Title

Risk of Gonococcal Infection During Vaginal Exposure is Associated With High Vaginal pH and Active Menstruation

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Authors

McLaughlin, Stephanie E
Ghanem, Khalil G
Zenilman, Jonathan M
et al.

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A woman's risk of acquiring an infection during gonococcal exposure is determined by her vaginal pH and menstrual cycle

Stephanie E. McLaughlin[1,2,a], J. McLeod Griffiss[3], Khalil G. Ghanem[1] and Jonathan M. Zenilman[1]

[1]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore MD, USA. [2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore MD, USA. [3]Department of Laboratory Medicne, University of California San Francisco, San Francisco CA 94121, USA

Corrresponding Author: Dr. J. McLeod Griffiss, San Francisco Veterans Administration Medical Center (111W1), 4150 Clement St., San Francisco, CA 94121. Fax: 415 221-7542; telephone: 415 476-5371; crapaud@loursage.org

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[a]Present affiliation: Department of Medicine, New York University, New York, NY

Correspondence: J. McLeod Griffiss, MD, VAMC (111W1), 4150 Clement St, San Francisco, CA 94121. Tel.: 415 476-5371; Fax: 415 221-7542; (crapaud@loursage.org).

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Abstract

Understanding gonococcal infection resistance in women could lead to the discovery of novel control measures. We enrolled 61 female contacts of men with gonorrhoea seen at Baltimore City Health Department clinics from 1/22/2008-5/16/2012**.** Data concerning exposure and sexual practices, co-infections, physical signs on exam, patient symptom report, and menstrual history were collected. Fifty-seven percent of exposed women enrolled were infected. Multiple logistic regression demonstrated a significant association between acquiring *Neisseria gonorrhoeae* infection and vaginal exposure during the peri-menstrual period (odds ratio 18.1). Similarly, high vaginal pH and cervical discharge on physical exam were significantly associated with gonococcal infection at presentation (odds ratio 20.4 for vaginal pH >5.0). As such, a diagnostic schema utilizing vaginal pH>5.0 and presence of cervical discharge on pelvic exam could be used to recruit patients for clinical studies aimed at testing novel antimicrobial agents. Lastly, modifiable risk factors at exposure, vaginal pH and menstrual cycle, could be actively manipulated in order to reduce risk of *Neisseria gonorrhoeae* in women.

Background

Twenty-five to thirty percent of women who are exposed to *Neisseria gonorrhoeae* during vaginal intercourse with an infected man resist cervico-vaginal infection – a rate that is consistent across multiple studies (1-3). Understanding the reasons why some women resist infection might lead to novel control measures; however the reasons are currently unknown.

Methods

Cohort Study Design and Recruitment

We recruited female contacts of men known to have gonorrhoea from either of the two Baltimore City Health Department (BCHD) clinics. Each exposed woman was given a Partner Notification Card (PNC) by a male sexual partner previously diagnosed with gonorrhoea at one of the clinics. Sixty-one female contacts were enrolled. Historical, physical, and laboratory attributes were collected from each woman and recorded, with the goal of comparing these attributes between infected female contacts and contacts who resisted gonococcal infection.

Enrolled women provided information about sexual practices, including age at sexual

debut, number of lifetime partners, type of sexual practice, history of prior sexually transmitted infections (including past gonococcal infections), douching, date of exposure to *N. gonorrhoeae*, date of last sexual activity, and menstrual cycle history (including date of last menstrual period (LMP), as well as use of any hormonal contraception). Symptoms of infection by patient report, such as vaginal discharge or other urogenital symptoms were also recorded.

The study was approved by the Johns Hopkins University Institutional Review Board, and written informed consent was obtained from all participants prior to study enrollment.

Menstrual cycle was divided into three phases corresponding to levels of estrogen and progesterone: follicular, ovulation, and luteal. These designations were used in prior studies investigating the link between menstrual cycle phase and HIV risk(5). The “follicular” phase, which includes active menstruation during days 1-5, encompassed days 1-11. “Ovulation” included days 12-17. The “luteal” phase included days 18-35 (5). The luteal phase was subdivided further into “early" and "late luteal”; late luteal encompassed days 25-35 (6). Any participant with a menstrual cycle greater than 35 days was excluded from this analysis.

Physical Examination after Enrollment

Clinic staff did a pelvic examination on all women enrolled and signs of infection were noted. Vaginal and cervical samples were collected during exam.

