Review Article

Gonorrhoeae Must Compete With The Naturally Inhabitant of Microbial Community Right At The Outer Mucosal Surface To Authorize Infection

ABSTRACT

Aim: to describe the competition between N. gonorrhoeae with the naturally inhabitant of microbial community right at the outer mucosal surface to authorize infection.

Discussion: Gonorrhea is a sexually transmitted infection (STI) caused by the bacterium Neisseria gonorrhea. Official report mention that by the year 2020, there were an estimated 82.4 million new infections among adults globally and poses a risk of onward transmission to sex partners. It surely could affect both men and women; for male, the symptoms are more obvious, but for women it occurs more often without prominent clinical symptoms and usually lead to long-term health problems including pelvic inflammatory disease and infertility. The female reproductive tract with its abundant variety of epithelial cells act as its primary niche where initially it was inhabited by normal microbiota, characterized by a high abundance of Lactobacilli, and uniquely linked to the host's mucosal immunity and plays a critical role in the regulation of genital inflammation. Unfortunately, the dynamics regarding number and composition of vaginal microbiota has been shown to fluctuate over several internal and external factors, especially due to STI like gonorrhea. Its proposed evolution from an ancestral commensal bacterium, N. gonorrhoeae has retained features that are commonly found among commensal inhabitants, but it has also developed unique features that are crucial to its pathogenesis. The scope of its pathogenesis field elucidate competition, colonization and growth properties as main virulence determinants.

Conclusion: Competition between *N. gonorrhoeae* and the already exist natural microbiota of the vagina occur initially at the mucosal surface. This gonococcus has several intrinsic factors that can facilitate its competitiveness including adherence, even though not all available adhesion mechanisms are actually used by this organism during the course of infection/colonization of any specific site.

Keywords: Gonococcal, Lactobacillus, Epithelium, Cervix, Vagina, colonization, nutrition

1. INTRODUCTION

Gonorrhea is a sexually transmitted disease (STD) or sexually transmitted infection (STI) caused by Neisseria gonorrhoeae [1]. It is transmitted via the route of vaginal, oral and anal sex where contact with exudates or discharge from genital mucous membranes of infected people took place [2]. Naturally, the obligate pathogen N. gonorrhoeae infects only humans [1] and causing, most commonly, asymptomatic cervicitis in women [3] and symptomatic urethritis in men [4]. During sexual intercourse, gonorrhea is more likely to be transmitted among homosexual male [5] than in heterosexuals, in this particular group the rate of transmission is higher in men to women than from women to men [6]. The infection can also be transmitted from mother-to-child during childbirth [7] which estimated among untreated pregnant women, the mother-to-child transmission rate of infection is at approximately 30%.

This host-adapted human pathogen poses a risk of onward transmission to sex partners, accompany with silent asymptomatic ascending infection and even dissemination if left untreated. According to the World Health Organization report, in 2020 there were an estimated 82.4 million new infections among adults globally. Estimated probability of penile-to-vaginal transmission is approximately 50% per sex act, and of vaginal-to-penile transmission is approximately 20% per act.1–3 Probabilities of per-

condomless act transmission during oral (63% urethral-to-pharyngeal and 9% pharyngeal-to-urethral) and anal sex (84% urethral-to-rectal and 2% rectal-to-urethral) have been estimated from mathematical model [8]. Fortunately, it is a preventable and curable disease [1,2]. Regrettably, combination of the practice of promiscuity, unsafe sexual intercourse and stigma attached to sufferers making the iceberg phenomenon on its epidemiology persistent [4,6,8].

The female reproductive tract presents this pathogen a long canal covered with a variety of epithelial cells which initially inhabited by normal microbiota as part of a dynamic and complex ecosystem [9]. This normal inhabitant microbiota community, in quantity and quality, influenced by several physiological, genetic, and behavioral factors [10]. It is uniquely linked to a woman's mucosal immunity and plays a critical role in the regulation of genital inflammation [11].

A vaginal microbiota composition characterized by a high abundance of lactobacilli as the orchestrator and in combination with low overall bacterial diversity; this type of assortment is associated with lower inflammation [12]. On the other hand, a more diverse microbiota is linked to high mucosal inflammation levels [13], a compromised genital epithelial barrier integrity [14], and an elevated probability of sexually transmitted diseases [15] and other situations such as endometrial cancer grade [16]. Cervicovaginal microbiota composition predicts its clinical presentation, both for Neisseria infection [17] and or intraepithelial neoplasia [18]. The aim of this article is to review the competition between N. gonorrhoeae with the naturally inhabitant of microbial community right at the outer mucosal surface to authorize infection.

2. INHABITANT MICROBIAL COMMUNITY: NORMAL MICROBIOTA

According to sunarti [9], the intrinsic microbiota are the inhabitant microorganisms normally found in healthy people. These microorganisms are present at numerous location in the human body and basically may be pathogenic (competent of causing disease) but actually are not in the active process of doing so [19]. Some of the normal microbiota are permanent inhabitant [20] and its existence appraised to be indigenous [21]. Others may show transient properties of existence [22].

Even without exception to the same individual, the relative composition of the microbial normal flora can possibly differ. The difference is caused by changes due to (1) accustomed daily diet [23], (2) psychology condition, e.g., stress-depression [24], (3) sexual practices and its consequences such as hormonal dynamics, pregnancy and contraceptive use [25], (4) pharmacology treatment [26], and (5) other host-related factors. Ordinary predominant strain of microbial flora is actually existing in or within body niches and even can shared functional traits [9].

