CHAPTER 2

Review of Literature

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2.1. The Vaginal Microflora

The human microbiota is established post-birth and influenced by various factors. The study of the microbiota has elucidated the microbes that humans host and helped us to understand their pathobiontic or symbiotic relationship with the host. Culturable techniques, next-generation sequencing, metagenomic studies, and 16s RNA gene analysis with statistical and bioinformatic analysis have vastly improved our knowledge of microbial flora of a particular niche [157, 158]. In 1892, Dodercilin first studied the vaginal microflora through culturable techniques [159]. *Aagaard et al.* have shown the use of metagenomic approach to interpret and compare the vaginal microflora of pregnant and non-pregnant women. They showed the abundance of Lactobacillales, Bacteroides, Clostridiales, and Actinomycetales family in pregnant women [160]. Studies by *Ravel et al.* and *Gajer et al.* are the first pioneering studies on dividing the vaginal microflora into various community state types (CST) [161, 162] i.e., a microbial flora that is dominated by a particular species of bacteria.

Women's vaginal microbiota has been divided into five CST through CST (I to V) [163], although a recent meta-analysis study by *Mancabelli et al.* has elucidated three new CST's or vaginotypes [164]. Women tend to transit between these states throughout the menstrual cycle during their reproductive age due to their habitual behaviour as reported by *Song et al.* and *Chaban et al.* [165, 166]. However, *Romero et al.* emphasized less fluctuation of vaginal microflora during pregnancy [167]. *Huang et al.* evaluated that pregnant women show stable microbial flora throughout the VC and cervix [168] and majorly shift from one *Lactobacilli* sp. dominated CST to another [167]. Perimenopausal women showed a prevalence of CST 4 and CST 2 microbes as demonstrated by *Brotman et al.* [49].

CST 1 is dominated by *Lactobacillus crispatus* and CST 2 by *Lactobacillus gasseri* both producing D- and L-type lactic acid and H_2O_2 whereas, CST 5 comprises of *Lactobacillus jensenii* producing only D-type lactic acid and H_2O_2 [123]. CST 3 comprises of *Lactobacillus iners* producing only L-type lactic acid and minimal H_2O_2 [122]. CST 4 has a prevalence of anaerobic bacteria like *Clostridiales, Atopobium, Gardnerella, Mobiluncus, Megasphaera, Prevotella, Anaerococcus,* and *Streptococcus*

with less population of LAB [169]. CST 4 has been divided further into two subtypes by *Ma et al.*; type B has shown a higher prevalence of anaerobes with very low population of LAB and type A had a better prevalence of LAB but was yet dominated by anaerobic microbes [170]. Recently, *France et al.* redivided CST 4 into three sub-types; type A shows a high prevalence of bacterial vaginosis-associated bacterium-1 (BVAB1) and a low population of *G. vaginalis,* whereas type B shows vice versa population distribution. Both type A and B had a low population of *A. vaginae,* whereas type C showed a variable population of different anaerobic microbes [171]. *Freitas et al.* demonstrated the dominance of *Bifidobacterium* sp. in the vaginal microflora of healthy women [172]. Studies by *Ravel et al.* report communities with two dominant species of LAB [162], however a study by *Soares et al.* reported rare codominance of two LAB in the VC [173]. The codominance of two LAB *L. crispatus* and *L. iners* was reported in a study by *Oerlemans et al.* However, such a community showed swift fluctuation [174]. CST 1, 2, and 5 manifest healthy conditions of VC, whereas CST 3 and 4 represent vaginal microbiota of infected or immune-compromised women according to *Smith et al.* [36]. CST 4 microbial flora has been correlated to BV showing no symptoms of irritation or burns in VC, although this vaginotype has higher chances of acquiring STI, and cancer, showing rare cases of infertility [175]. Microbes belonging to CST 3 support chlamydial growth [169] and have been shown to transit to CST 4 type easily in comparison to CST 1,2, and 5 [176, 177]. The presence of *L. jensenii* was not reported in a study by *Wessels et al.* among women with multiple sexual partners showing their unstable nature [176]. Whereas, CST 1 and 2 microbes show higher stability than other CST's [161, 178].

