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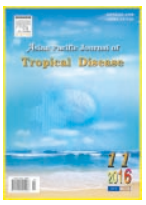

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Role of microbial flora in female genital tract: A comprehensive review

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ABSTRACT

The female genital tract is a complex of microbial colonization, which shows a prominent role in the development of either a healthy or diseased condition. The aim of the present review is to describe the diverse components of both the protective and defective mechanisms induced by microbial species present in the female genital tract. The protective mechanism was induced by indigenous microbial flora colonized in the female genital tract, which includes innate immunity, secretions containing cytokines, antimicrobial peptides and inhibitory substances like organic acids, H_2O_2, bacteriosin and toll-like receptors. On the other hand, abnormal microorganisms produce virulence factors and enzymes, which cause life-threatening infectious diseases including cancer. The review summarizes that depending upon the presence and/or absence of normal and abnormal microorganisms, the female genital tract shows either a healthy and/or infectious condition.

1. Introduction

A diverse group of microorganisms is associated with different parts of the human body from birth to death. The human body routinely harbours about 1 014 bacteria which are collectively called normal or indigenous microbial species. They are widely distributed in various parts of the body, including eyes, skin, nails, oropharynx, gastrointestinal and genital tracts. These microbial florae are stable during normal conditions and do not cause any harmful effects to the human body[1]. Anatomically, the female genital tract is a very favourable atmosphere for the occurrence of a number of microorganisms. Most of these normal microorganisms play a significant role in the defense mechanism for maintaining the healthy environment to that particular part or organ especially in female genital tract[2].

In 1892, Albert Doderlein first reported that the vaginal microbial flora was colonized with Gram-positive rods, popularly known as "Doderlein's bacilli". These bacteria are popularly known as *Lactobacillus* species and are predominant species of the genital microflora along with some aerobic and anaerobic bacterial species[3]. It is well established that lactobacilli are one of the defense mechanism against pathogenic organisms. Although several means of protection have been suggested, their mechanism of interaction is not fully understood. The improvement in the number and metabolic activity of lactobacilli plays an essential role in the prevention and treatment of different infectious diseases along with already available therapeutic interventions[4].

Historical data indicate that the majority (70%) of genital tract infections (GTIs) are caused by the abnormal microbial population[5]. Furthermore, abnormal microbial flora of the vagina and cervix causes various infectious diseases, such as bacterial vaginosis (BV) and sexually transmitted viral infections (human simplex virus and human papilloma virus), which can lead from moderate to severe infectious conditions, and sometimes they may also cause death[6]. The major cause of GTIs in females is bacterial infections, formerly known as nonspecific vaginitis which is defined

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as an infection of the female genital tract, characterized by the presence of an amine (putrescine and cadaverine) and clue cells with increased pH[7,8].

The complex mixture of both normal and abnormal microbial flora of the female genital tract shows their activity in either a protective or defective manner, respectively, as shown in Figure 1. Therefore, the present review was focused on how these normal and abnormal microbial florae and their products act as a boon and curse to female reproductive health management.

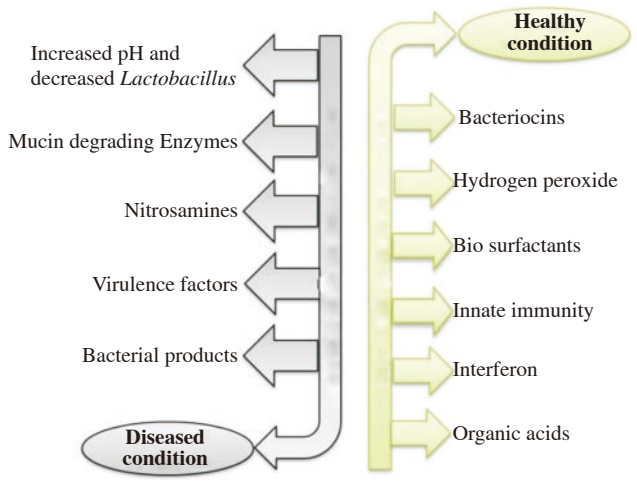

Figure 1. Protective and defective roles of normal and abnormal microbial flora of the female genital tract.