The exact number of good microorganisms in the body is difficult to determine; but of course the number is exceeding the number of cells in human body. According to Sender et al,27 whom estimates the total number of bacteria in the 70 kg "reference man" to be 3.8×1013. For human cells, the dominant role of the hematopoietic lineage to the total count (≈90%) and revise past estimates to 3.0×1013 human cells. They also update the widely-cited 10:1 ratio through an in-depth analysis, that the number of microorganisms in the human body is actually of almost the same order as the number of human cells, and their total mass is about 0.2 kg.27 The prevailing types of species in humans differ according to the body site or location, e.g., skin, hair-scalp, nose, oral cavity, stomach, ileum, colon and genitourinary tract [9].

2.1 Normal Microbiota of the Vagina

The density and composition of considered normal microorganisms in the healthy vagina is a complex ecosystem [11,12]. It is usually a sophisticated fusion of an obligate aerobic Lactobacillus species [28]; which some species of Lactobacilli, namely Lactobacillus crispatus, L. Acidophilus, L. gasseri, L. jensenii, and L. iners. L. crispatus and L. Jensenii [29]. Lactobacillus play a crucial role in protecting vaginal surfaces by secreting H2O2; an acidic substance that able to intercept the colonization of pathogenic microorganisms and also in the same time prevents their multiplication [30]. Lactic acid blocks histone deacetylases, thereby enhancing gene transcription and cellular DNA repair capacity [31]. According to Mijac et al,30 hydrogen peroxide producing lactobacilli could protect against the development of bacterial vaginosis, but not against vulvovaginal candidiasis and Trichomonas vaginalis.

The dynamics regarding number and composition of vaginal microflora has been shown to fluctuate over (1) age (neonates- childhood – adolescent – young adult – elderly), (2) routine menstrual cycle, (3) sexual activity (active-passive, promiscuity), (4) hygiene habits, (5) fashion related habits and (6) the practice of using intravaginal microbicides, e.g., nonoxynol-4. [22,24]

Related to the previously stated dynamics of the presence of vaginal microbiome that considered normal flora [9], studies confirmed that most healthy women have transient changes in vaginal flora [23], which although not permanent, can cause changes in the local microenvironment. Unfortunately, only a minority of healthy women had a lactobacilli-predominant flora [32]. Personal behavior including lifestyle [15,33], biological functions including hormones [23,25] and or other external conditions might contribute to the dynamic pattern of vaginal microflora [22]. Furthermore, the characterization of normal vaginal microflora and its contribution to maintain specific milieu in the vagina is still need to be investigated, especially among specific healthy women population.

The process of the development of normal flora is a lifelong continuous episode that starts immediately at birth process [20,34]. It is belief that the process of colonization starts during parturition when the neonate's intestine is seeded with mostly Gram-positive facultative anaerobes from the mother's vaginal microflora during normal delivery [35]. Close contact between mother to newborn is strongly contributes for the introduction of normal microflora to the newborn [36]. The vaginal microflora collected from mother's right after delivery was the same in composition as microflora found in the stools of neonates [37].

The vaginal microflora plays a pivotal role in early maternal-neonatal health condition [34-37]. Shift in microbiota composition and number (dysbiosis) during pregnancy are associated with negative reproductive outcomes, such as the likelihood of miscarriage due to elevated inflammation and infection [38] and preterm birth where it is realizable that some cases of preterm labor may be due to haematogenous proliferation of organisms, which was previously present in the vagina and is part of the normal microbiome, to the placenta and uterus [39]. Previous study regarding normal flora in pregnant women publicized that all-inclusive microbiome profiles could not be distinguished based on pregnancy condition [40]. However, the vaginal microbiomes of women with healthy ongoing pregnancies had lower diversity and also abundance, curtailed number of Mycoplasma spp. and Ureaplasma spp. and higher 'good' bacterial load when compared to non-pregnant women [40]. Lactobacillus spp. abundance was also greater in the microbiomes of pregnant women with Lactobacillus-dominated in comparison with the non-pregnant group [40,41].

2.2 Protective Role of Vaginal Microbiota Against STI

Normal cervicovaginal microbiota play an important role in sexual and reproductive outcomes [42], including protection from dangerous pathogen such as N. gonorrhea [17], as the composition of the cervicovaginal microbiota has been shown to modify susceptibility to several sexually transmitted pathogens [11,17,30,31]. For example, human vaginal population acquiescent by Lactobacillus crispatus is able to reduce the risk of STI's agent accession, including HIV [30,43]. In addition, women with bacterial vaginosis (BV), irrespective of whether the BV is symptomatic or not, a clinical condition characterized by (a) significant reduction in numbers of Lactobacillus species [44], (b) increased diversity of miscellaneous groups of obligate and facultative anaerobes [45], (c) increased risk of adverse reproductive and obstetric outcomes [38,39],and (d) consequential risk of acquiring and transmitting STI, including N. gonorrhoeae and HIV [1,2,8,43,46].

These factors indicate a mechanistic contribution of *L. crispatus* to protection from STI, presumably through the production of lactic acid and thus the maintenance of a low-pH vaginal microenvironment [28-30,32]. One of the biggest obstacles associated with re-shaping the composition of vaginal microbiome in order to prevent STIs is the strenuousness in maintaining the normal vaginal microbiota composition and function [32]. In women with symptomatic BV, to date, establishing a minimal but effective management of symptoms and "re-programme" the vaginal microbiome to a Lactobacillus-dominated state has been incomprehensible to accomplish [47]. Starting from this aspect, the discussion continues to a specific sexually transmitted infection, namely gonorrhea.

3. NEISSERIA GONORRHOEAE: ADHERENCE AND COMPETITION

The host-adapted human pathogen *N. gonorrhoeae* is the causative agent of gonorrhea [1,2]. Along with its proposed evolution from an ancestral commensal bacterium, *N.* gonorrhoeae has retained features that are commonly found among commensal inhabitants, but it has also developed unique features that are crucial to its pathogenesis [8,48].