Mycobiome is a broad class under the human microbiota comprising of fungi that colonize humans [179] and has been investigated inadequately due to their low population in the human body [180]. The culture-independent studies from the VC of healthy women have reported several fungal species from Ascomycota, Basidiomycota, and Oomycota phylum. Multiple studies have reported the presence of fungal species like *Candida, Aspergillus, Saccharomyces, Rhinocladiella, Dothideomycetes,* and *Cryptococcus* from VC of healthy adults [59]. *Candida albicans* of the ascomycota family is the most prevalent fungal species found in the VC post puberty. Other non-albican species found in VC are *C. glabrata, C. krusei, C. tropicalis, C. parapsilosis,* and *C. psuedotropicalis* [181]. The study by *Zhao et al.* showed two prominent CST's of VVC affected women, CST 1 was overpopulated with *C. glabrata* and CST 2 was overpopulated with *C.*

albicans. The CST 1 microflora showed a high population density of *Prevotella* sp. a causative of BV, whereas CST 2 showed a higher prevalence of *Ureaplasma urealyticum.* In both the groups the population of LAB was high i.e. ~80%, showing LAB and *Candida* sp. prefer similar growth conditions [182].

VC lavishly promotes the growth of host-friendly native microbiota from the *Lactobacilaceae* family [183]. Other microbes are also naturally present in the VC of healthy women maintaining a low population density. However, because the VC is constantly exposed to external/ habitual behaviour and hormonal changes the vaginal microbiota is prone to swift shifting. Thus, whenever the growth of native beneficial *Lactobacilli sp* is impeded, the other microbes overgrow showing virulence to the host by causing inflammation [184].

2.2. Effect of Hormones on Vaginal Microflora

The maturation of gonadal glands prior to puberty brings changes in the vaginal microflora of teenage girls showing more similarity to that of a woman post menarche. *Hickey et al.* reported a high population of *Lactobacillus* sp. in pre-pubertal girls showing a direct relationship between gonadal hormones (estrogen and progesterone) with the population of LAB in VC [185]. *Altmae et al.* suggest antagonist gonadotrophin hormone affects the uterine microbiota negatively reducing *Lactobacilli* density in the VC [186].

Gregoire et al. showed vaginal cells of rhesus monkeys produced glycogen when stimulated by the estrogen hormone [187]. The role of estrogen is extremely important in maintaining the homeostasis of VC as it stimulates glycogen production by the epithelium cells [22]. Post-puberty estrogen receptors present on the vaginal epithelium allow deposition of glycogen on VC supporting the growth of beneficial microbes, but the relationship between hormonal fluctuation, glycogen deposition, and microflora establishment was rarely described by *Wessels et al.* [188]. According to *Navarro et al.* ~ 0.1-32 µg/mL of glycogen is available in the VC. Vaginal LAB like *L. crispatus*, *L. gasseri*, and *L. jensenii* could utilize glycogen in a stimulated vaginal fluid at a pH of 4-5 indicating the ability of the microbes to utilize the complex carbohydrate. Although, previously the inability of the LAB to produce glycogen degrading enzymes was stated. *Navarro et al.* indicate the ability of vaginal LAB to utilize glycogen [189]. *Wessels et al* stated the immune system, the cervicovaginal fluid, and the response of the epithelial cells to invading pathogens is influenced by the hormonal balance in a woman's body during

their reproductive age [188]. Immune cells like NK, B, CD8, T cells, and macrophages fluctuate in number and phenotype throughout the menstrual cycle in different parts of the FRT [190].

The endometrium thickening of the uterus is maximum during ovulation, the population density of LAB is highest during this time due to the high production of estrogen hormone. However, *Zhang et al.* showed that women suffering from endometrium hyperplasia with constantly high levels of estrogen showed a lower population of LAB in comparison to normal women during their proliferative phase [191]. This signifies the importance of cyclic hormonal fluctuation which allows the beneficial microbes to be in a competitive nature for their survival. *Gajer et al.* [161] also reported shuffling of the vaginal microbiome due to hormonal fluctuation during the monthly cycle, however, the shuffling was dependent on the CST stability and many other habitual factors. Shifting of CST during proliferative and secretory phases of menstrual cycle shows the important role played by the hormones in maintaining vaginal health. *Keane et al.* emphasized the change of CST from normal to BV during the early proliferative stage of the menstrual cycle. However, more than 75 % of women in the same study maintained their CST throughout the menstrual cycle of a month. *Witkin et al.* also suggested the effect of hormones on inducing the growth of non-lactobacilli species during the proliferative stage, but a similar population of LAB was maintained in VC [8, 192]. During menses the diversity of vaginal microflora increases as noted by *Kaur et al.* [193]. The antibacterial environment maintained by low pH in VC is disrupted due to menstrual blood flow. *Shen et al.* suggest the overgrowth of non-lactobacilli species occurs during this time due to the increased vaginal pH and supply of nutritious food sources from the menstrual blood [194].

