Chlamydia trachomatis Genital Tract Infections:

When Host Immune Response and the Microbiome Collide

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Abstract

Genital infections with *Chlamydia trachomatis* continue to be a major health problem worldwide. While some individuals clear their infection (presumed to be the result of an effective Th1 / gamma-interferon response), others develop chronic infections and some are prone to repeat infections. In females in particular, chronic asymptomatic infections are common and can lead to pelvic inflammatory disease and infertility. Recent studies suggest that the genital tract microbiota could be a significant factor and explain person-to-person variation in *C. trachomatis* infections. One hypothesis suggests that *C. trachomatis* can use its *trpBA* genes to rescue tryptophan from indole, which is a product of anaerobic members of the genital tract microbiota. Women with particular microbiota types, such as seen in bacterial vaginosis, have increased numbers of anaerobes, and this would enable the chlamydia in these individuals to overcome the host's interferon-gamma attempts to eliminate it, resulting in more repeat and/or chronic infections.

Keywords: interferon-gamma, indole, sex hormones, *Lactobacillus*, bacterial vaginosis.

Chlamydia trachomatis Infections

Chlamydia trachomatis is an obligate intracellular bacterium that is the causative agent of sexually transmitted infection in humans and is a widespread public health concern because of its high prevalence. Worldwide, an estimated 131 million sexually transmitted *C. trachomatis* infections occur each year [1]. A significant proportion (up to 80%) of infections in women are asymptomatic, and in the absence of active screening and

treatment, most infections go undetected. If left untreated, about 10% of *Chlamydia* infections in women will progress to pelvic inflammatory disease (PID) [2]. Other complications such as endometritis, ectopic pregnancy and infertility can also occur [3]. Individuals vary in their response to *C. trachomatis* infections. In some individuals, infections can self-resolve in about one year on average (20-54% of cases) [4–7]. However, in others (23-30% of cases), they can become repeatedly infected, two or more times, in the 12 months following initial diagnosis and treatment [8–12]. It is generally assumed that a strong T-helper 1 (Th1) and interferon-gamma (IFN-γ) response can lead to spontaneous resolution in a percentage of patients, however the majority of patients do not spontaneously resolve their infections [4–7,13]. It has also been shown in the murine model that antibiotic treatment can suppress the host protective immune response [14]. This observation has been referred to as the "arrested immunity hypothesis" [15], in which, according to a mathematical model, suggest that early treatment of *C. trachomatis* infection increases population susceptibility to reinfections [16], presumably by interfering with the development of protective immune response. In addition to parasite and host effects, it has recently been proposed that the bacteria in the vaginal microbiota might play a key role in the outcome of *C. trachomatis* infections. Antibiotics used to treat the *Chlamydia* might also interfere with a the healthy bacterial equilibrium in the genital tract [17–19], specifically causing a decrease in the prevalence of commensal *Lactobacillus spp*., that are known to have inhibitory effects on pathogenic bacteria. [20–23]. The most recent hypothesis suggests that the anaerobe component of the vaginal microbiota (not the lactobacilli per se) might play a key role. In this review we summarize the many factors that contribute to a healthy versus diseased state in which *Chlamydia* could successfully colonize the female genital tract (Figure 1).

Chlamydia Has a Unique Developmental Cycle.

C. trachomatis is classified into two biovars; the trachoma biovar which includes ocular and urogenital strains (A-K) and the lymphogranuloma venereum LGV biovar (L1-L3) [24]. The ocular strains (serovars A - C) are characterized by localized infection of the epithelial surface of the conjunctiva. Serovars D-K are responsible for infections in the urogenital tract, especially in the endocervix [25]. *Chlamydia* has a unique biphasic developmental cycle, which alternates between two distinct morphological forms, the elementary bodies (EB) and the reticulate bodies (RB) [26]. When infection occurs, the metabolically inert EBs, attach to and stimulate uptake by the host cell. Upon entry, the internalized EBs remain within a membrane-bound, host-derived parasitophorous vacuole, termed an inclusion, where they differentiate into larger, non-infectious but the metabolically active RB form [27–29]. RBs undergo proliferation by binary fission and inclusion growth. After 8 to 12 rounds of multiplication, the RBs convert back into EBs. At 30-84 hours post-infection, depending on the infecting species, the infectious EBs are released from the host cell, predominantly via lysis, to attach to the neighboring cells and initiate another cycle (Figure 2A). However, under stressful growth conditions caused by a range of factors, such as host immunological response, antibiotic treatment or nutrient starvation [30–36], the

developmental cycle is disturbed and the EBs convert to enlarged, non-infectious, aberrant bodies (AB), until the stress factor is removed [28] (Figure 2B). This so called persistent state is defined as a 'viable but non-cultivable growth stage' [29,30]. Chlamydial persistence *in vitro* has been characterized in many studies [30–36], however very few groups have been able to isolate aberrant forms from clinical samples [34], confirming that chlamydial persistence *in vivo* needs further investigation.