2. Normal flora of the female reproductive system

The female reproductive system (ovaries, fallopian tubes, uterus, cervix, vagina and vulva and cervix of the uterus) is normally colonised by a complex mixture of indigenous microbial flora. Some well-established experimental and clinical studies have been devoted to elucidating the microbiota of the vagina and to a lesser extent, the cervix and vulva[9].

Several weeks after birth, the vagina of a new-born girl remains under the influence of predominantly normal flora of *lactobacillus*. Due to the influence of maternal hormones, the pH of the infant vagina increases to neutrality and remains neutral until puberty. In childhood, the normal flora of the genital tract consists of a variety of cocci and rod-shaped bacteria and is highly susceptible to a variety of bacterial pathogens [*Streptococcus pyogenes* and *Neisseria gonorrhoeae* (*N. gonorrhoeae*)]. During puberty, lactobacilli again become prominent, although a smaller number of yeasts and other bacterial species are also present and are more resistant to infection. After menopause, the vaginal tract returns to a neutral pH and consists of mixed flora, again susceptible to infection[10].

2.1. Indigenous microbial flora of female genital tract – Lactobacillus

The prevalence of indigenous microbiota of the female genital tract consists of *Lactobacillus* spp., especially *Lactobacillus crispatus*, *Lactobacillus jensenii* and *Lactobacillus iners*[11,12]. The symbiotic association between vaginal lactobacilli and its host is regulated by the circulating hormones in a woman's body, which stimulates the vaginal epithelial cell to produce glycogen[13]. The secreted glycogen is converted to lactic acid by *Lactobacillus* resulting in an enhanced acidic pH (< 4.5) that inhibits the growth of many potential pathogens. The acidic environment of a healthy vagina does not generally permit the growth of many potential pathogens[14,15]. Thus, the occurrence of normal flora (*Lactobacillus*) is resistant to the female genital tract infection that is often associated with a women's health status. The common species found in the vagina of healthy females are *Lactobacillus iners*, *Lactobacillus crispatus*, *Lactobacillus gasseri* and *Lactobacillus jensenii*[16], and approximately 10^8 lactobacilli cells can be found per milliliter of vaginal fluid[17].

In addition to lactobacilli, some yeast cells, *Mycoplasma* and *Ureaplasma* species are colonized up to 20%[18,19]. The bacterial flora in the vagina is regulated by the normal flora, and is referred to as bacterial interference, the condition when the indigenous microbial flora competes exogenous flora. Bacterial interference is accomplished through various mechanisms, such as synthesis of antimicrobial substances, hydrogen peroxide and bacteriocin-like substances, which compete for both nutrition and binding sites rather than the exogenous microorganisms[14,20].

2.2 Protective role of Lactobacillus

In addition to the impact of the indigenous microbiota, some mechanisms are assumed to compete the growth of pathogenic microbes, but their interaction was not well established[21]. A large body of evidence stated that the *Lactobacillus* is one of the primary protective mechanism to maintain the indigenous microbiota[22]. The indigenous microbiota of *Lactobacillus* forms a massive growth of biofilm, which tightly attaches to the surface of the vaginal epithelia, creating the first line of defence against potential pathogens as shown in Figure 2 under the normal microbial flora[13], whereas abnormal microbial flora produces mucin degrading enzymes which facilitates the entry of pathogens and finally cause infections including cancers. Stratified squamous epithelium of the vaginal wall has moisture due to vaginal fluid secreted by cervical and vestibular glands through the vaginal wall[23]. The vaginal fluid contains significant concentrations of organic acids, peroxides and polypeptides which are sufficient to be considered as antibacterial activity against pathogenic microbes[4]. Figure 3 shows that the protective role of *Lactobacillus* through the production of inhibitory substances block the adhesion sites and degradation of toxin receptors[24].

