proportions of three diagnostic categories based on Nugent score: normal (0–3), intermediate (4–6), bacterial vaginosis (7–10).

Wave 1 were five times more likely to be categorized with BV in Wave 2, RRR = 5.0; 95% CI (2.56–9.75); $p < .001$. Most interestingly, women initially categorized with BV in Wave 1, were more than 10 times likely to again be categorized with BV in Wave 2, RRR = 10.5; 95% CI (4.45–24.7); $p < .001$.

Presence of *Candida*

Wave 1.—In Wave 2, the overall prevalence of *Candida* was $5.94\% \pm 1.16$ (Table 5). There was a slight increase with age, 5.70% (ages 57–64) to 5.89% (ages 65–74) and highest at 6.49% (ages 75–85) although it was not statistically significant ($n = 957$, Figure 4; logistic regression, age coef = 0.008, $t = 0.25$, $p = .81$, Table 6).

We tested the hypothesis that bacterial pathogens would rarely co-occur with *Candida* within the same woman because the two microbiota have different pH and ecological requirements. Indeed, in women with bacterial pathogens present (either intermediate or BV categorization), *Candida* tended to be half the prevalence found in women with normal flora (9% vs 4%; $\chi^2 = 24.10$, $p = .076$).

Wave 2.—In Wave 2, the overall prevalence of *Candida* was again low at $4.98\% \pm 1.08$ and decreased with each age group, 6.15%, 5.16%, and 0.96% (Table 6; Figure 4; logistic regression, age coef = $-.074$, $t = -2.68$, $p = .010$, Table 7). The odds of detecting *Candida* significantly decreased by 7% with each year of age, OR = 0.93, 95% CI (0.88, 0.98); $p = .010$.

The inverse relationship between bacterial pathogens and *Candida* was stronger in Wave 2. For women with a normal flora categorization, 13% had *Candida* present; but in women with an intermediate or BV categorization, *Candida* presence was only one-quarter that of normal (3% of each category; $\chi^2 = 113.08$, $p = .002$).

Wave comparison.—Although the prevalence of *Candida* was similar for Waves 1 and 2, it was only in Wave 2 that we

Figure 3. Wave differences in the effect of age on predicted probability (95% CIs) of diagnostic categories based on Nugent score (normal, intermediate, and bacterial vaginosis) in Waves 1 (ages 57–85) and 2 (ages 62–90).

Table IV. Estimates of Nugent Score differences between waves at 5-year Δ points

Predictor	Difference	SE
65 years	0.65***	0.20
70 years	0.81***	0.16
75 years	0.96***	0.16
80 years	1.11***	0.21
85 years	1.27***	0.28

*** $p < .001$

observed a statistically significant decrease with age; in Wave 1, *Candida* prevalence increased slightly with age (Tables 6 and 7; Figure 4). The co-presence of *Candida* and bacterial flora across Waves 1 and 2 had similar results; the presence of *Candida* occurs more with normal flora categorization (9% and 13%) than with intermediate or BV categorization.

Table V. Estimated US prevalence of *Candida* for Waves 1 and 2 separately (overall and split by age group)

	Sample size	Positive
Wave 1		
Total	957	5.94 (1.16)
Age groups		
57–64	319	5.70 (2.36)
65–74	358	5.89 (1.33)
75–85	280	6.49 (2.01)
Wave 2		
Total	790	4.98 (1.08)
Age groups		
62–90	316	6.15 (1.95)
70–79	325	5.16 (1.55)
80–90	149	0.96 (0.70)

Figure 4. Effect of age on predicted prevalence (95% CIs) of *Candida* in Waves 1 and 2.

Table VI. Estimated coefficients from the logistic regression of *Candida* on age, separately by Waves 1 and 2

Predictor	Wave 1 (N = 957)		Wave 2 (N = 790)	
	Regression coefficient	SE	Regression coefficient	SE
Age	0.01	0.03	−0.07**	0.03
Intercept	−3.29	2.23	2.22	1.96

** $p = .01$

DISCUSSION

Postreproductive Vaginal Environment

NSHAP is the first large-scale, nationally representative survey of postmenopausal women reporting on the prevalence of BV. Among older US women, aged 62–90 in 2010–2011, 38% had vaginal flora consistent with BV. An additional 41% were in the intermediate category, at risk for a BV infection and only 21% were categorized as normal using the Gram stain Nugent score criteria. This calls for further research on postmenopausal women to confirm that this vaginal colonization is typical and to determine its association with vulvo-vaginal symptoms, affecting sex, voiding, and comfort.