The Immune Response and Progression to Disease

Women who acquire new infections or are unable to fully clear their current infection, are more prone to diseases such as PID, tubal factor infertility and ectopic pregnancy [2,37–41]. Progression to these sequelae is thought to be the outcome of immuno-pathological inflammatory responses that result in tissue disruption, fibrosis and scarring [39,42,43]. The adaptive immune response to *C. trachomatis* infection is characterized by the production of significant numbers of T cells infiltrating the infected area. One of the most important cellular immune responses in the development of protective immunity against chlamydial infection is characterized by antigen specific IFN-γ- secreting CD4+ and CD8+ T cells. Studies in the murine model have reported that the resolution of a genital chlamydial infection depends on IFN-γ- producing CD4+ Th1 cells [44–47] to control or eliminate an infection. However, upon reinfection or chronic infection, host cell release of chemokines could lead to recruitment of *Chlamydia*-specific immune cells to magnify the response. The release of proteases, clotting factors and tissue growth factors from infected host cells and infiltrating inflammatory cells results in tissue damage and scarring [43]. These devastating sequelae are the result of chronic, immunopathogenic responses to the *Chlamydia* and are associated with Th1 and Th17 cells (reviewed by Hafner *et al.* [48]). High expression of Th2-type cytokines such as IL-10, IL-1α, IL-6, IL-4 and TNF-α (Th1) were also associated with disease and increased risk of pathological sequelae in trachoma and urogenital *C. trachomatis* infections [49–51]. These findings highlight the theory of 'the immunological paradigm for pathogenesis' where T cell responses necessary for host defence may also cause collateral tissue damage [3]. The reasons why *C. trachomatis* can cause extended infections, lasting from months to years in the face of an immune response, is not well understood, but suggests an array of sophisticated parasite adaptation and/or evasion mechanisms. There are many factors that influence the initial chlamydial infection and its progress, including the host immune response, hormonal changes throughout the menstrual cycle, bacterial communities colonizing the genital tract, as well as co-infections with other sexually transmitted infections (STIs; Figure 1).

Figure 1 goes here.

Tryptophan Depletion via IFN-γ Response and *C. trachomatis* Evasion Mechanism

IFN-γ is a critical cytokine for the innate and adaptive immune response against a wide range of viral and bacterial infections, including *Chlamydia*. Among its many roles in the host immune response, IFN-γ has been associated with protection and immune activation against viral, bacterial and protozoal infections [52–54], activation of macrophages, induction of Class ll MHC [53] and involvement in autoimmune diseases [55]. IFN-γ also activates the expression of indoleamine 2,3-dioxygenase (IDO). IDO is an enzyme which catabolizes the amino acid L- tryptophan into kynurenine (Figure 2B), depleting the host cell's pool of tryptophan [25,56–59]. IFN-γ is well known as an anti-chlamydial agent, causing inhibition of growth and development of the *Chlamydia*, primarily because *C. trachomatis* is a tryptophan auxotroph [25,58–60]. Although high concentrations of IFN-γ *in vitro* are lethal to the *Chlamydia*, sub-inhibitory concentrations [58] can drive the *Chlamydia* into its persistence form [30,59], as evident by aberrant non-infectious bodies (Figure 2B). It has been suggested that *C. trachomatis* has developed a mechanism to evade the host's immune defence strategy, by retaining a subset of the genes in the tryptophan synthesis pathway: the *trpA*, *trpB* and *trpR* genes (Figure 2B). It has been shown *in vitro* that these genes enable the urogenital strains of *C. trachomatis* to synthesize or rescue tryptophan from indole [24,70,74]. This set of genes was found to be upregulated in a cell culture infected with *C. trachomatis* and treated with IFN-γ [58]. These genes are located on a region of the chlamydial genome referred to as the 'plasticity zone'. This chromosomal region varies between chlamydial species and has undergone increased levels of genetic variation, compared to other regions of the chromosome [63]. Different *Chlamydia* species contain different parts of the tryptophan biosynthesis pathway, with the exception of *C. trachomatis* serovar MoPn and *C. pneumoniae*, that do not encode any of the *trp* genes [60]. *In vitro* experiments show that the effect of IFN-γ on *C. trachomatis* growth and survival varies between the different serovars, as well as their ability to recover after the tryptophan starvation stress [25,57]. For example, *C. trachomatis* genital strains can be rescued after IFN-γ treatment using exogenous indole. However, the ocular serovars, which have inactivating mutations in their *trpBA* genes, are therefore not able to synthesize tryptophan from indole [64].