2.2.1. Production of inhibitory substances

Lactobacilli produce a variety of substances that inhibit the growth of both Gram-positive and Gram-negative bacteria. The inhibitory substances include bacteriocins, hydrogen peroxide and organic acids[25]. These compounds may reduce not only the number of viable bacterial cells, but may also affect the bacterial metabolism and its toxin production. *Lactobacillus* spp. produced

antimicrobial peptides called bacteriocins, which are important defence mechanisms against competitive microorganisms[26]. The antimicrobial activity of *Lactobacillus* spp. is due to the reduced transmembrane potential ($\Delta\psi$) as well as pH gradient is produced by host cell immunity[27]. Bacteriocins do not have haematolytic or cytotoxic activity and do not induce vaginal irritation. They are suitable for human use. Recently, Dover *et al* reported that the antimicrobials derived from vaginal probiotics were non-toxic to both animal and human vaginal tissue[4].

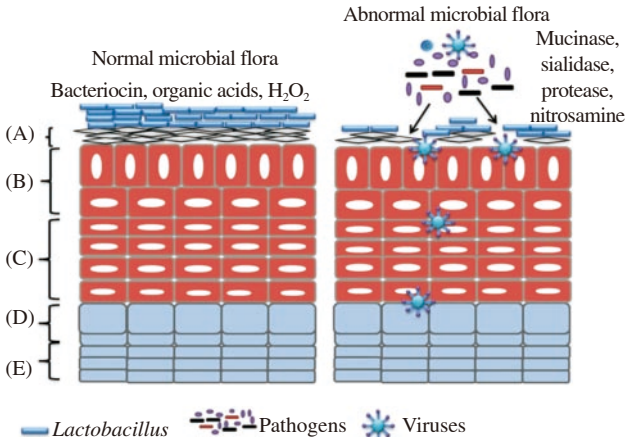

Figure 2. Role of normal and abnormal microbial flora in female genital tract.
A: Mucus layer; B: Superficial zone; C: Mid zone; D: Basal layer; E: Basement membrane with dermis.

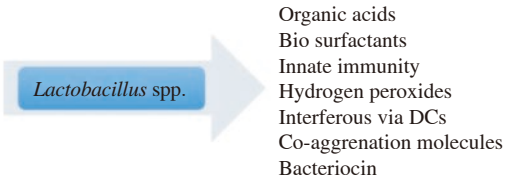

Figure 3. Protective role of *Lactobacillus* by the production inhibitory substances, interferons, hydrogen peroxide, bacteriocine, organic acids, *etc*.

Until today, nisin is the only one commercially available bacteriocin from *Lactobacillus*, and it was approved by the Food and Drug Administration, and generally recognized as safe[28]. Nisin also acts as a contraceptive agent due to its spermicidal activity[29]. Lactocin 160 is another bacteriocin produced by *Lactobacillus rhamnosus* 160 and potentially inhibits the growth of *Gardnerella vaginalis* (*G. vaginalis*) by destroying the cell membrane and generating the adenosine triphosphate efflux[14]. *Lactococcus lactis* subsp. lactis HV219 produces bacteriocin HV219 which potentially prevents the growth of both Gram-positive and Gram-negative bacteria[30]. Bacteriocin-like substances, another class of antimicrobial compounds, are produced by *Lactobacillus salivarius* and *Lactobacillus pentosus* TV35b which produces the bacteriocin-like substances and inhibits the growth of *G. vaginalis*[14].