Studies by *Moreno et al.* [195] showed less impact of the hormone on the endometrium microbiota, however high density of the *Lactobacilli* population in VC facilitated better chances of implantation. Post-implantation during pregnancy, the estrogen level shoots up eventually from 1st to 3rd trimester, showing stable CST during the initial months of pregnancy. Whereas, in the later stage of pregnancy fluctuation of CST in the VC is observed [193]. *Jakobsson et al.* showed that women going through IVF procedures with regular estrogen treatment showed the prominence of *L. gasseri, L. crispatus,* and *L. jensenii* in VC with the least prevalence of *L. iners* [196]. *Plezer et al.*

reported the ability of *L. gasseri* and *L. crispatus* to grow in follicular fluid stimulated by estrogen hormone [197]. Thus, both the CST 1 and 2 microbes are prominently present among healthy reproductive women during the first trimester due to a rapid increase of estrogen hormone. *Elovitz et al.* showed the prevalence of CST 3 and CST 4 with a lower population of CST 1 among women who suffered from PTB [198]. Organisms causing BV or anaerobic organisms prevalent in CST 4 were prevalent amongst pregnant women prone to PTB, due to high levels of pro-inflammatory cytokines production [198, 199]. The vaginal microbes of the mother are more prevalent in neonates born through normal delivery and are considered more beneficial than the ones transferred through surgical procedures. Fewer cases of asthma, obesity, and type 1 diabetes have been reported in a foetus born naturally [200]. Women with vaginal infections whilst giving birth could transmit harmful bacteria to neonates [201]. *Lactobacillus, Prevotella,* and *Sneathia* sp. were reported in the neonate's gut that may be transferred from the mother's VC [202].

Petricevic et al. showed that 80% of pregnant women harboured at least one species of *Lactobacillus* due to high levels of estrogen and progesterone during pregnancy. Whereas, only 40% of postmenopausal women showed the presence of LAB species in their VC [203]. Prior to menopause, a less diverse microbial flora with a high population of LAB has been observed. *Gandhi et al.* reported a higher population of *L. iners* among premenopausal women. Whereas, post menopause the diversity in the VC increases with a decrease in the population of LAB. They also elucidated a correlation of cytokine in VC with a population of LAB, with a higher level of IL-6, TNF-α, and IFN-γ among post-menopausal women [204]. However, hormone replacement therapy with estrogen hormone has shown an increasing population of LAB showing the evident link between estrogen hormone and its effect on the vaginal microbiome [193, 194]. A study by *Cauci et al.* reported a reduction in both *Lactobacilli* and other non-*Lactobacilli* organisms in the VC showing that the absence of a proper nutritional environment for the over-proliferation of any microbes [205]. *Shardell et al.* also showed 50% of postmenopausal women to have less population of LAB population in a cohort study [206].

2.3. Other Factors Influencing Vaginal Microflora

The vaginal health of an individual depends on self-factors like: genetic diversity, diet, and hygiene [207]; and associative factors like: sexual relationships [165]. Ethnicity or

geographical background influences the establishment of vaginal microbiota. A study by *Jin et al*. showed the distribution of LAB in Ugandan and Korean women. Although, geographically distant *L. crispatus* was the most prevalent strain among both the study groups. Korean women had a higher prevalence of *L. fermentum,* whereas the Uganda population had a higher population of *L. vaginalis, L. gasseri*, and *L. reuteri* [208]. Similarly, another study by *Pavlova et al.* on women from seven different countries showed similar results with the highest population of *L. crispatus, L. gasseri,* and *L. jensenii* [209]. This highlights the similar dominant population of a few LAB species among geographically distant women. Another study by *Ting et al.* showed ~70% prevalence of *Lactobacillus sp.* among Chinese women population from various geographical districts of China with *Gardnerella sp.* being the second most abundant species among them [210]. The comparative review article by *Kenyon et al*. showed the White population and Asian population had a lesser prevalence of BV, in comparison to the African and/or Hispanic women in UK and USA. However, women in China, Peru, and Iran in another study had ~50% prevalence of BV in comparison to the women from the UK, Spain, and Canada with ~<15% prevalence [211]. This difference in BV prevalence may be due to food availability, daily lifestyle, and lack of proper awareness among the adolescent/general population about healthy sexual practices.