Figure 2 goes here

Unique Challenges and Opportunities of Bacteria Living in the Female Genital Tract

The human body is a complex ecosystem, which functions in association with diverse microbial populations. The female vaginal tract, as with other mucosal sites in the body, is in contact with the external environment, providing a route of entry and colonization for microorganisms. A healthy host-vaginal microbiota is termed a holobiont, in which a functional equilibrium is established through mutualism between the host and the resident microbes. This healthy equilibrium acts to provide a barrier to both new colonization by pathogenic

organisms as well as overgrowth of non-commensal organisms [65,66]. In most reproductive age women, *Lactobacillus* species are numerically dominant in the lower genital tract [17,67–69]. Using molecular-based techniques to characterise the different communities, the microbiota can be conveniently classified into four or five community state types (CSTs) [69,70]. The groups are classified based on the dominant specific *Lactobacillus* species; (i) *L. iners,* (ii) *L. crispatus,* (iii) *L. gasseri* and/ or (iv) *L. jensenii*, while the last group (v) is characterized by a lower abundance of *Lactobacillus*, and a higher proportion of various anaerobic bacterial species [69,70]. *Lactobacillus spp*. are considered to have a key protective role in the vaginal flora by lowering the pH through lactic acid production [23], as well as inhibition of other pathogenic bacteria by producing a range of bacteriostatic and bactericidal compounds [71]. A *L. crispatus* dominant microbiota is generally considered to be the most stable flora, providing efficient protection against invading pathogenic microorganisms and is associated with the lowest pH levels among all CST groups [69]. In addition, women dominated by *L. crispatus* have been suggested to have higher lactic acid production and a healthier equilibrium, whereas *L. gasseri* and *L. iners* are more conducive to the occurrence of abnormal microflora [67,72]. Studies have shown that vaginal bacterial communities vary among women, are species rich, and include bacteria that were not previously detected by traditional culture-based methods [17,67,69,73] (Table 1). However, despite many of the studies revealing the composition of the species in the vaginal tract, a better understanding of the relationships between the different bacterial species and their function in this complex eco-system is still needed.

Composition of the Vaginal Microbiome as a Major Impact on Health and Disease

It is generally accepted that a healthy vaginal microbiota contains higher numbers of *Lactobacillus spp*. The replacement of the *Lactobacillus spp*. with facultative and/or strict anaerobes, such as *Gardnerella vaginalis*, *Prevotella spp., Peptostreptoccocus spp., Mobiluncus spp.*, and a wide range of other fastidious and uncultivable bacterial species (Table 1), can result in the switch from normal vaginal microbiota to dysbiosis and bacterial vaginosis (BV). In the 'diseased' state, such as BV, the bacterial community dynamics can change rapidly, and are usually individual specific [74]. BV is associated with higher pH conditions and an increased risk of acquiring sexually transmitted infections (STIs) such as herpes, HIV and *C. trachomatis* [17,75,76]. *Lactobacillus spp.* have been observed to inhibit *in vitro* growth of numerous bacterial species through the production of lactic acid [77,78], including time and concentration-dependent killing of *C. trachomatis* [20]. Interestingly, some of the bacterial species that are associated with BV are also lactic acid producers, including *Megasphaera, Streptococcus* and *Atopobium*. The production of lactic acid seems to be conserved in different bacterial communities regardless of their association with the host state of health or disease, implicating it as a key

factor in multiple ecological systems and functions. There are many factors that could affect the microbial communities in the vagina (Figure 1), causing significant changes over short periods of time [79]. For example, after antibiotic treatment, there was a rapid decrease in the presence of facultative and strict anaerobic bacteria, and an increase in *Lactobacillus spp*. However, this effect was a short-lived, lasting for only 2-4 weeks post treatment [74].

Table 1 goes here.

Influences on the Microbial Communities in the Female Genital Tract

The human microbiota is influenced by the host life style including diet, alcohol consumption, drugs and antibiotic use (Reviewed by Relman 2012) [80], smoking [81], hygiene, hormonal contraception, sexual life style and the number of sex partners [82] . The microbiome composition is also influenced by the host genotype, immune response and the environment (Figure 1).