Production of hydrogen peroxide is another important mechanism for antimicrobial activity of many *Lactobacillus* species. H_2O_2 is an oxidizing agent, toxic to most of the anaerobic microorganisms[31]. Dover *et al.* reported that 75% of healthy women (without any bacterial vaginosis) were colonised with hydrogen peroxide producing lactobacilli[4]. On the other hand, only 14% of the women with BV had H_2O_2-producing lactobacilli. In another study, polypeptides secreted from vaginal fluid of healthy women showed antimicrobial activity against gynaecological pathogens[23]. Lysozyme and calprotectin were antimicrobial polypeptides identified in vaginal secretions at very low concentrations. However, the efficiency of these polypeptides may be improved by synergistic action in which the polypeptides exhibited enhanced antimicrobial activity when they combined with lactoferrin and a leukoprotease inhibitor[32]. Lactic acid is one of the end product produced by glycogen metabolism of *Lactobacillus* spp., which maintains the acidic vaginal pH (3.8–4.5). The protonated form of lactic acid entering into the cytoplasm and decreasing the intracellular pH hampers vigorous cell functions and inhibits the growth of pathogenic bacteria[33]. *Lactobacillus* spp. also produce other inhibitory substances forming a mucus membrane-like structure around the vaginal cells and act as a barrier to reduce the attachment and entry of pathogens in the vaginal environment[34].

2.2.2. Blocking of adhesion sites

Competitive inhibition for bacterial adhesion sites on genital/intestinal epithelial surfaces is another mechanism of action for *Lactobacillus*[35]. Lactobacilli display a wide range of adhesions, which enables them to adhere to the surface of vaginal epithelium. These include lipoteichoic acids, as well as ill-defined proteinaceous and non-proteinaceous adhesions, some of which are located on fimbriae. *Lactobacillus acidophilus* and *Lactobacillus gasseri* appear to utilize glycoproteins as adhesions when binding to vaginal epithelial cells, the receptors being glycolipids[36,37].

2.3. Genital tract – innate immunity

The indigenous microbial flora of the genital tract also involves in prevention of microbial infections through innate and acquired immune. The innate immune system recognizes the pathogen-associated molecular patterns on microbial invaders rather than specific antigens like peptidoglycan (TLR2 ligand) present on Gram-positive pathogenic bacteria. The recognition of a pathogen-associated molecular pattern by an innate immune system triggers a sequence of events leading to the generation of pro-inflammatory cytokines and activation of the acquired immune system (T and B lymphocytes)[10,38]. Vaginal secretions have some soluble factors with innate immunity such as defensins, complement components, toll-like receptors (TLRs), mannose-binding lectins, secretory leukocyte protease inhibitor, nitric oxide and phagocytic cells[39]. Vaginal epithelial cells play a crucial role in the development of microbial infection by host-microbe interaction. Epithelial cells are having TLRs which recognize the pathogenic microbial components and triggers the development of antigen-specific acquired immunity by various signal transduction pathways[40]. Approximately, 11 types of TLRs have been identified so far, each of which has its own specificity[39]. Different types of TLRs and their modes of action were elucidated in Table 1 and Figure 4.

Table 1
Different types of TLRs and their function in immune system of the female reproductive system.

Type of TLRs	Occurrence	Mode of action
TLR1 and TLR2	Uterus, endometrium, cervix	Lipoprotein and peptidoglycan of Gram-positive bacteria
TLR3	Fallopian tube, uterus	Double-strand DNA of many viruses
TLR4	Endometrium stromal cells	Lipopolysaccharides of Gram-negative bacteria
TLR5	Fallopian tube, uterus	Flagellin of bacterial flagella
TLR6	Epithelial cells of lower genital tract	Diacyllipopeptides of *Mycoplasma,* lipoteichoic acid of Gram-positive bacteria, zymosan of fungi
TLR7 and TLR8	Vagina, cervix	Single-stranded RNA of viruses
TLR9	Vagina, cervix	Unmethylated CpG DNA sequences
TLR11	---	Uropathogenic bacteria