All microorganism that live in or on human host stand in need of the condition to colonize and gain access to nutrition in order to facilitate its growth, whether they are commensal organisms in origin that only once in a while cause anguish or definite pathology. The scope of its pathogenesis field elucidate competition, colonization and growth properties as main virulence determinants even though sometime they are frequently found also living together with other organisms in harmony and do not cause conspicuous pathology. However, for a certain pathogenic organism to accomplish definite anatomical impairment, it usually needs to overcome existing microbiota then takes over the balance of the composition of the original microbiota to then change conditions in a direction that is favorable for its own existence, occupy and colonize specific anatomical sites and encourage grow (except in the condition when pathogenesis take place via production of a toxin away from the locus of infection).

N. gonorrhoeae mainly colonizes and infect the mucosal surface infections of male and female reproductive tracts, while it can also occupy nasopharyngeal, rectum, and conjunctiva mucosa [49]. Its related pathology mostly results from the condition of variants that colonize strongly and penetrate poorly, thereby causing asymptomatic infection [50], and able to survive better in the portion of sub-microscopic damaged cervix [51] that is caused by its ability to infects the heterogeneous epithelia of the human cervix using distinct mechanisms [51] and in combination with the activation of innate immune responses, that favor the pathogen, at the sites of colonization [52] as *N. gonorrhoeae* does not contain sufficient and vigorous exotoxins [53].

The pathogen *N. gonorrhoeae* could survive 24 hours in ure1hral secretion on a glass slide and on a towel at 22°C, and 120 hours at 4°C, according to Elmros [54], but unable to survive in the condition of dehydration or exposed to non-physiological temperatures [55]. As both the commensal and pathogenic Neisseria spp. occupy the same niches [56], it is not easy to distinguish the state of colonization from active virulence condition [57,58]; the latter is obligatory to its pathogenicity that initiate host damage [58]. Since *N. gonorrhoeae* occupy mainly the genital, rectal and oral mucosal-epithelial surface [1,2], it is easily accepted that gonococcus expresses a repertory of elements that authorize its replication and also survival in such harsh and dangerous environmental niches, and also repertoires of factors that regulate and even helped them to evade from the host's immune system.

Capitalization on the host epithelial cell signaling pathways to establish infection is the main features of Gonococci's establishing infection at the mucosal epithelia of the human genital tract [50,51,59]. In order to facilitate infection at local site, Gonococci using three complimentary conditions:

1. adherence and colonization of the epithelia; according to Ray et al [60], adherence protects Gonococci from zinc-dependent growth restriction by host nutritional immunity proteins,

2. invasion of epithelial cells, where according to Yu et al [61] Gonococci actually invade non-polarized epithelial cells only through ezrin-driven microvilli elongation. Its entry into polarized epithelial cells prevented by the apical polarization of ezrin and F-actin, and

3. trafficking into the sub epithelial tissue. A study conducted by Stein et al [62] shows that Opa (a surface molecules) expression interferes with Gonococci transmigration across polarized human epithelial cells. Opa expression limits gonococcal ability to invade into sub epithelial tissues by forming tight interactions with neighboring bacteria and by inducing carcino-embryonic antigen–related cell adhesion molecules (CEACAMs) redistribution to cell junctions.

The pathologic process for each of these events be dissimilar between males and females, and within females at different anatomic locations [2,6,59]. What must be understood first is that these stages

begin with competition with the already existing normal microbiota (but unfortunately have changed their number and composition); this precede subsequent events that cannot be prevented by either

(1) anatomical barriers (starting from an initial understanding of the anatomy of the genital organs that characterized by during sexual intercourse, genital contact between the surface area of the cervicovaginal mucosa that is considerably larger than the surface of the penis and foreskin, facilitating greater potential exposure to STI pathogens. Semen may remain within the female genital tract for up to 3 days postcoitus, prolonging exposure to STIs, including Gonococcal and HIV [47] and the fact that initial gonococcal infection predominantly infecting columnar and transitional epithelia, although it can also adhere to the stratified squamous epithelium of the ectocervix [63]),

(2) existing normal microbiota (shifted in the cervicovaginal microbiota can modulate the penetration of STI through cervical mucus to access target cells [47]. During sexual activity, a perpetrator's genital microbiome can gain access of contact and exposed to the ally's oral, genital, and rectal microbiome [64]. Despite these disclosures (especially for repetitive and risky sexual intercourse), distinctive genital microbial communities are definitely perceived among women and men, a phenomenon that reflects that at least for opposite-sex intercourse, strong selective forces are employed by sex-specific microenvironments [65],

(3) the innate immune system. Infection with *N. gonorrhoeae* triggers an intense inflammatory response characterized by an influx of neutrophils in the genital tract, yet natural gonococcal infection does not induce a state of protective immunity Individuals with gonorrhea are usually not protected from reinfection. By exploiting this niche, *N. gonorrhoeae* exemplifies a well-adapted pathogen that proactively elicits from its host innate responses that it can survive and concomitantly suppresses adaptive immunity [52]. This apparent lack of an adaptive immune response to *N. gonorrhoeae* probably contributes to the continuing prevalence of this sexually transmitted infection, and challenges the development of a vaccine against it. [52,66]

However, all involve close interactions with host cells and alteration of host cell signaling pathways, generally leading to decreased epithelial cell exfoliation to promote colonization or invasion into the epithelial layer depends mainly on adherence and competition.