Hormones

The immune response, sex hormones, and the vaginal microbiota, are tightly connected to each other and influenced by fluctuations during the female menstrual cycle. Sex hormones co-ordinate cell trafficking and immune activation [83], and can contribute to the susceptibility of the innate and acquired immunity to bacterial and viral infections [84]. Symbiotic vaginal bacteria such as *Lactobacillus spp.* have also been shown to be under hormonal influence [85]. Studies have shown that during a window of 7-10 day during the secretory phase of the menstrual cycle, important components of innate, humoral and cell-mediated immunity are suppressed by estradiol and/or progesterone, enhancing the potential for viral and bacterial infections [86]. During this phase, estradiol levels transiently decline and then increase concurrently with progesterone. Estradiol and progesterone prepare the uterus for implantation during the second half of the menstrual cycle by providing an adequate vascular supply and subsequent nutrients [86]. Along with the reproductive function, sex hormones regulate the transport of immunoglobulin A (IgA) and IgG, the levels of cytokines, the expression of TLR genes [87] and the distribution of immune cells and antigen presentation in the genital tissues during the reproductive cycle [88]. Fluctuations in the Th1/Th2 cytokine profile across the menstrual cycle, with lower levels of IFN-γ during the luteal phase [89], could lead to a compromised immune system, while fighting against chlamydial infection. Furthermore, higher numbers of antibody-secreting B cells, along with higher levels of IgA and IgG, were detected in the pre-ovulatory phase compared to the post-ovulatory phase [90,91].

pH

Optimal pH levels vary among the different species of *in vitro* cultured commensal vaginal *Lactobacillus spp*. The optimal growth conditions and maximum bacteriocin production of the vaginal isolate, *L. salivarius* were recorded with an initial pH of 6.5 at 37°C [92]. The highest viable cell count for the vaginal isolate *L. crispatus*, was obtained at pH 5.5; whereas at pH levels below 3.5 or above 7.5, cell growth was inhibited [93]. In *L. acidophilus* culture, the best growth conditions and maximum lactic acid production were obtained with an initial pH of 6.5 at 37°C. Under these optimal growth conditions *L. acidophilus* was able to inhibit uropathogenic *Escherichia coli in vitro* [94]. Glycogen is also associated with pH and *Lactobacillus spp*. colonization in the genital tract. Quantification of glycogen from vaginal secretions has shown higher levels of free glycogen correlated with lower pH levels and higher levels of *L. crispatus* and *L. jensenii* colonization, but not *L. iners* [95]. At the onset of puberty, *Lactobacillus spp*. prevalence increases in the female genital tract and the pH level decreases [95]. These changes were suggested to be dependent on estrogen levels, because the low pH and *Lactobacillus spp.* colonization in the vagina occur at the same time as when estrogen levels are increasing [96]. Gorodeski *et al.* suggested that estrogen contributes to the acidification of the vaginal pH by upregulation of proton secretion via the apical membrane of vaginal ectocervical cells in their epithelial cell line model [97]. Several other studies also showed that estrogen contribute to the acidification of the vagina (Reviewed in [98]). However, a recent study showed that free glycogen levels, measured in the genital fluids of 7 pre-menopausal women, were negatively associated with pH and progesterone, while estrogen levels had no significant correlation with free glycogen [99].

The *C. trachomatis* Plasmid, Glycogen and the Vaginal Microbiome

Virtually all *C. trachomatis* isolates carry a highly conserved, 7.5 kb plasmid, which encodes eight coding sequences (CDS) [100–102]. Plasmid-deficient *Chlamydia* organisms are generally attenuated by removal of the plasmid [115]. *C. trachomatis* and *C. muridarum* plasmid-deficient strains failed to activate Toll like receptor-2 (TLR2) and show reduced virulence [105]. In a recent study investigating the transcriptional profiling of a plasmid-cured trachoma strain of *C. trachomatis*, in human epithelial cells, genes related to host cell inflammatory response and immune avoidance were not upregulated in comparison to the strain containing the plasmid, suggesting a crucial role for the plasmid in trachoma pathogenesis and virulence [106]. Plasmid-deficient strains of trachoma have been proposed as a live-attenuated vaccine when used to vaccinate non-human primates [107,108]. In addition, mature inclusions of the mutant strain with a deletion in the CDS5 and 6 regions on the plasmid resulted in altered morphology and a 'black hole' phenotype [100]. Recent progress also shows that the plasmid gene *pgp4* is responsible for the lytic exit of the organisms from the host cell during