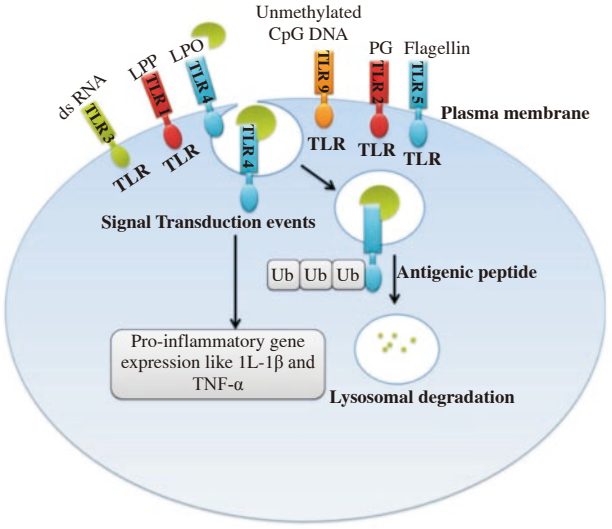

Figure 4. Mechanism of TLRs, important components of the vaginal innate immunity.
LPP: Lipo peptide; LPO; Lipopolysaccharides; PG: Peptidoglycan; ds RNA: Double standard RNA; Ub: Ubiquitin; IL: Interluekins; TNF: Tumor necrosis factors.

It is well established that the presence of TLR 1–3, 6, 7 and 11 exist in uterine natural killer cells[41]. Other reports demonstrated that the existence of TLR5 in smooth muscle and vascular endothelial cells indicates the role of TLRs in preventing the lower genital tract infections from the adverse pathological conditions[42,43]. Defensins are a class of non-specific antimicrobial peptides produced by the vaginal epithelial cells. Structurally, defensins have positively charged molecules, specifically binding to the surface of the bacteria which in turn leads to the disruption cell membranes and finally bacterial cell lysis[44,45]. Secretary leukocyte protease inhibitor, a potent protease inhibitor with broad spectrum antibacterial activity, has been shown to prevents the perinatal HIV-1 transmission from mother-to-child[46]. Mannose binding lectin is an antibacterial protein secreted from vaginal secretions, which recognizes mannose, fucose and N-acetyl glucosamine and carbohydrate moieties present on the surface of the bacteria. Binding induces the activation and deposition of complement cascade leading to either phagocytosis or direct cell lysis[47]. This was also evident by the women deficient with mannose-binding lectin production shown to be more prone to developing recurrent vulvovaginal candidiasis[48].

Heat shock proteins or stress proteins are a specific class of conserved proteins, which aid cell survival under adverse environmental conditions like extreme temperature and toxic chemicals, microbial pathogens or pathogenesis induced inflammation. Recently, one of the inducible heat shock protein 70 KDa (hsp-70) was recognised as an antimicrobial protein secreted from the vagina[49]. The synthesis of hsp-70 is greatly enhanced in response to microbial infection. The binding of extracellular hsp-70 with TLRs enhances the immune response against microbial pathogens[50]. In response to abnormal microbial flora, extracellular hsp-70 induces the production of nitric oxide in the vagina, which exhibits effective antimicrobial activity against a wide range of microorganisms[51]. Antibodies specifically recognized the antigenic epitopes on the surface of the microorganisms through opsonization could enter the vagina by circulation system. The vaginal and endocervical mucosal immune system also induces antibody (immunoglobulin G and immunoglobulin A) producing B lymphocytes[52], which provide a significant mechanism against infectious microbial flora without involvement of their systemic humoral immune response. Hence, the vaginal immune system has broad range and may differ from those in circulating antibodies[53].