The NSHAP estimate of the prevalence of BV in older women was within the range of smaller clinical studies of older women (Burton & Reid, 2002; Heinemann & Reid, 2005; Kaewrudee et al., 2009; Ocviyanti et al., 2010). But higher than other clinical studies of older women as there are conflicting data in the literature on the prevalence of BV in postmenopausal women (Cauci et al., 2002; Spinillo et al., 1997; Tibaldi et al., 2009). NSHAP BV prevalence is also higher than that of The National Health and Nutrition Examination Survey (NHANES), another large scale community-based study of younger reproductive-aged women using similar methods (Allsworth & Peipert, 2007; Koumans et al., 2007). This prevalence difference may be explained by the contrasting ages and thus vaginal environments of the study subjects. The higher prevalence of bacterial pathogens in the NSHAP sample may be correlated with changes in the vaginal environment as a result of postmenopause or vaginal atrophy. Further analyses correlating vaginal estrogenization using the maturation value (MV) and the Nugent score may elucidate this relationship.

There were significant differences between estimates from Waves 1 and 2. The prevalence of BV in Wave 2 was significantly higher than the 23% detected in Wave 1, using multiple statistical models. Moreover, the Wave 1 women re-interviewed in Wave 2 were ten times more likely to be categorized with BV 5 years later and five times more likely to have moved from intermediate to BV categorization. This increase between waves in BV and within subject had two sources: improved collection, handling, and shipping methods in Wave 2 and aging 5 years. In Wave 2, specimens were kept cold until they were processed and stained in the microbiology laboratory. This improvement to the methods increased the reliability and validity of Wave 2 results and enabled the detection of significant age effects.

From an aging perspective, the increase in BV may be the result of decreased estrogen levels, reduced glycogen content and increased vaginal pH common in this aging population (Hafez & Evans, 1978; Mac Bride et al., 2010). In addition, the increase in bacterial pathogens may result from decreased immune response to infection which can occur with aging. Indeed, in both waves women in the oldest age groups (75–85 and 80–90) had the highest percentage of BV and lowest percentage of normal flora decades after menopause transition. Future analysts can test both of these hypotheses with the variables available in the NSHAP data set.

To our knowledge, this is the first non-clinical nationally representative survey reporting the prevalence of *Candida* in postmenopausal women. In contrast to BV, the prevalence of *Candida* was lower as predicted by the increased pH and low glycogen levels naturally occurring in postmenopausal women (Hillier & Lau, 1997). Moreover, *Candida* was most prevalent when the balance of vaginal bacteria was normal (Nugent score 0–3) as the literature also confirms (Moodley, Connolly, & Sturm,

2002). *Candida* was present in 5% of women aged 62–90 in the United States based on Gram stain evaluation. This prevalence was slightly lower than the 6% detected in Wave 1. The NSHAP prevalence for both waves were within the reported ranges of *Candida* presence in menopausal women (6–7%) (Tibaldi et al., 2009).

The decreased presence of *Candida* in Wave 2 was an effect of both improved methods and aging 5 years. The lower sample storage temperatures in Wave 2 increased sample quality by preventing *Candida* growth. In addition, multiple statistical models confirmed a significant decrease with age, independent of wave effects. The odds of detecting *Candida* decreased at 7% with each year of age and were the lowest (0.96%) at the oldest age group. This decrease with age was not detectable in Wave 1 (Figure 3), likely because of sample handling differences.

Cooperation Rates and Collection

In both waves, we show that older women can successfully self-collect vaginal swabs in the home without clinical personnel present, and generate excellent specimens for analysis by a microbiologist. We had an impressive 74% cooperation rate in Wave 2 for recruited female participants, a 9% improvement over Wave 1 (68%). NHANES had a 80.5% cooperation rate among young reproductive-aged women (14–49 years of age) using a similar self-collected swab method and also had the benefits of clinical personnel as well as a mobile laboratory for immediate sample processing (Koumans et al., 2007).

Nonetheless, not all women provided samples. Reasons for not obtaining a swab in our older sample were physical difficulty, concern about pain because of vaginal atrophy and occasionally privacy concerns. Vaginal swab collection of community-dwelling older women in the home is a contribution to field studies that do not have a mobile laboratory or for respondents who may have difficulty traveling to the field site. It also broadens the population studied to include women who are uncomfortable having someone else examine their genital area. The self-collected vaginal swab remains an accepted and highly sensitive method for vaginal microbiology collection in clinical or field studies or for women without access to a gynecological clinic (Chernesky et al., 2005; Kashyap, Singh, Bhalla, Arora, & Aggarwal, 2008).