the developmental cycle, and thereby is necessary for the infection of neighboring host cells [109]. Despite the great progress in the chlamydial plasmid studies, many of its biological characteristics are still not fully understood. The older literature shows that the chlamydial plasmid is linked to glycogen synthesis and accumulation [110]. *C. trachomatis* possess all the essential genes for glycogen synthesis (*mrsA, glgC, glgA, glgB*), as well as genes required for glycogen degradation (*glgX* and *glgP*) [111]. Although the glycogen synthase gene, *glgA*, is located on the chlamydial chromosome, the plasmid apparently controls its transcription levels [112]. Naturally occurring plasmid-less, and plasmid-cured strains of *C. trachomatis* are lacking visible glycogen accumulation and present reduced transcript levels of the *glgA* gene [112]. When a wild type *C. trachomatis* strain was grown in a medium containing various glucose concentrations, low concentrations limited the *Chlamydia* growth *in vitro*, as well as its ability to accumulate glycogen [103]. Glycogen is thought to support *Lactobacillus spp*. colonization *in vivo*, as many studies report correlation of increasing glycogen levels in female vaginal secretions, reaching a peak at mid-cycle, with a subsequent increase in lactic acid producing microbes in the vagina and an acidic pH [66,95,113]. However, most of the *Lactobacillus spp.* isolates found in the vaginal flora cannot utilize glycogen [114]. It was found recently that α-amylase is present in the vaginal fluids, and can break down glycogen to small carbohydrates, which the *Lactobacillus spp.* can use as an energy source [115]. A more recent study by the same group reported that a low pH actually limits the α-amylase enzyme by more than 50%, but was still able to maintain *Lactobacillus* growth [116].

Indole as a Key Factor in the Microbial Equilibrium

Indole is an important molecule due to its diverse biological roles associated with biofilm, drug resistance, plasmid stability and microbial communities [117–122]. Many bacterial species, both Gram-negative and Gram-positive, can produce indole in large quantities. Although it has been known for over 100 years [123] that bacteria can produce indole, only recently have studies discovered its biological role as an intercellular signaling molecule [117,122,124]. Indole levels increase drug resistance in *Salmonella enterica*, which cannot produce indole, through the induction of four multidrug efflux pumps [119]. It was also found that in the presence of the antibiotic ampicillin, the production of indole in *E. coli* was 22-fold higher than the control [125]. However, indole represses the genes encoding the *mexGHI-opmD* multidrug efflux pump in *Pseudomonas aeruginosa*, and also represses the genes involved in quorum sensing-regulated virulence factors [118]. A report by Lee [117] shows that indole represses biofilm by decreasing motility in *E. coli* through SdiA, however, in direct contrast, it increases biofilm production in *P. aeruginosa.*

Differences in virulence, drug resistance, and biofilm formation, induced by indole, can have major consequences on health and disease. For example, the role of indole was recently investigated in the gastro-intestinal tract, and was found to have probiotic properties [126]. Bansal *et al.* found that indole increases epithelial-cell tight junction resistance and attenuates indicators of inflammation, such as decreasing TNF-α gene expression and the proinflammatory chemokine IL-8. They also showed that exposure to physiologically relevant indole concentrations had a beneficial result on the human enterocyte cell line HCT-8, including strengthening the mucosal barrier and mucin production. Their previous work [127] showed that indole secreted from commensal *E. coli* in human feces also reduced pathogenic *E. coli* chemotaxis, motility and attachment to epithelial cells *in vitro*. In contrast, it is known that indole and other indole compounds, play a role in carcinogenesis as they have genotoxicity properties, and have been linked to colonic cancer [128–130]. These findings emphasize the important role of all factors including, bacterial composition, signal molecule production, immune response and various host factors in a single ecosystem.