3. Abnormal microbial flora of the female genital tract

The microbial population of the vaginal ecosystem is not static, which always changes according the influence of the endogenous and exogenous factors[54]. Stage of the menstrual cycle, frequency of sexual intercourse, specific sexual partners, pregnancy, use of contraceptive agents, vaginal douching and utilization of antibiotics or other medications with immune or endocrine activities are the effective variables for changing the vaginal ecosystem. These variables lead to the altered milieu, cause fluctuations in the native vaginal ecosystem and regulate the vaginal microbes. The best example under this category is the damage of the primary protective layer of the vagina (lactobacilli) when it has been undergoing with use of antibodies for non-vaginal infections and abnormal sexual intercourse[55]. Abnormal flora may also occur because of sexually transmitted infections, such as *Trichomonas vaginalis* (Tv), *Haemophilus influenzae* and *Streptococcus pneumoniae* or by massive growth or increased virulence of an organism that is a constituent part of normal vaginal flora, *e.g. Escherichia coli*. Changes in vaginal flora do not necessarily imply diseases or result in symptoms. Diseases will develop from the interplay between microbial virulence and the immune response of the host system.

The *Candida albicans* (*C. albicans*) is one of the acid tolerant yeast cells present in the vagina of women of reproductive age. Typically, *C. albicans* species are asymptomatic when their concentration is less. However, under specific conditions, such as local immunosuppressive events, *C. albicans* can multiply and convert into a more invasive hyphal form known as candidiasis[56]. Vulvovaginal

candidiasis is the most common cause of vaginitis in the female genital tract, caused by *Candida* species, especially *C. albicans* (89%)[57]. *Candida* may be isolated from vaginal secretions in one-fourth of asymptomatic women, which are known as endogenous infections[58]. *Candida* vaginitis has been associated with antibiotic therapy, use of hormonal contraceptives, estrogen therapy, diabetes and chronic stress[59].

Due to the alterations in the ecosystem of the female genital tract, most of the indigenous microbial flora is replaced with abnormal flora, which leads to the development of diseased conditions like cervicitis, urethritis [*N. gonorrhoeae, Chlamydia trachomatis* (CT)*, Mycoplasma hominis (M. hominis), Mycoplasma genitalium*] vaginitis [Tv, *Gardnerella, Mobiluncus* and *Candida* species], herpes disease (herpes simplex virus type 1 and 2) candidiasis and death-causing diseases like cervical cancers (human papilloma virus-16, 18)[60,61].

3.1. Pathogenesis and clinical manifestation of abnormal microbial infections

Typically, there are two types of microbial infections in the female genital tract. One is sexually transmitted diseases (STDs), and the second one is non-sexually transmitted diseases. Both types are the most common infectious diseases and the rates are increasing worldwide.

3.1.1. STDs

STDs have been labeled as a covert epidemic among adolescents who are experiencing the highest prevalence of STDs in developing countries. Most of the STDs, regardless of pathogens, are asymptomatic, especially in adolescents. Sexually transmitted GTIs are urethritis, cervicitis, vaginitis and vaginosis, genital ulcers and lesions. Long-term complications of pelvic inflammatory diseases (PID) consist of infertility, chronic pelvic pain and ectopic pregnancy[62].

CT and *N. gonorrhoeae* are the sexually transmitted genital pathogens reported among adolescent girls. Most of the multisite, randomized chlamydial (62%) and gonorrheal infections (51%) were asymptomatic[63]. The main reason for more prevalence of these infections is that sexually active adolescents are unaware of their risk for these infections. Although often asymptomatic, chlamydial and gonorrheal infections are present in various STD syndromes, and depending on the site of infection may develop cervicitis and vaginitis, and may also ascend into the pelvis, causing PID. It is well established that aerobic and anaerobic organisms, in addition to CT and, *N. gonorrhoeae,* are implicated in PID. It has been hypothesized that the ascending spread of these sexually transmitted organisms facilitates access of normal vaginal flora into the upper genital tract affecting PID[64].

