CHAPTER 1

Introduction

Chapter 1: Introduction

1.1. Female Reproductive System

The female reproductive tract (FRT) in humans comprise of the uterus, ovaries, fallopian tubes, cervix, and vagina (Fig 1.1). At the fourth week of gestation, the pelvic region of an embryo has a wolffian duct, a mullerian duct, the metanephros, cloacae, and gonadal ridge. Post 5th week of gestation, the primordial germ cells migrate from the yolk sac's endodermal lining through the hindgut's dorsal mesentery to the gonadal ridge. In the absence of testosterone hormone, the wolffian duct degenerates; whereas the mullerian duct forms in the absence of anti-mullerian hormones [1, 2]. The health status of the FRT influences intermittent physiological processes like fertilization, implantation, foetal growth, and parturition. Additionally, the health of the tract also has an impact on sexual health and other physiological processes like menstruation and regular mucus outflow [3].

The mullerian duct eventually forms the uterus, fallopian tubes, ovaries, and two-third of the upper-vaginal canal (VC) [4]. The remaining one-third of the VC is formed from the cloaca. The cloaca is separated by the urorectal septum to form the urogenital sinus. On the twelfth week of gestation, the mullerian duct tip invades the urogenital sinus forming the vaginal plate and urethra [5]. The primordial follicular cells in the female foetus release oestrogen from granulosa cells that stimulate the formation of the clitoris, labia, mons pubis, and vaginal opening till the 36^{th} week of gestation. The genital tubercule forms the clitoris and vestibular bulbs, the urogenital sinus forms the Skene's and Bartholin's gland and urethra. The urogenital fold and the labioscrotal fold form the labia major and minor [6]. The ectoderm cell layer forms the mons pubis, clitoris, and labia, which has sweat glands, sebaceous glands, hair follicles, and keratinized squamous epithelial cells. The endodermal layer forms the mucosa of vulva vestibule and the mesodermal layer forms the vagina having non-keratinized squamous epithelial cells [7]. The VC with the vaginal opening forms a tubular organ which is the opening of the FRT (3-5″) that leads to the cervix and connects to the uterus. This portion of the tract is open to the exterior environment and is constantly affected by external factors and the monthly menstrual cycle [8]. The VC in general maintains a temperature of 37 ℃ (body

temperature) and has an acidic pH (3.8-4.2). The acidity in the VC is particularly unusual considering the neutral pH maintained in the rest of the FRT [9].

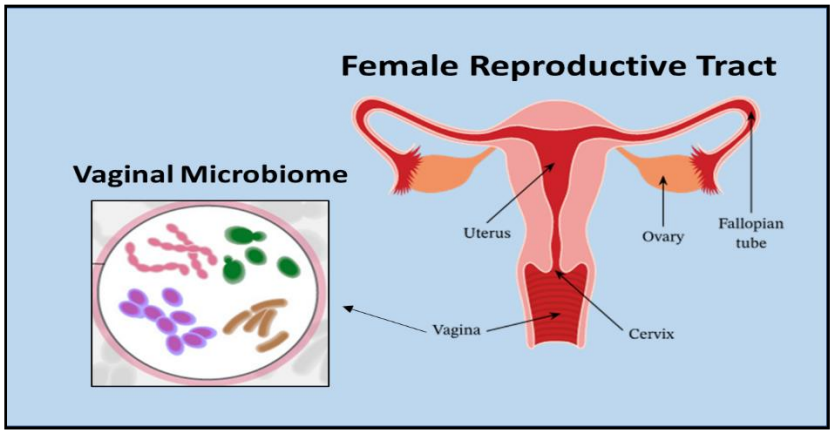

Fig1.1.: The female reproductive tract

1.2. Menstrual Cycle

On approaching puberty (10-16 years), the hypothalamus releases gonadotrophin hormones in a pulsating manner that stimulates the anterior pituitary gland to release the follicle-stimulating hormone (FSH) and the luteinizing hormone. The LH hormone stimulates the theca cells in ovarian follicles to produce progesterone and androstenedione. The androstenedione on reaching granulosa cells is converted to estrogen/estradiol by the aromatase enzyme [10, 11]. The first physiological change in the female body occurs during this stage also known as thelarche. During this stage, breast development is seen due high production of progesterone and estrogen. Post six months the pubarche stage initiates, during this phase the pubic hair in women becomes dense, coarse, and dark in colour. The uterine environment during this phase goes through a constant progression and regression in cellular structure, depending upon the level of estrogen hormone in blood plasma. The hormonal ascend changes the shape of the uterus from tear-drop to pear-shaped. Menarche is the first menstrual cycle that occurs 1.5-3 years post thelarche. The high estrogen in plasma poses negative feedback on the gonadotropin hormones release suppressing their production. This eventually causes regression of estrogen hormone rupturing the uterine walls, thus initiating the first menstrual cycle. However, the progesterone level in plasma remains low till the first ovulation has occurred which is approximately 6-9 months post menarche [12].