3.1 Adherence

Following transmission, *N. gonorrhoeae* establishes contact with the mucosal epithelium in order to establish replication and ultimately transmit to the surrounding new hosts [67]. *N. gonorrhoeae* is ultimately a mucosal colonizer [68], attaching to various epithelial surfaces [63]. The prime episode of authorizing infection and the first step in pathogenesis is the bacterial adherence to the epithelium of the mucosa [60-62], which is mediated through marked bacterial surface structures that include Type IV pili (an external located outer membrane architectures that are important for facilitating early bacterial-cellular cohesion, common transformation adeptness, twitching motility and immune evasion through antigenic and phase deviation) [69], certain lipooligosaccharide (LOS)-a major constituent within the outer membrane [70], opacity (Opa) proteins [71], and the bacterial major membrane porin, also referred to as PorB [72]. Adherence to the epithelial surface and subsequent pilus retraction allow the invading gonococci close to the cell surface [60-62]. All of these become the *N. gonorrhoeae*'s armamentarium named constant surface variation [72].

After initial adherence, *N. gonorrhoeae* replicates and initiates pili induced-clustering micro colonies [73], followed by biofilms formation- a source of features linked to microbial fitness [74], and likely competes with the resident microbiota.

3.2 Competition

The human vaginal ecosystem is dominated by Lactobacillus species [1, 2]. Lactobacilli are gram-positive rods that, in vitro, produce substances with antimicrobial properties, including lactacidin, acidolin, lactacin B, and hydrogen peroxide (H_2O_2) [12,32,44]. H_2O_2-producing lactobacillus strains play a pivotal role in controlling the microenvironment of the vagina [75] and in inhibiting the

overgrowth of potentially pathogenic organisms [76,77]. In vitro, H2O2-producing lactobacilli are effective “antibiotic” for external pathogens such as STI’s agent and even against multidrug-resistant urogenital pathogens [78], perhaps because of the reaction of H2O2 with myeloperoxidase and halides present in vaginal fluid and its biological consequences in the extracellular milieu are induced by both oxidant formation and ionic interactions [79].

With regard to sexually transmitted diseases (STDs), there was a strong association between bacterial vaginosis and N. gonorrhoeae [46]. In the context of Gonococcus, *once N. gonorrhoeae* adheres to the mucosal epithelium, efficient colonization requires extracellular bacterial replication and nutrient acquisition from the surrounding extracellular milieu [59]. It has not been meticulously resolved which exact microenvironments condition are met during the process colonization and thus the exact nutrient composition of each ecological niche that *N. gonorrhoeae* may inhabit during urogenital, rectal, and oropharyngeal infection is remain unknown and of course this is a gap that needs to be explore in the future to find out how these bacteria utilize the local availability of nutrients for their own benefit.

In laboratory culture milieu, *N. gonorrhoeae* actually need a complex nutrient related media requirements and with specific handling [55,80]. Specifically, for the growth medium, bacteria cannot grow in culture without a supplemented fountain of sufficient glutamine, glucose, iron, thiamine, phosphate and even carbon dioxide [55,81].

Moreover, outside the laboratory atmosphere, in natural conditions in its predilection niche, in order to meet its nutritional requirements, *N. gonorrhoeae* must interact and possibly compete with resident microbiota for available nutrients. Indeed, *N. gonorrhoeae* must seek for vital nutrients like iron, zinc, copper and manganese that are limited by the human host as a defense against bacterial pathogens in a process termed nutritional immunity and metal intoxication. The *N. gonorrhoeae* has unique ways to subvert and evade from the harsh condition of metal intoxication and nutritional immunity, particularly by producing transporters that bind and extract metals from human metal-sequestering proteins [82, 83].

As Neisseria spp. lack siderophores [84], *N. gonorrhoeae* scavenges and hijacks bulwarked iron during infection [85], in other word directly from host-bound complexes, obtaining metals through a series of membrane transport complexes by transporting them into the bacterial cell [86]. This probably the logical explanation on how Gonococcal obtain their nutrient requirement.

Futhermore, the influx of neutrophils to the infection site that appears microscopically during symptomatic colonization, driven by localized inflammation [71], may promote nutrient acquisition by causing leakage of serum components, tissue damage, and exposing N. gonorrhoeae to the abundant intracellular nutrient pools following phagocytosis, thus providing nutrients for bacterial growth [55,87].

4. CONCLUSION

Competition between N. gonorrhoeae and the already exist natural microbiota of the vagina occur initially at the mucosal surface. This gonococcus has several intrinsic factors that can facilitate its competitiveness including adherence, even though not all available adhesion mechanisms are actually used by this organism during the course of infection/colonization of any specific site. To this end, we herein summarize current knowledge pertaining to the gonococcal competitiveness while establishing infection of the human cervix.