Genital herpes was characterized by grouped vesicles mixed with small ulcers caused by members of Herpesviridae family type 1 and 2 and herpes simplex virus (HSV-1 and 2). Transmission may also occur through skin contact during periods of asymptomatic shedding[65]. Genital warts are another type of sexually transmitted disorders caused by human papillomaviruses (HPV). More than100 types of HPV are identified, of which high risk-HPV (HPV 16, 18) causes cervical cancer and low risk HPV (6, 11) causes genital warts[66]. A wart is a small, rough, solid blister existing on the surface of the skin on genital organs and spread through skin to skin contact. Genital warts are diagnosed visually are confirmed by biopsy[67]. Globally, cervical cancer is the most prevalent gynaecological malignancy caused by high-risk HPV (16 and 18)[66]. It has been well established that some microbial agents functions as a risk factors and are directly or indirectly interacting with HPV in the development of cancerous lesions of the uterine cervix through the production of carcinogenic metabolites or by increasing the susceptibility of the inflamed epithelium[34].

The development of cervical lesions is also associated with flagellated protozoan known as Tv since 1950s. It is one of the STDs caused by an anaerobic, unicellular, flagellated protozoan, which is mostly associated with increased vaginal discharge (yellowish-green), frothy and malodorous, rarely extending beyond the vagina[68]. Tv infection (trichomoniasis) may act as a risk factor for the transmission of HIV, cervical neoplasia, tubal infertility and post-hysterectomy infection[69]. Conventional diagnosis of trichomoniasis is typically made on the basis of vaginal wet-mount examination. Nucleic acid amplification tests are the most sensitive diagnostic tests available for detection of Tv[70]. There is a controversial issue regarding the infectious role of CT in the progression of cervical lesions. However, strong epidemiological data suggests that CT acts as a potential cofactor in the development of cervical intraepithelial neoplasia along with other factors, like smoking and sexual promiscuity[71].

Syphilis is another sexually transmitted infection more commonly seen in immunocompromised hosts, caused by the spirochete (*Treponema pallidum*). Primarily it is transmitted through sexual contact and may also be transmitted from mother to fetus during pregnancy or at birth, resulting in the development of congenital syphilis. Syphilis develops different stages (4 stages) and symptoms vary with each stage[72]. The major symptoms of syphilis may be characterised with painless, single, firm, non-itchy chancre, followed by gummas, neurological or cardiac problems[73]. A non-treponemal rapid plasma reagin test and a venereal disease research laboratory test are the two important sensitive tests for the diagnosis of syphilis. If either of these tests is positive, a confirmation must be followed by either a fluorescent treponemal antibody absorption test or a microhemagglutinin-*Treponema pallidium* test[74].

3.1.2 BV

BV is one of the genital infections and is characterized by alteration or disturbance of indigenous microbial flora of the vagina, in which it reduces the predominant flora *Lactobacillus* and increases the abnormal anaerobic milieu. Of these infections, BV is the most prevalent but the least well understood with an asymptomatic condition[75]. Epidemiological studies suggest that risk factors for BV include multiple sexual partners and a history of

sexually transmitted infections. Vaginal douching or other washing practices are also associated with BV, although there are no exact relationships with this association[76].

Women with BV are characterized by modifications in indigenous microbiota, especially by declining the lactobacilli and a larger number of anaerobic and facultative anaerobic bacteria[77]. Previously, it was reported that only 25% of women maintain *lactobacillus* as their predominant vaginal flora throughout the menstrual cycle[78]. The most common outward symptoms of BV include a thin, homogeneous, milky white vaginal discharge, a foul or fishy odor that may intensify following sexual intercourse, and sometimes vaginal itching during sexual intercourse. However, as many as 50% of all women with BV are asymptomatic in most of the conditions[79].