Menstrual cycle is a monthly cyclic cycle of 28-30 days, during which the female body prepares for fertilization leading to pregnancy [13]. The cycle is distinctively divided into three phases. The follicular phase is the first phase which differs in length from women to women. The FSH receptors on the immature follicles activate increasing estrogen production. During this phase, the uterine cavity prepares for fertilization with more layers of endometrium cells, enlarged uterine glands, and spiral arteries spreading through the endometrium. The hormonal balance also changes the elasticity of the cervical fluid to a watery consistency making the environment more friendly for sperm delivery. The primordial follicle matures to a graafian follicle/mature follicle, which then releases the oocyte during the second stage called ovulation. The ovulation period ranges from 5-7 days during which fertilization is supported. Post ovulation the secretory phase of 12 days initiates. Progesterone is excessively released by the corpus luteum or the dead follicle cells during ovulation with the production of estrogen as well. Progesterone causes swelling of the endometrium for implantation of the possibly fertilized ovum and relaxes the uterine muscles as well. The consistency of cervical mucus thickens during this assuming no role in sperm delivery. At the later stage of secretory phase, negative feedback from progesterone and estrogen drops the production of FSH and LH hormones. This sudden drop causes the blood vessels in the uterine cavity to constrict and eventually rupture initiating menstrual bleeding [13, 14]. Generally, the menstrual blood flow occurs for 3-5 days with ~30ml of blood expelled from the VC. However, heavy menstrual flow of more than 80ml is considered abnormal. Women with cycles of less than 21 days are polymenorrheic, whereas women with cycles of more than 35 days are oligomenorrheic [15].

From seven million primordial follicles during gestation the follicle number reduces to two-to-three million during birth. During the reproductive age, approximately 400 follicles mature on a monthly basis, leaving the rest follicles to become inactive or functionless [16]. Fertility or the ability to conceive declines with age although the regular menstrual cycle in women continues for a longer period. This occurs due to the failure of the neuroendocrine system to maintain the hypothalamus-pituitary-ovarian endocrine axis resulting in inefficient reproductive ability. Perimenopause is the age prior to menopause when the ovarian follicles deplete in number with degraded genetic constitution. Variability is also noticed in ovulation pattern, menstrual cycle length (short or long), and hyper or hypo estrogenism during this period. Eventually, at the of 45-55

years, the follicles deplete with no estrogen and progesterone production. A high level of FSH and LH hormone levels is observed with no negative feedback from the gonadotrophin hormones post menopause [17-19]. The absence of menses for consecutive 12 months indicates menopause in a woman. Hot flash: high heat with heavy perspiration; Urinary incontinence; Vaginal atrophy: dryness, irritation, itching, and dyspareunia; Decreasing libido, and Mental dejection are varied signs and symptoms of menopause [20].

1.3. Physio-biochemical Changes in the Vaginal Canal

The vulval tissue at puberty becomes more prominent and thickens with an increase of pubic hair. The vaginal stratified squamous epithelium layer also thickens post puberty. The VC has three layers of stratified squamous epithelial cells divided into- basal, super-basal, and corneum layer resting on the lamina propria of connective tissue [21]. The basal layer is mitotically active and divides to form new layers of epithelium cells. The super-basal layer connects the basal and corneum cells. Cells of the corneum layer have loose integrity due to lack of cadherin, they show permeability to micro and macro-nutrients as well as moisture unlike basal and super-basal layers [22]. These cells store glycogen and remain thick during puberty aiding the growth of microbial flora in the VC [23, 24]. The layer has glycolipid envelopes to which microbes adhere and proliferate. The superficial layer consists of dead cells that have no nucleus or organelles. Epithelial cells have specific receptors for exogenous microorganisms, on being encountered they release cytokines and chemokines which act as the first layer in physical and immunological protection. These cells allow the beneficial bacteria to adhere to these receptors discouraging the attachment of pathogenic microbes [25, 26].

The cervical vaginal fluid (CVF) is a semi-transparent complex fluid with 95% water and 5% solid matter containing proteins, carbohydrates, lipids, nucleic acids of dead epithelial cells; mucins; and microbial flora [27]. Healthy discharge is loaded with antibodies like IgG, IgM, IgA; lysozyme; lactoferrins; bacteriocins; organic acids; and peroxidases preventing infections by constantly washing and self-cleansing the VC [28, 29]. Wira *et al.* [30] showed that the secretion from epithelial cells inhibits the growth of HIV, *Candida* sp., and *N. gonorrhea* but not the growth of *Lactobacilli* sp. Progesterone and estrogen hormones affect the IgA and IgG levels in the cervicovaginal mucus with high antibodies during menstruation and low during ovulation [31]. During pregnancy,

the cervical mucus forms a plug in the cervical region to prevent pathogens to transcend upwards to the uterus [32]. In general, the transparent to whitish discharge is normal. Whereas, a white clumpy discharge with a particular smell; change of colour; loaded with anaerobes and gram-variable motile bacteria is a sign of infection [33].

