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 *Neisseria 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 *N. 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]. Management of gonorrhea according to Centers for Disease Control and Prevention (CDC) that recommends a monotherapy, single dose of 500 mg of intramuscular ceftriaxone. Alternative regimens are actually available when ceftriaxone cannot be used to treat urogenital or rectal gonorrhea. Although medication will stop the infection, it will not repair any permanent damage done by the disease.

"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 3.8×10^{13}. For human cells, the dominant role of the hematopoietic lineage to the total count (≈90%) and revise past estimates to 3.0×10^{13} 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, L. iners. L. crispatus* and *L. Jensenii* [29]. Lactobacillus play a crucial role in protecting vaginal surfaces by secreting H_2O_2; 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 Trichomoniasis 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 [9]. 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 [47, 64]. 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 [11], occupy and colonize specific anatomical sites and encourage grow [47](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 [60].

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, H_2O_2-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 H_2O_2 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

Due to its silent spread and possibility of severe complication, increasing public awareness regarding this STI must always be conducted; and this include their mechanism of invasion that starts from adherence and followed by competition. 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.

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