The diagnosis of the BV has been complicated due to its complex polymicrobial population and primarily it involves the laboratory culturing of microbial population. Laboratory culture of *Gardnerella* species in vaginal secretions is not a valid diagnostic test for BV, because lower concentrations of *Gardnerella* species may be present in many women without BV[80]. The Amsel practice describes BV by the presence of three of four factors which include the presence of a homogenous vaginal discharge, increased vaginal pH (> 4.5), a positive "whiff" test result (vaginal discharges are mixed with 10% KOH), and the formation of clue cells[81]. Gram staining of the vaginal secretions is also one of the diagnostic method based on standardized interpretive microbial score[82]. Several molecular approaches have been used to study the vaginal bacterial flora. Amplification of the bacterial 16s rRNA gene or other phylogenetically informative genes are most commonly employed to identify the bacterial communities associated with BV[83]. Gillet *et al.* reported that there was a significant association between the presence of clue cells on Papanicolaou smears and cervical intraepithelial neoplasia. They also speculated that BV enhances the rate of cervical intraepithelial neoplasia, possibly by the production of some carcinogenic compounds such as nitrosamines[84].

There is a substantial association between abnormal vaginal flora of clinically diagnosed patients with BV and HIV seropositivity[85,86]. Although this association is independent of risk factors such as age, number of sexual partners, use of condom and contraceptive method. Finally, they concluded that the women with BV have more prone to heterosexual transmission of HIV. One of the prospective study suggests that there is an increased risk of HIV seroconversion in women diagnosed with BV in the absence of vaginal *Lactobacillus*[82]. In addition to this, in the absence of vaginal *Lactobacillus*, the cervical vaginal lavage fluid enhances the HIV activity in women with BV majorly associated with *M. hominis* and *G. vaginalis*[86-88]. The above epidemiological study suggests that the treatment of BV and vaginal colonization with *Lactobacillus* should be evaluated as potential interventions to reduce the risk of STDs including HIV[86].

The cervical mucus is one of the protective barrier to the vaginal and cervical epithelial cells and likely to prevent the acquisition of herpes, especially genital herpes. Olmsted *et al.* demonstrated that cervical mucus has the ability to trap HSV in its viscous gel *in vitro*[89]. However, the majority of BV associated abnormal microbial flora are known to produce increased levels of mucin-degrading enzymes (mucinase, sialidases)[90,34] as shown in Figure 2. Hence, it is possible that increased degradation of a mucus layer in women with BV may facilitate the entry of HSV-2 to epithelial cells[91]. The synergy between abnormal vaginal flora and HSV-2 infection has been incrementally exposed. Previous cross-sectional studies reported that there is a significant association between BV and HSV-2[92], while longitudinal studies suggest a linkage between antecedent HSV-2 infection and BV incident[93]. Recently, Stoner *et al.* reported a BV recalcitrance among HSV-2 infected women[65]. Cherpes *et al.* also reported an increased incidence of HSV-2 in women with BV when compared to those without BV or normal vaginal flora[91].

3.2. Microbial infections associated with gynaecological cancer

Female gynaecological cancers are the most common and fatal cancers worldwide, which including ovarian, cervical and endometrial carcinomas. Epidemiological studies suggest that several factors could be involved in the development of gynaecological cancers, including lifestyle, genetic predisposition and infectious agents like bacteria and viruses[94].

Microorganisms involved in STDs have been associated with a higher risk for gynaecological cancers especially ovarian cancer[95]. It is important to note that the cells infected with chlamydia have more prone to convert neoplastic transformation because the pathogen causes persistent inflammation leading to tissue damage and has an anti-apoptotic activity on infected cells[96]. Baczynska *et al*. established that *Mycoplasma* species (*M. hominis* and *Mycoplasma genitalium*) cause inflamed tubal epithelial cilia through production of metabolic products like hydrogen peroxide and superoxide radicals[97]. This appears to be reliable since recent studies suggest that the most aggressive ovarian carcinoma can be initiated from the fallopian tube epithelium[98,99].

4. Conclusions

In light of the above evidences, the female genital tract consists of a complex system of either immune protection or infectious agents, depending on the normal and abnormal microbial flora, respectively. The protective role of indigenous lactobacilli is greatly influenced by the other bacterial species within the microflora of the genital tract.

Conflict of interest statement

We declare that we have no conflict of interest.

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