Dendritic and myeloid cells possess receptors: Toll like receptors (TLR), Nucleotide oligomerization domain like receptors (NOD-NLR), Retinoic acid-inducible gene-I-like receptors (RIG-RLR), and C-type lectin receptors (CLR). The receptors recognise intruding microbes by pathogen associated molecular patterns (PAMP) produced by the pathogenic cells and activate T cells [34]. Single nucleotide polymorphism in genes related to TLR receptors affects the recognition of *A. vaginae* and may facilitate BV [35]. Genetic variants of pattern recognition receptors (PRR) genes like-TLR4, TLR9, IL-1R, and IL-1R2 may cause variable host response to a microbial attack [36]. IL-1β gene polymorphism is seen in women with recurring BV, this inhibits the formation of IL-8 and recruitment of neutrophils to mount response on BV pathogens [37].

1.4. Role of Sex Hormones in the Vaginal Canal

The microbial flora of the VC has a significant correlation with the hormonal balance maintained in a female's body. Post birth of a female foetus, the presence of glycogen has been reported in the VC till the breast-feeding stage [38]. The presence of estrogen in breast milk stimulates the vaginal epithelium cell of the neonate to secrete glycogen. Post the breast-feeding stage, the vaginal pH of young female children is almost neutral (6.5-7), harbouring a wide variety of enteric and anaerobic microbes like *Diphtheroid, Escherichia,* and *Staphylococcus* sp. [39]. The VC of young girls has shown a higher prevalence of coli organism in comparison to the bacilli, without exhibiting any symptoms of vaginitis [40].

At the onset of puberty, oestrogen receptors present on the vaginal epithelium cells allow heavy deposition of glycogen on the VC. Human α- amylase enzyme breaks down the glycogen into smaller saccharides, thereby increasing carbohydrate abundance in vaginal mucus [41, 42]. The microbial flora utilizes these saccharides to form products like lactic, propionic, and acetic acid as well as alcohols [43, 44]. The acids produced by beneficial microbes reduce the pH and cause the vaginal lumen to be acidic in nature [44]. The lactic acid bacteria are the most common inhabitants of VC post puberty in the VC.

They account for 70% of the microbial population in a healthy VC by maintaining a beneficial relationship with the host (Fig 1.2 and 1.3) [45]. During pregnancy, high and constant production of estrogen leads to heavy deposition of glycogen on vaginal walls which is maintained throughout the pregnancy. The pH in VC remains static during this time with more density of *Lactobacilli* sp. due to less hormonal fluctuation [46].

Following menopause, vaginal pH increases to (6.5-7), indicating that no oestrogen is produced [47]. The total abundance of *Lactobacilli* sp. declines, whereas the population of other anaerobic flora increases [48]. CST 4A microbes like *Anaerococcus, Peptoniphilus, Prevotella,* and *Streptococcus* sp. are found in higher abundance [49]. On the other hand, women undergoing estrogen therapy post-menopause or women with diabetes, show a higher population density of LAB in general [50].

The hypothalamus in humans releases corticotrophin-releasing hormone (CRH) under stress, which in turn increases the release of other hormones like cortisol and adrenaline [51]. High cortisol is known to affect the release of glycogen by epithelium cells diminishing the proper maintenance of a healthy VC microflora [52]. Moreover, studies on mice have shown that induction of stress increases adrenaline and noradrenaline levels increasing susceptibility to chlamydia infection [53]. Stress also enhances the occurrence of infections like: vulva-vaginal candidiasis (VVC) [51], sexually transmitted infections (STI), and bacterial vaginosis (BV) [54].