How *Chlamydia* Can Utilize the Microbiome to Overcome Host Immune Attack

When chlamydial particles, which contain LPS, contact a target epithelial cell in the genital tract, they stimulate an innate IFN-γ cascade which the host utilizes in an attempt to eliminate the invading pathogen. The triggered IFN-γ cascade, among other processes, upregulates the host cell enzyme IDO, which converts available tryptophan to kynurenine. This has the effect of depleting the intracellular pools of the key amino acid, tryptophan. Of course, tryptophan is an essential amino acid and the human genital strains of *C. trachomatis* are, somewhat surprisingly, tryptophan auxotrophs. *Chlamydia* has evolved two mechanisms to overcome this host immune attack. First, they slow down their metabolism and hide out by entering a persistence state [28–30]. While the initial trigger is unknown, the organisms are prevented from undergoing binary fission and their differentiation from RBs to EBs is subsequently halted, however, there is continued genome replication and mRNA synthesis [29,30,36,58]. This results in chlamydial aberrant bodies inside the inclusion that are able to survive without tryptophan due to their lowered metabolism. The second mechanism, applicable only to the genital strains of *C. trachomatis* in the female genital tract, involves using the pool of extracellular indole produced by some bacterial species (e.g. *Prevotella spp., Peptostreptococcus spp., Peptoniphilus spp., Porphyromonas spp.*), that are present in the fornix, cervix and lower region of the vaginal canal [70,131]. The *C. trachomatis* genital strains (D-K) have retained just the single enzyme, TrpBA, in the tryptophan biosynthetic pathway, which enables them to convert indole (produced by the microbiota) to tryptophan. This proposed mechanism is well described by Morrison [132] and was supported by several other reports arguing that some bacteria from the vaginal microbiome, especially those that are associated with BV, are able to produce indole

using their *tna*A gene, which, in a symbiotic-like arrangement, has driven the chlamydial urogenital strains to retain their ability to synthesize back rescue tryptophan [25,34,58,61]. In addition, *in vivo* examination of women infected with *C. trachomatis* revealed higher levels of indole in their endocervical secretions (159-278mM) [34]. When screening the vaginal microbial flora of reproductive age women, it was shown that women with specific anaerobic bacterial communities, combined with higher pH and lower prevalence of lactic acid producing bacteria, are associated with an increased risk of STIs, including *Chlamydia* [61,69]. While this eukaryotic strategy of restricting tryptophan for invading bacterial pathogens as a means of eliminating them, is not unique, *Chlamydia*'s strategy of utilizing the other microbiota bacteria as a source of indole to overcome this host strategy does seem to be particularly resourceful.

Indole has many important roles as an interkingdom signaling molecule and has also been shown to have contradictory effects when associated with various species of bacteria. *Lactobacillus spp*. are an essential part of the gastrointestinal tract flora, due to their health promoting properties. Studies show that *Lactobacillus spp.* strains, dead or alive, can absorb indole efficiently, and even cause lower genotoxicity of indole after the interaction [129]. In addition, a high abundance of gut *Lactobacillus* was found to be associated with IDO1 inhibition and an increase in Th17 levels in simian immunodeficiency virus (SIV)-infected macaques [134]. IDO is known for its immunosuppressive function and long-term self-tolerance effects [135]. Consequent to downstream effects of the IDO activity, tryptophan depletion inhibits T cell proliferation and induces apoptosis [136].

What is the evolutionary benefit of the *trpBA* set of genes to the *Chlamydia* urogenital strains? While only a small relative percentage of the commensal vaginal tract bacteria can produce indole, and the species that do produce indole are mostly commensal gut bacteria (Table 1). Studies in animals show that *Chlamydia* can reside in the gastrointestinal tract for long periods of time, probably in a chronic or persistent state, and often without any pathological or even immunological response (reviewed by Rank *et al.* [137]). Horizontal transfer between hosts, via the oral-fecal route has been recorded as well, while shedding infectious chlamydial particles through feces is highly prevalent in livestock and other animal models. Whereas most data in regards to rectal *C. trachomatis* infections was reported in high risk patients such as men who have sex with men, recent studies report a high prevalence in women as well [138,139]. A study in two Canadian sexual health clinics found that 82.4% (n= 433) of the *C. trachomatis* positive patients were rectal positive, while 30% of *Chlamydia* cases were positive solely at the rectal site [139]. In addition, in this study the authors found that there was no association between patients reporting anal intercourse and having rectal *Chlamydia* infection, suggesting transfer from the genital tract to the rectal site. Indole concentrations in the gastrointestinal tract are high, due to

commensal gut bacteria (such as *E. coli*) that are able to produce indole via their *tnaA* gene [126,140]. One explanation for the existence of the *trpBA* genes in the urogenital strains of *C. trachomatis* could be related to their site/s of infection. While still speculative, it is possible that *Chlamydia* has found a niche residing in the gastrointestinal tract, hiding away from the immune system in a persistent form, while being able to survive using indole provided by the gut microbiota. This might result in a reservoir of chlamydial particles in the gastrointestinal tract/rectal site (particularly in females) that could potentially cross-infect the vaginal site. Whether indole is the key factor which is responsible for the evasion of *C. trachomatis* from the host immune mechanism via IFN-γ, or it is the combined conditions in the female genital tract that allows the *Chlamydia* to take advantage of the fragile equilibrium of bacteria and metabolic products in this disease state, is unknown. A high abundance of lactobacilli will result in lower levels of indole, lower pH, and the ability to efficiently eliminate chlamydial infection via the IFN-γ host response. However, an altered microbiome with less lactobacilli and more non-lactic acid producers, will lead to higher indole concentrations, which subsequently could enable the *Chlamydia* to overcome IFN-γ effect.