CONSENT

Not needed

ETHICAL APPROVAL

Not needed

REFERENCES

1. Springer C, Salen P. Gonorrhea. [Updated 2023 Apr 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK558903/
2. Mahapure K, Singh A. A Review of Recent Advances in Our Understanding of Neisseria gonorrhoeae. Cureus, 2023; 15(8): e43464. https://doi.org/10.7759/cureus.43464
3. Hananta IP, van Dam AP, Bruisten SM, Schim van der Loeff MF, Soebono H, de Vries HJ. Gonorrhea in Indonesia: High Prevalence of Asymptomatic Urogenital Gonorrhea but No Circulating Extended Spectrum Cephalosporins-Resistant Neisseria gonorrhoeae Strains in Jakarta, Yogyakarta, and Denpasar, Indonesia. Sex Transm Dis. 2016 Oct;43(10):608-16. https://doi.org/10.1097/OLQ.0000000000000510.
4. Vigneswaran HT, Baird G, Hwang K, Renzulli J, Chan PA. Etiology of symptomatic urethritis in men and association with sexual behaviors. R I Med J (2013). 2016 Jun 1;99(6):37-40.
5. Fairley CK, Hocking JS, Zhang L, Chow EP. Frequent Transmission of Gonorrhea in Men Who Have Sex with Men. Emerg Infect Dis. 2017 Jan;23(1):102-104. https://doi.org/10.3201/eid2301.161205.
6. Lin JS, Donegan SP, Heeren TC, Greenberg M, Flaherty EE, Haivanis R, Su XH, Dean D, Newhall WJ, Knapp JS, Sarafian SK, Rice RJ, Morse SA, Rice PA. Transmission of Chlamydia trachomatis and Neisseria gonorrhoeae among men with urethritis and their female sex partners. J Infect Dis. 1998;178(6):1707-12. https://doi.org/10.1086/314485.
7. Vaezzadeh K, Sepidarkish M, Mollalo A, As'adi N, Rouholamin S, Rezaeinejad M, Mojtahedi MF, Hosseini SMM, Taheri M, Mahjour S, Mohammadi M, Chemaitelly H, Rostami A. Global prevalence of Neisseria gonorrhoeae infection in pregnant women: a systematic review and meta-analysis. Clin Microbiol Infect. 2023 Jan;29(1):22-31. https://doi.org/10.1016/j.cmi.2022.08.008.
8. Kirkcaldy RD, Weston E, Segurado AC, Hughes G. Epidemiology of gonorrhea: a global perspective. Sex Health. 2019 Sep;16(5):401-411. doi: https://doi.org/10.1071/SH19061.
9. Sunarti LS. Microbial Normal Flora: Its Existence And Their Contribution To Homeostasis. Journal of Advances in Microbiology, 2022;22 (9): 1-15 https://doi.org/10.9734/JAMB/2022/v22i930483.
10. Lehtoranta L, Ala-Jaakkola R, Laitila A, Maukonen J. Healthy Vaginal Microbiota and Influence of Probiotics Across the Female Life Span. Front Microbiol. 2022;13:819958. https://doi.org/10.3389/fmicb.2022.819958.

11. Dabee S, Passmore JS, Heffron R, Jaspan HB. The Complex Link between the Female Genital Microbiota, Genital Infections, and Inflammation. Infect Immun. 2021;89(5):e00487-20. doi: https://doi.org/10.1128/IAI.00487-20.
12. Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. Microb Cell Fact 2020;19: 203. https://doi.org/10.1186/s12934-020-01464-4.
13. Bayigga L, Nabatanzi R, Ssekagiri A, Kateete DP, Sekikubo M, Anderson DJ, et al. Diverse vaginal microbiome was associated with pro-inflammatory vaginal milieu among pregnant women in Uganda. Human Microbiome Journal, 2020; 18:100076. https://doi.org/10.1016/j.humic.2020.100076.
14. Delgado-Diaz DJ, Jesaveluk B, Hayward JA, Tyssen D, Alisoltani A, Potgieter M, et al. Lactic acid from vaginal microbiota enhances cervicovaginal epithelial barrier integrity by promoting tight junction protein expression. Microbiome. 2022 Aug 31;10(1):141. https://doi.org/10.1186/s40168-022-01337-5.
15. Lewis FMT, Bernstein KT, Aral SO. Vaginal Microbiome and Its Relationship to Behavior, Sexual Health, and Sexually Transmitted Diseases. Obstet Gynecol. 2017;129(4):643-54. https://doi.org/10.1097/AOG.0000000000001932.
16. Hakimjavadi H, George SH, Taub M, Dodds LV, Sanchez-Covarrubias AP, Huang M, et al. The vaginal microbiome is associated with endometrial cancer grade and histology. Cancer Res Commun. 2022 Jun;2(6):447-55. doi: https://doi.org/10.1158/2767-9764.CRC-22-0075.
17. Lovett A, Seña AC, Macintyre AN, Sempowski GD, Duncan JA, Waltmann A. Cervicovaginal Microbiota Predicts Neisseria gonorrhoeae Clinical Presentation. Frontiers in Microbiology, 2021;12: 790531. https://doi.org/10.3389/fmicb.2021.790531.
18. Lee YH, Kang G-U, Jeon SY, Tagele SB, Pham HQ, Kim M-S, Ahmad S, Jung D-R, Park Y-J, Han HS, et al. Vaginal Microbiome-Based Bacterial Signatures for Predicting the Severity of Cervical Intraepithelial Neoplasia. Diagnostics. 2020; 10(12):1013. https://doi.org/10.3390/diagnostics10121013
19. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157(1):121-41. doi: https://doi.org/10.1016/j.cell.2014.03.011.
20. Daft JG, Ptacek T, Kumar R, Morrow C, Lorenz RG. Cross-fostering immediately after birth induces a permanent microbiota shift that is shaped by the nursing mother. Microbiome. 2015 Apr 25;3:17. https://doi.org/10.1186/s40168-015-0080-y.
21. Blaser MJ, Webb GF. Host demise as a beneficial function of indigenous microbiota in human hosts. mBio. 2014 Dec 16;5(6):e02262-14. https://doi.org/10.1128/mBio.02262-14.
22. Lamont RF, Sobel JD, Akins RA, Hassan SS, Chaiworapongsa T, Kusanovic JP, Romero R. The vaginal microbiome: new information about genital tract flora using molecular based techniques. BJOG. 2011;118(5):533-49. https://doi.org/10.1111/j.1471-0528.2010.02840.x.
23. Song SD, Acharya KD, Zhu JE, Deveney CM, Walther-Antonio MRS, Tetel MJ, Chia N. Daily Vaginal Microbiota Fluctuations Associated with Natural Hormonal Cycle, Contraceptives, Diet, and Exercise. mSphere. 2020;5(4):e00593-20. https://doi.org/10.1128/mSphere.00593-20.
24. Amabebe E, Anumba DOC. Psychosocial Stress, Cortisol Levels, and Maintenance of Vaginal Health. Front Endocrinol (Lausanne). 2018 Sep 24;9:568. https://doi.org/10.3389/fendo.2018.00568.
25. Krog MC, Hugerth LW, Fransson E, Bashir Z, Nyboe Andersen A, Edfeldt G, et al.. The healthy female microbiome across body sites: effect of hormonal contraceptives and the menstrual cycle. Hum Reprod. 2022;37(7):1525-1543. https://doi.org/10.1093/humrep/deac094.
26. Pryor R, Martinez-Martinez D, Quintaneiro L, Cabreiro F. The Role of the Microbiome in Drug Response. Annu Rev Pharmacol Toxicol. 2020;60:417-435. https://doi.org/10.1146/annurev-pharmtox-010919-023612.
27. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016 Aug 19;14(8):e1002533. https://doi.org/10.1371/journal.pbio.
28. Pendharkar S, Skafte-Holm A, Simsek G, Haahr T. Lactobacilli and Their Probiotic Effects in the Vagina of Reproductive Age Women. Microorganisms. 2023; 11(3):636. https://doi.org/10.3390/microorganisms11030636.