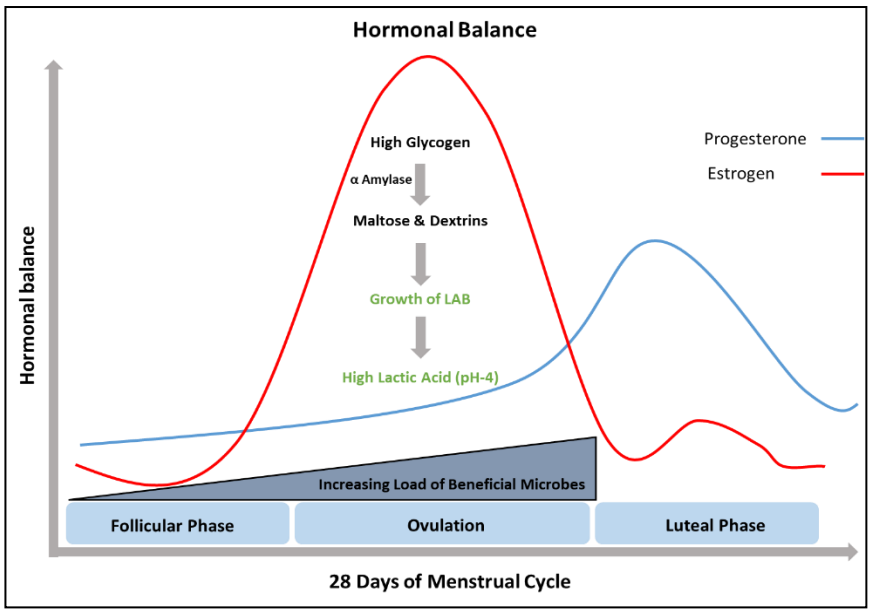

Fig 1.2.: Relationship of estrogen with the microbial flora of the vaginal canal. Das *et al.* (415)

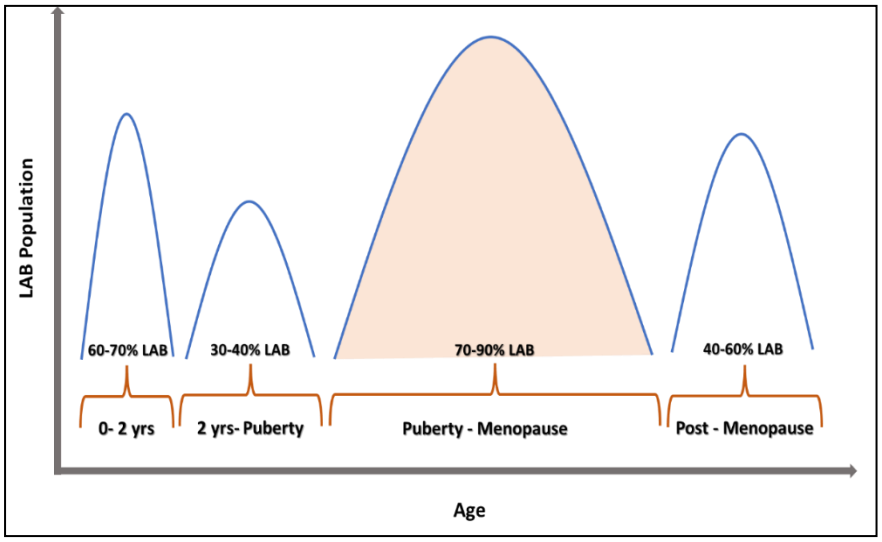

Fig 1.3.: Population Fluctuation of Lactic Acid Bacteria in a Woman's Life Time. Das *et al.* (415)

1.5. Pathogenic Microbes in the Vaginal Canal

The human body hosts trillions of microbes that maintain a mutualistic relationship with the host. The skin, mouth, gastrointestinal tract, and urogenital tract harbours a unique and distinctive microbial flora of its own. These microbes maintain commensalism with the host and do not pose any immunological threat. They evade the immunogenic response of the body and build a safe microbial population that causes no pathogenesis toward their niche [55]. The VC is known to asymptomatically shelter a range of viruses, protists, and archaea [56]. The canal generally harbours a large number of aerobic and anaerobic bacteria and fungi. The commonly found aerobic gram-positive bacteria are- *Diphtheroid, Lactobacilli, Staphylococcus,* and *Streptococcus*; and gram-negative bacteria are- *Escherichia, Klebsiella, Enterobacter, Proteus,* and *Pseudomonas*. Similarly, the anaerobic bacteria found in VC are- *Bacteroides, Bifidobacterium, Clostridium, Eubacterium, Fusobacterium, Peptococcus, Peptostreptococcus,* and *Propionibacterium* [57, 58]. Multiple studies have also reported the presence of fungal species like *Candida, Aspergillus, Saccharomyces, Rhinocladiella, Dothideomycetes,* and *Cryptococcus* in the VC of healthy adults [59]. Moreover, a wide variety of viruses from Adenoviridae, Herpesviridae, Papillomaviridae, Polyomaviridae, and Anelloviridae family have also been reported in women without showing symptoms of any infection [60]. The imbalance of the LAB population in the VC has shown to cause quantitative overgrowth of opportunistic or residential microbes. The overgrowth of these microbes

causes vaginal dysbiosis, leading to vaginitis. Vaginal infections caused by such microbes are:

a. **Vulva-Vaginal Candidiasis (VVC),** also known as candidiasis is caused by the increasing population of *Candida* sp. and low population density of *Lactobacilli* sp. [44]. *Candida* sp. is an opportunistic pathogen that overgrows through epithelium invasion [61]. The common reasons for VVC are use of antibiotics, chemotherapy, HIV infection, pregnancy, birth control pills, hormone replacement therapy (HRT), iron deficiency, and diabetes [62]. 75% of women suffer from VVC once in their lifetime, whereas women 5-9% suffer from the infection thrice within 12-month interval causing recurrent VVC [63, 64]. The common symptoms of VVC are itching, burn, pain, redness, curd like discharge, and shedding of epithelium cells. Severe symptoms of VVC occur when *Candida* sp. forms biofilms and inhibits *Lactobacilli* sp. from attaching to the vaginal epithelium cells. Initial self-diagnosis followed by vaginal swab collection at a clinic for microscopic/ culture based analysis is recommended [65].
b. **Bacterial Vaginitis** (BV), is generally caused by overgrowth of *Gardenella sp.*, when the population of *Lactobacilli* sp. in the VC reduces. A higher population of other anaerobic species in VC like: *Atopobium, Prevotella, Peptostreptococcus, Mobiluncus, Sneathia, Leptotrichia*, and Mycoplasma also cause BV in women. These microbes are known to survive and proliferate in the absence of hydrogen peroxide producing LAB [66, 67]. The common reasons for this vaginal infection are unsafe sexual contact, use of antibiotics, and unhygienic habits. 70% of women in the entire world encounter this infection once in their life [68]. The infection is characterized by abnormal grey to white discharge and fishy odour from the VC [69]. The microbial flora of women infected with BV is grouped from normal- intermediate-severe. Severe BV is characterised by a very low population of *Lactobacilli* sp. and high population of BV related organisms, with presence of clue cells in the VC. Whereas, intermediate BV shows absence of clue cells. Chances of acquiring BV is higher in the first week of the menstrual cycle when the population of *Lactobacillus* sp. in the VC is low [70]. BV increases the chances of acquiring STI and causes preterm birth (PTB), dysuria, dyspareunia, and vaginal pruritus [71]. *Marrazzo et al* showed that the vaginal secretion of BV infected women may be considered as a STI among monogamous women [72].

Previously, the Amsel test criteria was used for diagnosis of BV; fishy odour and greyish discharge were personally detected or reported by patients, furthermore the pH of vaginal discharge and clue cells detection were diagnosed by the physicians to confirm the occurrence of BV. However, few women manifest alkaline vaginal pH with high prevalence of anaerobic bacteria without showing any symptoms of BV. Thus, the Nugent test was introduced, where a gram stain of the vaginal swab was performed and the symptoms were diagnosed to confirm BV [73].

c. **Aerobic Vaginitis** (**AV**) is generally caused by Group B Streptococcus *(S. agalactiae), Enterococcus faecalis, Escherichia coli*, and *S. aureus*. The infection is characterized by yellowish discharge and a burning sensation in the vagina [74]. Shedding of parabasal epithelial cells, thinner vaginal mucus, and an increased number of leukocytes with lysozyme enzymes are other characteristics of AV infection. Vaginal pH is generally greater than 6, with high cytokine and interleukin post AV infection [75]. The succinate level is also notably higher in the vaginal fluid during AV. 8–11% of pregnant women and 5–24% of non-pregnant women report this infection annually [74]. *Donders et al* described that AV differs from BV with the relation of a minimal number of aerobic cocci and bacilli in VC [75]. Wet mount test of vaginal swabs studied under the microscope is used for diagnosis of AV.

d. **Syphilis** was one of the first STIs to be identified; it is caused by gram-negative *Treponema pallidum.* Diagnosis of the infection is done through microscopic techniques and serological tests. Fever, rash in the genitals, swollen lymph gland, muscle ache, fatigue, and condylomatalata or benign cutaneous lesion on skin are few symptoms of the infection [76, 77]. 16, 000 cases of syphilis was reported in the year 2013 according to the WHO [78].

e. **Chlamydia** infection is caused by the gram-negative intracellular parasite *Chlamydia trachomatis,* which invades vaginal epithelium cells [79]. Chlamydia has symptoms like pelvic inflammatory disease, tubular infertility, ectopic pregnancy, cervicitis, and still birth. The diagnosis is done through cytological tests, culturable methods, antigen tests, or enzyme immunoassays [80], nucleic acid amplification and nucleic acid hybridization kits [81]. Chlamydia is the most

prevalent STI among women, it showed ~55% prevalence among women in the year 2015 [82].

a. **Gonorrhea** caused by a gram-negative bacterium *N. gonorrhea,* infects mucosa of VC causing pus, discharge, lower abdominal pain, and dyspareunia. In case of severe infection, it causes PID, ectopic pregnancy, and inflammation of fallopian tube [83]. In 2016 the WHO showed 0.9% prevalence of gonorrhea among women worldwide [84]. Gram's staining of urethral swab, culture on Thayer martin media or EIA for gonococcal enzymes are used for diagnosis of gonorrhoea [85].