Concluding Remarks

C. trachomatis continues to be a major health burden worldwide, with STI infection rates increasing rather than decreasing. Hence, there is a continuing need for improved therapeutics, including an effective vaccine as well as novel treatment options. Perhaps not surprisingly, given that *C. trachomatis* is a successful colonizer of the female genital tract, it has evolved to make use of the host's microbiota at this site to supplement its own capabilities to overcome host immune attack. This seems to be a unique adaptive strategy, but one that is clearly successful for this obligate intracellular pathogen. As we continue to understand how this holobiont interaction functions, it does open up new possibilities for treatment (see Outstanding Questions). Vaccines could potentially target points in this tryptophan rescue pathway (e.g. using the *Chlamydia*-specific TrpBA protein as a vaccine candidate), perhaps in conjunction with traditional membrane protein vaccine target strategies. However, it does also suggest that strategies to manipulate the vaginal microbiota, such as modifying the balance between beneficial lactobacilli and indole-producing anaerobes, might be useful. The use of indole antagonists, applied directly to the vaginal site, in conjunction with manipulation of the microbiota, might also be beneficial. Finally, the question of cross infection of the vaginal site via the gastrointestinal tract (which contains high levels of indole producing bacteria) and the potential for the gastrointestinal tract to be a reservoir for *C. trachomatis* should be considered. [141]

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Table 1. Common Commensal, Symbiotic, and Pathogenic Vaginal Bacteria and Their Association with Health or Disease

Species	Healthy[a]	Dysbiosis	Indole[b]	Role	Species	Healthy	Dysbiosis	Indole	Role
Peptostreptococcus anaerobius	+2	+3	-	Commensal bacteria	***Prevotella ruminicola***	+1	+2	-	Gut bacteria
Propionibacterium acnes	+1	+3	+	Commensal skin bacteria	***Prevotella intermedia***	+2	+4	+	Commensal oral and vaginal bacteria
Escherichia coli	+2	+3	+	Commensal gut bacteria	***Prevotella nigrescens***	+2	+3	+	Commensal oral and vaginal bacteria
Enterococcus faecalis	+2	+3	+	Commensal gut bacteria	***Prevotella buccalis***	+1	+3	-	Oral bacteria
Staphylococcus saprophyticus	+3	+1	-	Commensal vaginal bacteria	***Prevotella bivia***	+1	+5	-	Vaginal pathogen
Klebsiella pneumoniae	+1	+5	-	Pathogen	***Prevotella disiens***	+1	+5	-	Vaginal pathogen
Bacteroides fragilis	+1	+4	-	Commensal gut bacteria	***Prevotella oris***	+2	+2	-	Commensal oral and vaginal bacteria
Bacteroides bivius	+2	+4	-	Vaginal pathogen	***Prevotella oralis***	+2	+2	-	Commensal oral and vaginal bacteria
Bacteroides ureolyticus	+1	+3	-	Vaginal pathogen	***Prevotella timonensis***	+2	+3	-	Pathogen
Mobiluncus curtisii	+1	+4	-	Vaginal pathogen	***Prevotella amnii***	+1	+4	-	Vaginal pathogen
Mobiluncus mulieris	+2	+3	-	Vaginal pathogen	***Prevotella corporis***	+2	+3	-	Oral bacteria
Atopobium vaginae	+1	+4	-	Vaginal pathogen	***Sneathia amnii***	+2	+4	-	Vaginal pathogen
Megasphaera elsdenii	+1	+3	-	Commensal gut bacteria	***Sneathia sanguinegens***	+2	+4	-	Vaginal pathogen
Mycoplasma hominis	+1	+5	-	Vaginal pathogen	***Ureaplasma urealyticum***	+2	+4	-	Vaginal pathogen
Gardnerella vaginalis	+1	+5	-	Vaginal pathogen	***Clostridium sphenoides***	+1	+4	+	Commensal gut bacteria
Peptoniphilus harei	+2	+3	-	Commensal bacteria	***Clostridium fallax***	+1	+4	-	Commensal gut bacteria
Peptoniphilus lacrimalis	+2	+3	-	Commensal bacteria	***Clostridium perfringens***	+1	+4	-	Commensal gut bacteria
Peptoniphilus asaccharolyticus	+2	+3	+	Commensal bacteria	***Clostridium sporogenes***	+1	+3	-	Commensal gut bacteria
Peptoniphilus indolicus	+1	+3	+	Vaginal pathogen	***Clostridium septicum***	+1	+5	-	Commensal gut bacteria
Finegoldia magna	+2	+3	-	Commensal skin bacteria	***Clostridium sordellii***	+1	+5	+	Commensal vagina bacteria
Streptococcus anginosus	+3	+1	-	Commensal bacteria	***Clostridium innocuum***	+1	+3	-	Commensal gut bacteria
Streptococcus intermedius	+3	+1	-	Commensal bacteria	***Parvimonas micra***	+2	+4	-	Oral and vaginal pathogen
Streptococcus mitis	+3	+1	-	Commensal	***Porphyromonas***	+1	+4	+	Vaginal pathogen