29. Valenti P, Rosa L, Capobianco D, Lepanto MS, Schiavi E, Cutone A, et al. Role of Lactobacilli and Lactoferrin in the Mucosal Cervicovaginal Defense. Front Immunol. 2018;9:376. https://doi.org/10.3389/fimmu.2018.00376.
30. Mijac VD, Dukić SV, Opavski NZ, Dukić MK, Ranin LT. Hydrogen peroxide producing lactobacilli in women with vaginal infections. Eur J Obstet Gynecol Reprod Biol. 2006; 129(1): 69-76. https://doi.org/10.1016/j.ejogrb.2005.11.036.
31. Ciszewski WM, Sobierajska K, Stasiak A, Wagner W. Lactate drives cellular DNA repair capacity: Role of lactate and related short-chain fatty acids in cervical cancer chemoresistance and viral infection. Front Cell Dev Biol. 2022;10:1012254. https://doi.org/10.3389/fcell.2022.1012254.
32. Forney LJ, Foster JA, Ledger W. The vaginal flora of healthy women is not always dominated by Lactobacillus species. J Infect Dis. 2006;194(10):1468-9; author reply 1469-70. https://doi.org/10.1086/508497.
33. Romero-Gamboa DG, Díaz-Martínez LA, Díaz-Galvis ML, González-Blanco DP. Impact of genital hair removal on female skin microenvironment: barrier disruption and risk of infection, a literature review. Medicas UIS. 2019;32(3): 27-33. https://doi.org/10.18273/revmed.v32n3-2019004.
34. Coelho GDP, Ayres LFA, Barreto DS, Henriques BD, Prado MRMC, Passos CMD. Acquisition of microbiota according to the type of birth: an integrative review. Rev Lat Am Enfermagem. 2021;29:e3446. https://doi.org/10.1590/1518.8345.4466.3446.
35. Coscia A, Bardanzellu F, Caboni E, Fanos V, Peroni DG. When a Neonate Is Born, So Is a Microbiota. Life (Basel). 2021;11(2):148. doi: https://doi.org/10.3390/life11020148.
36. Browne HP, Shao Y, Lawley TD. Mother-infant transmission of human microbiota. Curr Opin Microbiol. 2022;69:102173. doi: https://doi.org/10.1016/j.mib.2022.102173.
37. Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: mom matters. Trends Mol Med. 2015;21(2):109-17. https://doi.org/10.1016/j.molmed.2014.12.002.
38. Saadaoui M, Singh P, Ortashi O, Al Khodor S. Role of the vaginal microbiome in miscarriage: exploring the relationship. Front Cell Infect Microbiol. 2023;13:1232825. https://doi.org/10.3389/fcimb.2023.1232825.
39. Bayar E, Bennett PR, Chan D, Sykes L, MacIntyre DA. The pregnancy microbiome and preterm birth. Semin Immunopathol. 2020;42(4):487-499. https://doi.org/10.1007/s00281-020-00817-w.
40. Freitas AC, Chaban B, Bocking A, Rocco M, Yang S, Hill JE, Money DM; VOGUE Research Group. The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. Sci Rep. 2017;7(1):9212. https://doi.org/10.1038/s41598-017-07790-9.
41. Gupta P, Singh MP, Goyal K. Diversity of Vaginal Microbiome in Pregnancy: Deciphering the Obscurity. Front Public Health. 2020;8:326. https://doi.org/10.3389/fpubh.2020.00326.
42. Kroon SJ, Ravel J, Huston WM. Cervicovaginal microbiota, women's health, and reproductive outcomes. Fertil Steril. 2018;110(3):327-336. doi: https://doi.org/10.1016/j.fertnstert.2018.06.036.
43. Chávez-Torres M, Gómez-Palacio-Schjetnan M, Reyes-Terán G, Briceño O, Ávila-Ríos S, Romero-Mora KA, Pinto-Cardoso S. The vaginal microbiota of women living with HIV on suppressive antiretroviral therapy and its relation to high-risk human papillomavirus infection. BMC Microbiol. 2023;23(1):21. https://doi.org/10.1186/s12866-023-02769-1.
44. Kim JM, Park YJ. (2018). Lactobacillus and Urine Microbiome in Association with Urinary Tract Infections and Bacterial Vaginosis. Urogenital Tract Infection. 2018;13(1): 7-13. https://doi.org/10.14777/uti.2018.13.1.7.
45. Swidsinski S, Moll WM, Swidsinski A. Bacterial Vaginosis-Vaginal Polymicrobial Biofilms and Dysbiosis. Dtsch Arztebl Int. 2023;120(20):347-54. https://doi.org/10.3238/arztebl.m2023.0090.
46. Bautista CT, Wurapa E, Sateren WB, Morris S, Hollingsworth B, Sanchez JL. Bacterial vaginosis: a synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections. Mil Med Res. 2016;3:4. https://doi.org/10.1186/s40779-016-0074-5.
47. Tuddenham S, Ravel J, Marrazzo JM. Protection and Risk: Male and Female Genital Microbiota and Sexually Transmitted Infections. J Infect Dis. 2021;223(12 Suppl 2):S222-S235. doi: https://doi.org/10.1093/infdis/jiaa762.