b. **Trichomoniasis** is caused by the anaerobic protozoan *Trichomonas vaginalis* [86], it is characterised by foamy thin green-yellow discharge, dysuria, itching, burning, abdominal pain, and vaginal pH >5. TV causes scaring of vaginal epithelium and increase the risk of HIV infection [87]. TV can phagocytose healthy bacteria cells, epithelial cells, and fungi found in the VC. The pathogen is diagnosed by microscopic test (wet mount or Pap smear), culture of the pathogen in Diamond's or Trichosel media, molecular identification through PCR based techniques, and molecular kit for antigen identification [88]. This disease has a prevalence of 8% among women globally [89].

c. **HPV** is a double stranded DNA virus that causes warts in the VC and impacts fertility causing PID and PROM. HPV is the leading cause of intraepithelial neoplasia and vaginal cancer [90, 91]. HPV is diagnosed by vaginal inspection for warts on vaginal epithelium, pap smear of cervical cells, colonoscopy, and through HPV DNA test through PCR of genital cells [92]. Asian and African continent has a higher prevalence of HPV infection among women; 90% of cervical cancer is caused by the HPV virus [93].

f. HSV-1 and HSV-2 are linear ds-DNA virus causing herpes. HSV-2 is more related to genital herpes causing dysuria, local pain, and adenopathy. The virus transfers through skin contact, sexual encounters, and parturition [94]. The infection is diagnosed through genital ulcers, culture assays, EIA, and DNA identification method [95].

g. HIV is a single stranded RNA virus that is sexually transmitted and causes acquired immunodeficiency [58]. According to the WHO, 0.8% of adults are living with HIV. This virus weakens the immune system by hosting immune cells like dendritic cells and macrophages. Serological tests, antigen/antibody tests,

antibody tests, and nucleic acid tests (NAT) are used for the diagnosis of the virus. Women affected with HIV have a high risk of transferring the virus to foetus through breast feeding, parturition, and during the first trimester of gestation. Swollen lymph nodes, STI/STD, vaginitis/vaginal infections, cervical cancer, genital warts, and pelvic inflammatory diseases [96] etc are some symptoms of AIDS [96].

1.6. Medications for Vaginal Infections

Vaginal infections are generally treated by the use of antibiotics (oral/vaginal), topical creams/gels, and estrogen (tablets/creams) [97]. Candidiasis is generally treated by the use of azole drugs [98]. For *C. albicans* infection three doses of 150 mg oral fluconazole treatment in a span of 9 days is recommended. This can be followed by 150 mg fluconazole in a week for 6 months. Nystatin vaginal tablets for two weeks are used for VVC caused by *C. glabrata* which are resistant to azole drugs [65]. Oteseconazole is a new antibiotic used for clearance of azole resistant *C. albicans, C. glabrata,* and *Candida krusei* that tend to cause RVVC [63]. Ibrexafungerp is a newly approved triterpenoid agent for the treatment of RVVC [99]. Azole (clotrimazole, miconazole, and butoconazole) topical creams for 3 days are also recommended for VVC patients [100].

Clindamycin and metronidazole are the commonly used oral/ vaginal antibiotics for the treatment of BV [101]. Metronidazole tablets of 500 mg twice daily for seven days are recommended for BV. The use of vaginal gel for five consecutive days at a concentration of ~37 mg or intravenous application of 5mg/ml is recommended for BV treatment [102]. Clindamycin at a dosage of 300 mg twice a day for seven days is recommended for BV [103]. 2% clindamycin vaginal gel for seven nights and 0.75% metronidazole for five nights is also recommended for BV. These drugs tend to cause nausea and irregular bowel movements [104].

No clinical strategy has been established for the treatment of AV. Antibiotics for microbial agents, estrogen cream for atrophy, and hydrocortisone steroid cream for inflammation help in the improvement of AV [105]. AV can be treated by kanamycin, clindamycin, and fluoroquinolones [105, 106]. Enteric microbe like *E. coli, K. pneumonia, E. faecalis, E. cloacae,* and *E. faecium* are susceptible to carbapenem and amikacin [105]. Fenticonazole is a topical agent that causes inhibition of *G. vaginalis, S. aureus, S. agalactiae, E. coli* and *Candida* sp [107]. Fenticonazole applied high in the

vagina with a 5mg dose for seven days reduces mixed infection caused by overgrowth mixed pathogen population [108].