				bacteria	***gingivalis***				
Streptococcus acidominimus	+3	+2	-	Commensal bacteria	***Porphyromonas levii***	+2	+3	-	Commensal vaginal bacteria
Streptococcus constellatus	+3	+1	-	Commensal bacteria	***Porphyromonas asaccharolytica***	+3	+3	+	Commensal oral gut and vaginal bacteria
Streptococcus sanguis	+3	+1	-	Commensal bacteria	***Bifidobacterium biavati***	+3	+1	-	Commensal vaginal bacteria
Streptococcus salivarius	+3	+1	-	Commensal bacteria	***Lactobacillus crispatus***	+5	+1	-	Symbiotic vaginal bacteria
Streptococcus agalactiae	+3	+1	-	Commensal bacteria	***Lactobacillus iners***	+4	+1	-	Symbiotic vaginal bacteria
Streptococcus morbillorum	+3	+2	-	Commensal bacteria	***Lactobacillus gasseri***	+4	+1	-	Symbiotic vaginal bacteria
Streptococcus uberis	+3	+2	-	Commensal bacteria	***Lactobacillus jensenii***	+5	+1	-	Symbiotic vaginal bacteria
Aerococcus christensenii	+2	+3	-	Vaginal bacteria	***Lactobacillus acidophilus***	+4	+1	-	Symbiotic gut, oral and vaginal bacteria
Anaerococcus octavius	+1	+3	-	Commensal oral and skin bacteria	***Lactobacillus rhamnosus***	+4	+1	-	Symbiotic urogenital bacteria
Anaerococcus tetradius	+1	+3	-	Vaginal pathogen	***Lactobacillus fermentum***	+4	+1	-	Symbiotic oral, gut and vaginal bacteria
Anaerococcus prevotii	+1	+3	-	Commensal oral and skin bacteria	***Lactobacillus plantarum***	+4	+1	-	Symbiotic oral, gut and vaginal bacteria
Anaerococcus vaginalis	+1	+3	-	Vaginal pathogen	***Lactobacillus brevis***	+4	+1	-	Symbiotic gut and vaginal bacteria
Leptotrichia amnionii	+1	+4	-	Vaginal pathogen	***Lactobacillus casei***	+4	+1	-	Symbiotic gut oral and vaginal bacteria
Fusobacterium nucleatum	+1	+3	+	Commensal gut bacteria	***Lactobacillus vaginalis***	+4	+1	-	Symbiotic vaginal bacteria
Treponema pallidum	+1	+5	-	Vaginal pathogen	***Lactobacillus delbrueckii***	+4	+1	-	Symbiotic vaginal bacteria
Chlamydia trachomatis	+1	+5	-	Vaginal pathogen	***Lactobacillus salivarius***	+4	+1	-	Symbiotic gut and vaginal bacteria
Neisseria gonorrhoeae	+1	+5	-	Vaginal pathogen	***Lactobacillus reuteri***	+4	+1	-	Symbiotic oral gut and vaginal bacteria

[a]Whether a species contributes to health or dysbiosis is indicated by a scale from +1 to +5. At the lower end of the scale, +1 indicates that this species is not associated to health or dysbiosis at all, while +5 means the species is highly associated with health or dysbiosis.

[b]Whether a species is capable of producing indole is indicate by '+', those that are not are shown by '-'.