48. Zhu W, Cardenas-Alvarez MX, Tomberg J, Little MB, Duncan JA, Nicholas RA. Commensal Neisseria species share immune suppressive mechanisms with Neisseria gonorrhoeae. PLoS One. 2023; 18 (4): e0284062. https://doi.org/10.1371/journal.pone.0284062.
49. Lenz JD, Dillard JP. Pathogenesis of Neisseria gonorrhoeae and the Host Defense in Ascending Infections of Human Fallopian Tube. Front Immunol. 2018 Nov 21;9:2710. https://doi.org/10.3389/fimmu.2018.02710.
50. Song W, Yu Q, Wang LC, Stein DC. Adaptation of Neisseria gonorrhoeae to the Female Reproductive Tract. Microbiol Insights. 2020; 13: 1178636120947077. https://doi.org/10.1177/1178636120947077.
51. Yu Q, Wang LC, Di Benigno S, Gray-Owen SD, Stein DC, Song W. Neisseria gonorrhoeae infects the heterogeneous epithelia of the human cervix using distinct mechanisms. PLoS Pathog. 2019; 15(12): e1008136. https://doi.org/10.1371/journal.ppat.1008136.
52. Liu Y, Feinen B, Russell MW. New concepts in immunity to Neisseria gonorrhoeae: innate responses and suppression of adaptive immunity favor the pathogen, not the host. Front Microbiol. 2011;2:52. https://doi.org/10.3389/fmicb.2011.00052.
53. Mahapure K, Singh A. A Review of Recent Advances in Our Understanding of Neisseria gonorrhoeae. Cureus, 2023; 15(8): e43464. https://doi.org/10.7759/cureus.43464
54. Elmros T. Survival of Neisseria gonorrhoeae on surfaces. Acta Derm Venereol. 1977;57(2):177-80.
55. Quillin SJ, Seifert HS. Neisseria gonorrhoeae host adaptation and pathogenesis. Nat Rev Microbiol. 2018;16(4):226-240. https://doi.org/10.1038/nrmicro.2017.169.
56. Baerentsen R, Tang CM, Exley RM. Et tu, Neisseria? Conflicts of Interest Between Neisseria Species. Front Cell Infect Microbiol. 2022;12:913292. https://doi.org/10.3389/fcimb.2022.913292.
57. Calder A, Menkiti CJ, Çağdaş A, Lisboa Santos J, Streich R, Wong A, et al. Virulence genes and previously unexplored gene clusters in four commensal Neisseria spp. isolated from the human throat expand the neisserial gene repertoire. Microb Genom. 2020;6(9):mgen000423. https://doi.org/10.1099/mgen.0.000423.
58. Kurzyp K, Harrison OB. Bacterium of one thousand and one variants: genetic diversity of Neisseria gonorrhoeae pathogenicity. Microb Genom. 2023;9(6):mgen001040. https://doi.org/10.1099/mgen.0.001040.
59. Walker E, van Niekerk S, Hanning K, Kelton W, Hicks J. Mechanisms of host manipulation by Neisseria gonorrhoeae. Front Microbiol. 2023;14:1119834. doi: https://doi.org/10.3389/fmicb.2023.1119834.
60. Ray JC, Smirnov A, Maurakis SA, Harrison SA, Ke E, Chazin WJ, Cornelissen CN, Criss AK. Adherence Enables Neisseria gonorrhoeae to Overcome Zinc Limitation Imposed by Nutritional Immunity Proteins. Infect Immun. 2022;90(3):e0000922. https://doi.org/10.1128/iai.00009-22.
61. Yu Q, Wang LC, Di Benigno S, Stein DC, Song W. Gonococcal invasion into epithelial cells depends on both cell polarity and ezrin. PLoS Pathog. 2021;17(12):e1009592. https://doi.org/10.1371/journal.ppat.1009592.
62. Stein DC, LeVan A, Hardy B, Wang LC, Zimmerman L, et al. Expression of Opacity Proteins Interferes with the Transmigration of Neisseria gonorrhoeae across Polarized Epithelial Cells. PLOS ONE, 2015; 10(8): e0134342. https://doi.org/10.1371/journal.pone.0134342
63. Unemo , Seifert HS, Hook EW, Hawkes S, Ndowa F, Dillon JR. Gonorrhoea. Nat Rev Dis Primers 2019;5: 79. https://doi.org/10.1038/s41572-019-0128-6
64. Kalia N, Singh J, Kaur M. Microbiota in vaginal health and pathogenesis of recurrent vulvovaginal infections: a critical review. Ann Clin Microbiol Antimicrob 2020;19:5. https://doi.org/10.1186/s12941-020-0347-4
65. McLeod DV, Day T. Sexually transmitted infection and the evolution of serial monogamy. Proc Biol Sci. 2014;281(1796):20141726. doi: https://doi.org/10.1098/rspb.2014.1726.
66. Liu Y, Liu W, Russell MW. Suppression of host adaptive immune responses by Neisseria gonorrhoeae: role of interleukin 10 and type 1 regulatory T cells. Mucosal Immunol. 2014;7(1):165-76. https://doi.org/10.1038/mi.2013.36.
67. Jarvis GA, Li J, Swanson KV. Invasion of human mucosal epithelial cells by Neisseria gonorrhoeae upregulates expression of intercellular adhesion molecule 1 (ICAM-1). Infect Immun. 1999 Mar;67(3):1149-56. doi: https://doi.org/10.1128/IAI.67.3.1149-1156.1999.