Syphilis is majorly treated with a single/double dose of 2.5-million-unit benzathine penicillin G through intramuscular shots depending on the stage of syphilis with 90-100% recovery. Tetracycline (100 mg quadruple time orally for 14 days), doxycycline (100 mg twice daily orally for 14 days), ceftriaxone (1-2 g daily intramuscularly or intravenously for 14 days), azithromycin (2 gm single dose intramuscular shot), and procaine penicillin (2.4 million-unit intramuscular shot) with probenecid (500 mg quadruple times 14 days) are additive treatment post the use of penicillin for complete recovery of syphilis [109].

Azithromycin 1 g orally for one dose followed by doxycycline 100 mg orally quadruple times for 7 days is the first line of medication for chlamydia. Alternatively, erythromycin, ofloxacin, and tetracycline 500 mg orally quadruple times for 7 days is also recommended by WHO [78].

The recommended treatment for gonorrhea by Centres for Disease Control and Prevention is either an oral or intramuscular (IM) dose of antibiotics. Ceftriaxone (250mg), Ceftizoxime (500 mg), Cefoxitin (2 g), and Cefotaxime (500 mg) single IM dose is recommended. Cefixime and cefpodoxime (400 mg), probenecid and cefuroxime (1 mg), and cefpodoxime proxetil (200 mg) are other alternative oral antibiotics recommended according to the physician's choice and degree of infection [110].

Nitroimidazole drugs like metronidazole are preferred for trichomoniasis treatment due to their availability and price. Tinidazole has been approved for treatment, this drug in lower concentration shows better efficiency with lower side effects in comparison to metronidazole. Both the drugs at 2 gm single dose or 500 mg single dose for five-seven days is recommended [111].

Podophyllotoxin and imiquimod are approved by the Food and Drug Administration (FDA) for the treatment of non-oncogenic HPV viral infection. Other compound which are anti-inflammatory and antiproliferative against the virus are sine-catechins and trichloro-acetic acid. For oncogenic HPV invasive surgical procedures are recommended, however, they do not reduce the risk of reoccurrence. Chemotherapy with cisplatin is widely recommended for HPV cancer, even drugs like aclitaxel, ifosfamide, topotecan, capecitabine, paclitaxel, pemetrexed, irinotecan, and vinorelbine are used with

chemotherapy. Moreover the FDA has approved monoclonal antibodies pembrolizumab, nivolumab, and ipilimumab which target the programmed cell death of HPV cancer cells [112].

Famciclovir (250 mg thrice for 10 days), valaciclovir (1000 mg twice for 10 days), and (400 mg thrice for 10 days) are the recommended dosages of oral antiviral drugs. An intravenous formulation of acyclovir (5mg/kg) for five to seven days is given under serious conditions. Penciclovir (1%) topical formulations that work against this virus but is rarely recommended [94]. Docosanol, cidofovir, foscarnet, vidarabine, and trifluridine are other nucleoside analogs that have shown potential to inhibit the replication of HSV virus [113].

Treatment or clearance of HIV has not yet been discovered; however antiretroviral therapy (ART) is used for patients infected with the virus. These ART's help in managing the fatal disease to remain as a chronic benign disease. Nucleoside/nucleotide reverse transcriptase inhibitor (e.g., Abacavir, didnosine, zidovudine, tenofovir, lamivudine, emtricitabine, stavudine) for inhibition of RNA virus to transform to DNA. HIV-1 proteases (e.g., Indinavir, saquinavir, ritonavir, nelfinavir, atazanavir, amprenavir, lopinavir, darunavir, and tipranavir) targeting the viral assembly are most widely used ART. Integrase enzyme inhibitors (Raltegravir), membrane fusion inhibitors (Enfuvirtide), and virus attachment inhibitors (Maraviroc) have also been developed [114, 115].

1.7. Beneficial Microbes in the Vaginal Canal

The presence of maltose, malt-hexose, and malt-triose in the VC from the breakdown of glycogen enhances the growth of various microbes. Among the lot, *Lactobacillus* sp. accounts for 70% of the microbiome population [116]. The high population of LAB utilizes the sugars in the VC producing lactic acid through fermentation. This acid is responsible for the low pH (3.8-4.2) in a woman's VC post puberty [117]. A wide range of bacteria and fungi inhabit the VC maintaining a commensal relationship with this species. LAB attaches itself firmly to the epithelial cell layer and maintains a high population preventing the overgrowth of other microorganisms making it the "protector of VC" [118]. Although the cervix and vagina have different embryological origins they show similar LAB dominated microbiota [119].

The VC is known to harbour various species of LAB like: *L. crispatus, L. gasseri, L. jensenii, L. mucosae, L. johnsonii, L. murinus, L. oris,* and *L. acidophilus* etc [120]. *Lactobacilli* sp. are known to produce both chiral forms of lactic acid, whereas humans only produce L form of lactic acid [121]. The presence of only L-lactic acid producing LAB has shown to cause more incidences of VVC, BV, and chlamydial infections [122]. Thus, species that produce both forms of lactic acid are more beneficial and stable. *Lactobacillus crispatus* and *Lactobacillus gasseri* are the most common vaginal LAB known to produce both D and L type of lactic acid and hydrogen peroxide [123]. *Lactobacillus jensenii* another vaginal LAB produces only D type of lactic acid and hydrogen peroxide. Whereas, *Lactobacillus iners* produces only L type lactic acid and minimal hydrogen peroxide [122, 124].