Diet also shows its effect on vaginal microflora, low nutritional food with high glycaemic fat and sugar has been shown to increase the incidence of BV and VVC [212]. A study by *Noormohammadi et al.* and *Neggers et al.* has reported that BV is promoted by high sugar and fat diet. The higher fat content in food increases the pH of the vaginal canal and shows a negative impact on the mucosal immune system. On the other hand, a diet with a high quantity of vegetables, beans, and grains reduces the chances of BV. The rate of BV is inversely proportional to folate, calcium, and vitamin E concentration. Red meat shows a higher prevalence of BV whereas, eggs and fish increase folate content in food and are beneficial for vaginal health [212-214]. A study by Verstraelen *et al.* showed deficiency of iron leads to higher chances of BV during early pregnancy, thus increasing chances of pre-term delivery. The deficiency of iron harms the innate and cellular immunity of the body bringing about these fatal outcomes [215]. *Bodnar et al.* report the correlation of vitamin D deficiency with BV in the first trimester among pregnant women. The BV condition improved in women with vitamin supplements in a dose dependant

manner. On reaching 80 nmol/L concentration in blood the prevalence of BV declined [216].

Unhealthy habits, like smoking, have also been correlated to the incidence of BV. According to *Brotman et al*. smoking population has displayed a lower population density of *Lactobacilli* sp. in VC due to its negative effect on the estrogen hormone. Moreover, residues of benzo-α-pyrene diol epoxide a carcinogen from cigarettes were detected in the vaginal mucus [217]. On the contrary, *Tuzil et al.* showed that smoking did not affect the population of *Lactobacillus* sp. or the vaginal pH, however, the population of *Gardnerella* sp. and *Mobiluncus* sp. was increased due to smoking with the increase of Nugent score, thus increasing the risk of BV [218]. *Benn et al*. showed that women smoking during pregnancy showed a higher rate of wheezing in born neonates [218]. *Onywera et al.* showed that alcohol consumption was one of the associative factors for causing HPV infection [219].

Usage of medicines or contraceptives also has a range of negative effect on vaginal microflora. *Champer et al.* have elucidated that the high population of LAB in vaginal microbiota is disturbed by the usage of antibiotics increasing the chances of VVC and BV [220], and the use of oral or vaginal probiotics helps to maintain the natural microflora. Women using spermicides show atypical vaginal microbiota with a higher population of enteric microbes and gram-negative rods, with less population of *Lactobacilli* sp. as reported by *Gupta et al.* [221]. The use of spermicides and cervical caps showed a higher nugent score in women. *Lewis et al.* elucidated that women have less prevalence of BV on the usage of oral contraceptives in comparison to using other methods of contraception also previously demonstrated by *Gupta et al.* [222]. *Mitchell et al*. showed that the use of contraception increases vaginal epithelium disruption affecting the attachment of beneficial LAB, thus, leading to higher chances of HIV acquisition [223]. Oral contraception has shown a negative impact on the immune cells of cervical-vaginal fluid, also increasing the chances of HIV acquisition [224]. *Fichorova et al*. showed that the use of oral contraceptives and depot medroxyprogesterone acetate increased the interleukins in the vaginal microenvironment making the environment more susceptible to HIV. The progestin hormone in contraceptives reduces the clearance of HPV from genital tracts leading to a higher risk of cervical cancer. [225].