68. Muenzner P, Hauck CR. Neisseria gonorrhoeae Blocks Epithelial Exfoliation by Nitric-Oxide-Mediated Metabolic Cross Talk to Promote Colonization in Mice. Cell Host Microbe. 2020 May 13;27(5):793-808.e5. doi: https://doi.org/10.1016/j.chom.2020.03.010.
69. Jacobsen T, Bardiaux B, Francetic O, Izadi-Pruneyre N, Nilges M. Structure and function of minor pilins of type IV pili. Med Microbiol Immunol. 2020;209(3):301-8 https://doi.org/10.1007/s00430-019-00642-5.
70. Christodoulides M. Preparation of Lipooligosaccharide (LOS) from Neisseria gonorrhoeae. Methods Mol Biol. 2019;1997:87-96. https://doi.org/10.1007/978-1-4939-9496-0_6.
71. Alcott AM, Werner LM, Baiocco CM, Belcher Dufrisne M, Columbus L, Criss AK. Variable Expression of Opa Proteins by Neisseria gonorrhoeae Influences Bacterial Association and Phagocytic Killing by Human Neutrophils. J Bacteriol. 2022;204(4):e0003522. https://doi.org/10.1128/jb.00035-22.
72. Virji M. Pathogenic neisseriae: surface modulation, pathogenesis and infection control. Nat Rev Microbiol 2009;7, 274–86. https://doi.org/10.1038/nrmicro2097
73. Taktikos J, Lin YT, Stark H, Biais N, Zaburdaev V. Pili-Induced Clustering of N. gonorrhoeae Bacteria. PLoS One. 2015;10(9):e0137661. https://doi.org/10.1371/journal.pone.0137661.
74. Płaczkiewicz J, Adamczyk-Popławska M, Lasek R, Bącal P, Kwiatek A. Inactivation of Genes Encoding MutL and MutS Proteins Influences Adhesion and Biofilm Formation by Neisseria gonorrhoeae. Microorganisms. 2019; 7(12):647. https://doi.org/10.3390/microorganisms7120647
75. Miko E, Barakonyi A. The Role of Hydrogen-Peroxide (H2O2) Produced by Vaginal Microbiota in Female Reproductive Health. Antioxidants (Basel). 2023;12(5):1055. https://doi.org/10.3390/antiox12051055.
76. Sgibnev AV, Kremleva EA. Vaginal Protection by H2O2-Producing Lactobacilli. Jundishapur J Microbiol. 2015;8(10):e22913. doi: https://doi.org/10.5812/jjm.22913.
77. Tachedjian G, O'Hanlon DE, Ravel J. The implausible "in vivo" role of hydrogen peroxide as an antimicrobial factor produced by vaginal microbiota. Microbiome. 2018;6(1):29. https://doi.org/10.1186/s40168-018-0418-3.
78. Scillato M, Spitale A, Mongelli G, Privitera GF, Mangano K, Cianci A, Stefani S, Santagati M. Antimicrobial properties of Lactobacillus cell-free supernatants against multidrug-resistant urogenital pathogens. Microbiologyopen. 2021;10(2):e1173. https://doi.org/10.1002/mbo3.1173.
79. Hawkins CL, Davies MJ. Role of myeloperoxidase and oxidant formation in the extracellular environment in inflammation-induced tissue damage. Free Radic Biol Med. 2021;172:633-51. https://doi.org/10.1016/j.freeradbiomed.2021.07.007.
80. Brendefur Corwin LM, Campbell P, Jakobsen K, Müller F, Lai X, Unemo M, et al. Improvement in Neisseria gonorrhoeae culture rates by bedside inoculation and incubation at a clinic for sexually transmitted infections. Ann Clin Microbiol Antimicrob. 2023;22(1):27. doi: https://doi.org/10.1186/s12941-023-00576-0.
81. Menkiti C, Snyder L. Improvement of Neisseria gonorrhoeae culture media to enable growth without CO_2. (2023). https://doi.org/10.1101/2023.08.01.551449.
82. Branch AH, Stoudenmire JL, Seib KL, Cornelissen CN. Acclimation to Nutritional Immunity and Metal Intoxication Requires Zinc, Manganese, and Copper Homeostasis in the Pathogenic Neisseriae. Front Cell Infect Microbiol. 2022;12:909888. doi: https://doi.org/10.3389/fcimb.2022.909888.
83. Liyayi IK, Forehand AL, Ray JC, Criss AK. Metal piracy by Neisseria gonorrhoeae to overcome human nutritional immunity. PLoS Pathog. 2023;19(2):e1011091. doi: https://doi.org/10.1371/journal.ppat.1011091.
84. Page MGP. The Role of Iron and Siderophores in Infection, and the Development of Siderophore Antibiotics. Clin Infect Dis. 2019;69(Suppl 7):S529-S537. https://doi.org/10.1093/cid/ciz825.
85. Stoudenmire JL, Greenawalt AN, Cornelissen CN. Stealthy microbes: How Neisseria gonorrhoeae hijacks bulwarked iron during infection. Front Cell Infect Microbiol. 2022 Sep 15;12:1017348. https://doi.org/10.3389/fcimb.2022.1017348.
86. Maurakis S, Cornelissen CN. Metal-Limited Growth of Neisseria gonorrhoeae for Characterization of Metal-Responsive Genes and Metal Acquisition from Host Ligands. J Vis Exp. 2020;(157):10.3791/60903. https://doi.org/10.3791/60903.

87. Criss AK, Seifert HS. A bacterial siren song: intimate interactions between Neisseria and neutrophils. Nat Rev Microbiol. 2012;10(3):178-90. https://doi.org/10.1038/nrmicro2713.