Laboratory Data Collected

Vaginal pH of 56 subjects was measured at the time of the examination with pH indicator strips (range: 4.0 to 7.0; Fisherbrand pH Test Paper)(7).

Wet mounts from vaginal swabs were examined for inflammation (white blood cells), yeast, *Trichomonas vaginalis*, and clue cells. Whiff tests were done for evaluation of Bacterial Vaginosis (BV) by Amsel criteria (8). Nucleic acid amplification tests (NAATs) were used to test for concurrent *Chlamydia trachomatis* infection.

Cervical cultures for *N. gonorrhoeae* were obtained along with additional urine and cervical swabs for gonococcal NAATs. Cultures were plated on modified Thayer-Martin medium and processed according to the clinics' Standard Operating Procedures. Gonococcal NAATs were run on urine specimens from all women whose cultures were negative. Gonococcal NAATs (Hologic/GenProbe Aptima Combo 2) were run on urine specimens from all women whose cultures were negative.

Only women who had both negative gonococcal cultures and NAATs were considered GC negative.

Statistical Analysis

Associations between gonococcal infection and single behavioral factors, physical signs and symptoms or laboratory data gathered at presentation were investigated using X^2, Fisher's exact or simple logistic regression with individual variables of interest in STATA

14 (STATA Corp, College Park, TX).

Information concerning gonococcal exposure more than thirty-five days post-exposure was considered less reliable; some risk factor analyses were restricted to those who reported within 35 days of exposure. Similarly, analyses of vaginal pH, which varies over the menstrual cycle (4), were restricted to those women who reported within 35 days.

Multiple logistic regression (MLR) was then used to investigate the relationship between gonococcal infection and covariates associated with risk at time of exposure (Models 2a and 2b) and covariates (attributes) at time of presentation to clinic (Model 1). Covariates for these models were considered based on single logistic regressions (behavioral factors, physical signs and symptoms, and laboratory data), previous literature, and biological plausibility. Individual variables with *p* values of 0.2 or less from single logistic regression were considered in MLR models. Additional variables were included if there existed a convincing biological basis for an association or if one had been reported.

MLR analysis was restricted to African American women who presented within 35 days of exposure (as there were very few non-black participants). Manual backward model

selection with uncentered variance inflation factor analysis was used to derive the most parsimonious models. Some biologically based co-variants, such as age and time to presentation to the clinic, were included in the model although not meeting a *p* value of 0.05 in the MLR.

Results

Diagnosis of gonococcal infection in contacts

We evaluated 61 women with reported contact to GC. Fifty-seven point four percent had a cervical culture that grew *N. gonorrhoeae,* and another 4.9% had urine NAATs that were positive for *N. gonorrhoeae.* The sensitivity of culture in this setting was 92%, and 62% of exposed women became infected (as diagnosed by culture or NAAT).

Age, sexual debut, lifetime partners and delays in reporting

Among all 61 women, those who resisted infection did not differ in age from those who became infected (means (ranges): 25 (19-38) and 24 (18-39), respectively).

Among all enrolled women, there was no difference between infected and uninfected women in age at sexual debut (means (ranges): 14.8 (11-18) and 15.2 (11-18),

respectively) or number of lifetime partners (means (ranges): 22.3 (1-200) and 19.6 (3-200), respectively).

Additionally, among all women there was no difference in the number of days between exposure and reporting to the clinic between those infected (mean: 24.3 days; median: 11 days) and uninfected (mean: 25.8 days; median: 8 days).

Sexual Behaviors

There was no difference between the two groups in sexual practices, prior gonococcal infections, nor regular douching distinguished. Oral sex was reported by 46% of all 61 women, and anal sex by 4.9%. No symptoms distinguished the groups with varied sexual behaviors (Table 1). Surprisingly, vaginal discharge was more frequently reported by uninfected women (13/23) than infected women (14/38), although the difference was not significant ($p = 0.185$; Fisher's exact test).

In all women, only one of the 28 women who acknowledged receptive oral sex had a positive pharyngeal culture; pharyngeal cultures were not specified in the study design and were not routinely done at the BCHD clinics. Three women reported having had anal sex and two had positive rectal cultures. As with pharyngeal cultures, rectal

cultures were obtained only from women who reported anal intercourse.