1.8. Use of Probiotics in Vaginal Health

Generally recognized as safe (GRAS), LAB has been deemed fit for human consumption. These microbes are an inherent part of indigenous food culture [125, 126]. *L. acidophilus, L. reuteri,* and *L. casei* are a few strains of LAB that are elaborately used in food and dairy industry [127]. The bacteriocins produced by the LAB are utilized as preservatives [128]. LAB have also been employed for the treatment of diseases like diarrhoea, gastroenteritis, colitis, oral infections, cancer, and irritable/inflammatory bowel syndrome [129]. These microbes are generally used in the form of paste, gels, powder, ointments, and capsules [130, 131]. *Tygat et al.* [132] have illustrated that *Lactobacilli* sp. have pili like structures that helps them attach to epithelium layer. They produce hydrogen peroxide, D/L-lactic acid, bacteriocins, and anti-microbial peptides/ [133] that provide them the status of probiotic [134]. *L. plantarum* and *L. rhamnosus* are the most popular LAB probiotic available in the market [135].

Use of antibiotics for urogenital infection causes reoccurrence of infection, abolition of healthy microflora, and antibiotics resistance on drug overuse, thus, emphasizing usage of probiotics as a healthier and safer choice [136]. Othman et al. [137] suggested that 81% of vaginal infections were cured by using probiotics only, but called for more clinical trials to confirm their efficiency. Probiotics administered vaginally or orally reduces vaginal dysbiosis by producing lactic acid and H_2O_2, without the further use of antibiotics [138]. However, pH lowering vaginal tablets show lesser efficiency in comparison to the beneficial microbes in maintaining vaginal health, as the probiotics

additionally reduce the cytokine levels in VC ensuring the reestablishment of healthy microbial flora [139].

L. rhamnosus strains have been substantially used in clinical trials for testing probiotics against vaginal infection, followed by the use of *L. fermentum* [140]. The use of *L. reuteri* RC14 and *L. rhamnosus* GR1 with antibiotics worked twice more effectively in comparison to the group only administered with antibiotics [141]. *L. rhamnosus* GR1 and *L.reuteri* RC14 showed less fungal population from the vaginal discharge of patients post clinical trials, a showing higher recovery rates from VVC [142]. Similarly, *Chew et al.* showed efficient inhibition of non-albican species causing VVC using the same microbe [143]. *Sabbatini et al.* demonstrated *Lacticaseibacillus rhamnosus* ATCC 53103 to completely inhibit the biofilm formation of *G. vaginalis* and also showed its bactericidal effect on the pathogen [144]. *L. casei* GR-1 another probiotic has shown the reduction of recurrent UTI [145]. *Rangasamy et al.* showed the safe usage of *Limosilactobacillus reuteri* 29B as an intravaginal probiotic in the mouse model [146]. *Che Ou et al.* showed clearance of HPV virus with use of *L. rhamnosus*, *L. reuteri,* and metronidazole antibiotic supplemented orally [147]. The use of probiotics during late pregnancy has been shown to improve vaginal microbiota, showing less density of *A. vaginae, G. vaginalis*, and *Veillonellaceae*. It also reduces anti-inflammatory cytokines like IL-4 and IL-10 which are responsible for pre-term birth (PTB) [148]. *Zuccotti et al* [149] have reported that pre and probiotics taken during and post pregnancy reduces the chances of eczema in neonates showing a positive effect on the child. Probiotics in the mother's diet have also shown high secretory IgA and fewer cytokines in the mother's milk helping to increase the body weight of neonates [150]. *L. rhamnosus, L. salivarus,* and *L. reuteri* oral dosage in pregnant women showed two-three times less GBS infection [151].

Vaginal specific probiotics, belonging from community state type (CST) 1 and 2 have also been tested clinically for their probiotic potential. *Stapelton et al* [152] showed less recurrence of UTI and *Czaja et al* [153] also reported less recurrent UTI among the trial group supplemented with *L. crispatus* CTV-05. *L. crispatus* supplied intra-vaginally with metronidazole reduced the population of *G. vaginalis* and *A. vaginae* in another clinical trial [154]. Orally supplied *L. gasseri* with clindamycin and metronidazole antibiotics limited the recurrence of VVC and BV infection [155]. Moreover, *L. gasseri,*

L plantarum, and *L. fermentum* supplied orally with metronidazole improved recurrent rate of both AV and BV [156].