Specimen Adequacy Rates and Improvements in Vaginal Swab Methods

Our measure of sample quality (specimen adequacy rate) increased from Waves 1 to 2 by 9% for the Nugent score of bacteria flora and 4% for detection of *Candida*. In Wave 2, there were notable methods improvements that increased sample quality; our refinements to field collection, storage, and shipping protocols are detailed in the Methods section of this article under *Wave 2 Refinements* and in O'Doherty et al.,

this volume. Refinements to sample collection, handling, and shipping likely helped to produce better quality specimens in Wave 2. Additionally, for the respondents re-sampled from Wave 1, familiarity with the self-swab collection technique may also have resulted in a higher quality specimen.

Nugent Score in Older Women

We chose the Gram stain Nugent score method for BV diagnosis in our survey population for the following reasons: to enable comparison of NSHAP Waves 1 and 2, to compare our data with other large-scale survey research study (NHANES), for the long-term storage and reference of specimens on stained slides, and finally, because it is comparable to other clinical diagnostic methods, such as Amsel's and Spiegel's criteria (Udayalaxmi, Kotigadde, & Shenoy, 2011). The Nugent score is the gold standard for the diagnosis of BV, it was originally developed and validated to analyze vaginal flora of reproductive-aged women. We, however, are studying postmenopausal women who are known to have lower estrogen levels, higher vaginal pH, fewer lactobacilli, and less robust immune function. This hormonal and aging difference increases the prevalence of bacterial pathogens, and may necessitate modifying the range and interpretation of the Nugent score classification to better fit older women (Cauci et al., 2002). Currently, there is no standardized recommendation for the validation of BV in this age group so we used the best method available.

Another limitation to the Nugent score is that it does not measure all bacterial species common in postreproductive vaginal flora. For example, we informally observed Gram-positive cocci in chains and pairs (presumably streptococci), in some of our specimens, as have been reported in small clinical samples of older women (Mac Bride et al., 2010). Future categorization of these streptococci may further elucidate the shift in vaginal flora that occurs with age. Clinical symptoms of vaginal infection, such as discharge or burning during urination, were not collected during the NSHAP survey study. These diagnostic variables may have helped to better clarify the categorical interpretation of the Nugent score.

It is our understanding that this is the first large-scale non-clinical representative study of BV and *Candida* in postmenopausal women living in the United States, and is a first step towards validating the methods used. We chose not to culture the specimens because our goal was to measure the relative presence of microorganisms in the vaginal environment, not optimizing their growth for identification. Future studies of older women inspired by this publication may utilize molecular analysis to more specifically identify the vaginal microbes present in this age group.

Future Analyses

This manuscript focuses on the prevalence of BV and *Candida* and their correlation with age; however, we realize

that there are more analyses to be done. More conclusions can be made from demographics, socioeconomic status, and marital status, which will give a contextual understanding of the biomeasures. Additional analyses will correlate estrogen HRT, medications, health profiles, and sexual behavior which may help to better understand the risk factors for vaginal infection in this age group. Preventative education may be more effectively disseminated by public health agencies and clinical personnel with better understanding of the causes of and risk factors for vaginal microbial overgrowth.

Ultimately, we would like to know how common these biological changes are in the general population and what their effect is on sexuality as well as overall health. For instance, the biological process driving vaginal atrophy may be better explained by analysis of covariates such as estrogen HRT use, hormone levels, hysterectomy, ovariectomy, MV, chemotherapy, and pain during sex data. Additionally, hormone analyses may provide insight into influences on libido and vaginal function. Analysts of the NSHAP data set who are interested in sexual practices, particularly sexual intercourse and masturbation, will benefit by including measures of vaginal function in older women, just as measures of erectile dysfunction are relevant to older men.

As the "baby boomer" generation has begun to cross the 65-year age threshold, a large portion of the older population is becoming increasingly available for additional data collection and validation of both clinical and scientific research models. This publication encourages data collection in this age group and further analysis of the NSHAP data to elucidate not only the biological, sexual, and behavioral mechanisms affecting vaginal flora in older women, but also the potential affect that it may have on quality of life.

KEY POINTS:

- Among community-dwelling postmenopausal women in the United States, the prevalence of BV was 38% (23% in Wave 1) and increased with age in both waves, 41% had an intermediate classification (39.8% in Wave 1) and only 21% (36.9% in Wave 1) had normal bacterial flora, which decreased with age in both waves.
- In both waves, the presence of *Candida* more commonly occurs in women with normal bacterial flora (9% in Wave 1 and 13% in Wave 2) than those with an intermediate or BV categorization.
- While the prevalence of *Candida* was rare in both waves (6.0% in Wave 1 and 5.0% in Wave 2), a decrease with age was observed in Wave 2, enabled by improved collection, storage, and shipping methods.
- A 5-year increase in age, as well as refined methods in Wave 2, contributed to the change in BV and *Candida* observed between Waves 1 and 2.

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