Coinfections:

There was no significant association between *Trichomonas* or yeast infection and gonococcal infection (in all women enrolled); although, numbers of confections were low (*Trichomonas*, 8; yeast, 3). Seven of the eight women who were infected with *C. trachomatis* were concurrently infected with *N. gonorrhoeae*.

Bacterial Vaginosis:

Bacterial vaginosis by aggregate Amsel Criteria (3 of 4 criteria) was not associated with gonococcal infection (in all enrolled women). However only vaginal pH >4.5 was associated with gonococcal infection; although the difference was not significant (p = 0.165; Fisher's exact test, Table 2).

Cervical Discharge and Inflammation on Wet Mount.

Clinicians observed cervical discharge in 20/61 women —16 of whom were infected. Those infected were significantly more likely to have cervical discharge on pelvic exam (p = 0.05, single logistic regression).

Similarly, gonococcal infection at presentation to clinic was significantly associated with the degree of inflammation, as judged by the presence and number of leukocytes seen on wet mount of the cervix and graded as 1-4 by the clinician. All 11 wet mounts with the highest degree of inflammation (4) were obtained from infected women ($p = 0.018$; X^2 analysis, Figure 1). There was not a significant association between inflammation on wet mount and a patient's report of vaginal discharge ($p = 0.68$; X^2).

MLR of Attributes at Presentation associated with gonococcal infection (Model 1):
The odds of gonococcal infection in exposed women was 3.6 for each successive increase in unit pH, after controlling for participant age and time to presentation (p=0.022). When pH was dichotomized into exposed women with pH at presentation above 5.0 and those equal to or below 5.0, the odds of gonococcal infection in those with high pH was 20.4 (as compared to low pH) after adjusting for age and time to presentation (p=0.017).

The closer measurement of vaginal pH was to the time of exposure, the greater was the difference between infection resistance and infection acquisition (Table 3). When analysis was restricted to women who reported within five days, the difference was highly significant (p = 0.009; Fisher's exact test, Table 3)

MLR of Hormonal Risk Factors at time of Exposure associated with gonococcal infection - Menstrual Cycle (Model 2):

During active menstruation the odds of gonococcal infection in exposed women was 77.3 (p=0.032) after controlling for cycle phase (follicular, ovulation, luteal), participant age, pH, and time to presentation. Similarly in this model, women who were exposed to gonococci during ovulation showed a trend toward higher risk of infection (odds 23.7, p=0.08), after adjusting for age and time to presentation to clinic. When looking at the grouped peri-menstrual period (Model 2b), days 26-35 (late luteal) plus days 1-5 (active menstruation), odds of gonococcal infection was 18.1 (p=0.023) after adjusting for ovulation, age, and time to presentation. In Model 2b, there was a non-significant trend toward association between ovulation with gonococcal infection (odds 6.70, p=0.139) after adjusting for peri-menstrual cycle phase, age, and days to clinic.

No difference between gonococcal infection and resistance was seen between those enrolled using hormonal contraceptives (HC) and those not using HC (X^2, p=0.635). This was also true for those using progesterone only HC, medroxyprogesterone depo injection and levonorgesterol-releasing IUDs, (X^2, p=0.451); 70% of those using HC used progesterone-only methods (7/10). The other three women enrolled used

combined estrogen/progesterone pills of varying formulations. That rate of total hormonal contraception use, however, was quite low at 16% (10/61) among all women enrolled.

Discussion

Previous studies suggest that about 35% of women who are exposed to *N. gonorrhoeae* through vaginal intercourse with an infected man resist cervical infection(1,3,9). We confirmed this rate of resistance; 38% of the women enrolled resisted infection even when tested by more sensitive NAAT assays.

We sought modifiable biological bases for infection resistance by comparing women in the same core mixing group (10) who did or did not become infected after exposure.

Interestingly, we found no association between symptom report (such as vaginal discharge), sexual practices, prior gonococcal or other STIs, age, age at sexual debut, or lifetime partners and gonococcal infection after exposure. This may be due to the absence of consistent, related symptoms reported by the exposed, and subsequently infected, women. The incentive to report would be lessened in the absence of

symptoms. However, several signs observed by clinicians on physical exam were associated with gonococcal infection - cervical discharge, inflammation on wet mount, and elevated pH.

As a diagnostic test pH>5.0 and presence of cervical discharge on physical exam (when determined in parallel) had a sensitivity of 81% in all women enrolled and 85% in women presenting to the clinic within 5 days of exposure. Vaginal pH is, however, dynamic(4); when measured within five days of exposure, these diagnostic criteria could be used without any further laboratory or microscopic examination to determine likely gonococcal infection. Additionally, heavy inflammation on wet mount had a 100% positive predictive value when diagnosing gonococcal infection in those exposed. Either of these two methods could be used in selection of study participants when testing novel antimicrobial therapies for gonococcal infections.

Furthermore, we found a strong association between low pH and gonococcal resistance in those exposed. Wiesenfeld, *et al.,* reported in 2003 that BV, which is associated with a high vaginal pH, was a strong predictor of both gonococcal and chlamydial infections (11). However, Ness, *et al.,* reported that although both BV and the two STIs commonly co-occurred in African American women, BV did not increase the risk of incident

infection with either organism (12). Finally, Das and Sris, with use of a prospective case-control study, found a highly significant association of high vaginal pH with gonococcal and chlamydial infection in the absence of BV among predominantly white women (13). None of these studies involved women who had resisted infection after known exposure as they only included women with known diagnoses of gonococcal infections and did not compare them with similar non-infected female controls.

Our study was restricted to women who had been exposed to an infected partner, involved both those who resisted infection and those who did not, and predominantly involved African American women. However, the results of the study are very similar to those reported by Das and Sris (13). Only 12.3 % of the cases in the Das and Sris study had a diagnosis of BV (13); whereas 28% of the women in our study had BV as diagnosed by Amsel criteria (8). The prevalence of BV would be expected to be higher in our study, as almost all of the women were African American and incidence of BV is higher in African American women (14).

Our data and those of Das and Sris (13) and Ness, et al. (11), make clear that it is the high vaginal pH associated with BV, and not necessarily clinical BV, that heightens the risk of incident gonococcal infections. Our data confirm the highly significant

association between higher vaginal pH and *N. gonorrhoeae* infection, independent of a diagnosis of BV.

A limitation of our study was the measurement of vaginal pH at the time of examination, an average of 25 days after exposure. Vaginal pH is fairly stable in individual women between menstrual periods **(*)**, but rises with menses (4). When during the luteal phase the pH begins to rise is not known, but it returns to pre-menstrual levels rapidly after the onset of menses (4). Although the number of women for whom we could calculate menstrual cycle phase at the time of exposure was small, there was an association between risk of infection and exposure during the time surrounding menstruation when the vaginal pH rises as much as 2 pH units (4).

In addition to high pH, the availability of iron likely contributes to the increased risk of gonococcal infection surrounding menstruation, as iron is necessary for gonococcal growth and proliferation (15). During the late luteal phase levels of lactoferrin in vaginal mucus rapidly decline and are negligible by day 25 of the cycle (16). Lactoferrin binds iron, and low levels of lactoferrin in turn lead to higher levels of iron in vaginal mucus. Low concentrations of lactoferrin and the presence of menstrual blood would provide abundant iron and may contribute to increased risk of infection peri-menstrually (Figure

3).

An acidic vaginal pH is maintained, in part, by estrogen-regulated epithelial cell proton pumps (17). Estrogen levels are lowest during the late luteal phase. As estrogen levels fall, the vaginal pH increases. During the menstrual phases in which estrogen levels are higher (ovulation and early luteal phase), vaginal pH is lower (4), due, in part, to the upregulation of the vaginal epithelial cell proton pumps (17).

The increase in risk of infection during ovulation suggested by these data could be due to a variety of factors: increased expression of gonococcal Opa (18,19) and its receptor, host CEACAM (20), or up-regulation of the lutropin receptor (21), as both mechanisms have been implicated in gonococcal attachment and uptake by cervical epithelial cells. Additionally, changes in cervical mucus that permit transit of spermatazoa may also permit gonococcal infection of the cervix (22). However, definitive data surrounding the effect of ovulation on risk was limited due to inexact menstrual cycle information (as day of menstrual cycle at exposure was calculated from patient report) as well as small sample size.

In summary, our data showing increased risk of gonococcal infection with high vaginal

pH, menstruation, and ovulation could be used to design non-antibiotic based methods of controlling gonococcal infection. Risk of gonococcal infection could be lowered by manipulation of the vaginal pH. Additionally, hormonal regulation with various contraceptive agents could be utilized to prevent gonococcal in two ways: further pH modulation (23) and suppression of active menstruation, and perhaps ovulation.

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