Social concepts of polygamy, polygyny, and polyandry markedly increase the risk of sexual diseases and compromise the reproductive health of women. *Bove et al.* reported the prevalence of reproductive health issues in ~40% of polygamous women [226]. Cleansing of VC is a common concept among women. The use of sexual moisturizers/lubricants is also known to affect the vaginal microbiota by showing a cytotoxic effect against *Lactobacillus* sp. and weakening the vaginal epithelium as shown by *Fashemi et al.* [227]. *Pavlova et al.* estimated the effect of various vaginal douches against LAB and vaginal pathogens. Vinegar containing vaginal washes selectively inhibited pathogens causing BV and VVC, but not the LAB species. Douches with povidone-iodine, sodium lauryl sulfate, propyl-paraben, di-sodium EDTA, octoxynol-9, citric acid, and sodium citrate, etc were found to inhibit all vaginal microbes [228]. Another comparative study between women from Kenya and USA showed that American women tend to use commercial products and vinegar with water over using simple water. Whereas, Kenyan women used simple water, salt water, or soap water for vaginal washing. Women from USA showed a higher prevalence of BV causing microorganisms in comparison to the other group [229]. This elucidates the use of simple water or salt water is preferable for vaginal cleansing.

Unprotected and unusual sexual contact is a major cause of vaginal dysbiosis. The use of condoms for protective sexual encounters has always been prescribed. *Ma et al.* showed that the use of condoms increased H_2O_2 producing *Lactobacillus sp.* population by ~15% and decreased the nugent score by ~15% in comparison to women using intrauterine devices [230]. Prostrate specific antigens and semen released in the VC post unprotected sexual contact, increase the risk of BV acquisition. Alkaline semen of pH 7.2 may disrupt the acidic pH of VC [231] [232]. *Marrazzo et al.* showed the use of vaginal lubricants and sharing of sexual toys among homosexual women resulted in high BV prevalence. Widely used lubricants showed a higher pH of ~6-7 which had a negative effect on the vaginal microflora [233]. Anal sex causes exposure to anal enteric bacteria and oral sex to oral alkaline bacteria. *Ballini et al.* explain the variable outcome of having vaginal contact, post unusual sexual practices. Transmission of syphilis, chlamydia, VVC, meningitis, gonorrhea, and shigellosis are seen even in the absence of coitus posing a risk of vaginal infection [234]. On the other hand, male circumcision has been shown to reduce the risk of BV in their female partners, referring to it as a good step for sexual hygiene [235].

2.4. Role of Lactic Acid Bacteria in Vaginal Microenvironment

The VC shows a low pH of <4 with minimum of 110 mM lactic acid. *Hearps et al*. showed that lactic acid in its protonated lactate form stimulates the production of anti-inflammatory cytokine IL-1RA by vaginal epithelial cells. The acid also interacts with the TLR agonist and inhibits the production of pro-inflammatory cytokines (IL-6 and TNF-α) and chemokines [(IL-8, regulated on activation, normal t expressed and secreted (RANTES) and macrophage inflammatory protein (MIP)], inhibiting any inflammatory response. Lactic acid also inhibits LPS induced activation of immune cells (macrophages and monocytes). This reduces the infiltration of T cells and inhibits HIV acquisition [236].

LAB species *L. crispatus, L.* gasseri, and *L. jensenii* produce both D and L type of lactic acid, whereas *L. iners* produces only L-type lactic acid. On the other hand, the human body can only produce the L form of lactic acid through the methylglyoxal pathway [121]. For maintaining intracellular pH in vaginal epithelial cells, an extracellular matrix metalloproteinase inducer (EMMPRIN) is produced. EMMPRIN acts as a cofactor for monocarboxylate transporter (MCT) 1 and 4 which helps in transporting L-lactic acid outside the epithelial cells. EMMPRINs are also known to induce the production of matrix-metalo-peptidase (MMP-8) [237]. Shortage of extracellular D-lactic acid which is exclusively produced by LAB has been correlated to high EMMPRIN and MMP-8 in the VC. This highlights the importance of D to L type ratio balance in the VC as elucidated by *Witkins et al*. Imbalance in the ratio with low D-type acid causes a high level of MMP-8 leading to a breakdown of the extracellular matrix causing upper urogenital tract infections, scars on endo-cervical tissues that lead to PROM, and BV [238]. A study by *Nasioudis et al.* also showed a positive correlation of D-lactic acid with α-amylase, secretory leukocyte peptidase inhibitor (SLPI), and neutrophil gelatinase-associated lipocalin [239]. Estrogen helps in the availability of glycogen for α-amylase to act on, thus helping the growth of LAB. Whereas, SLPI and NGAL inhibit the growth of BV associated microorganisms [41]. *Amabebe et al.* stated *Lactobacilli* sp. produces both chiral forms of lactic acid to help in the acetylation of DNA and repairing of the corneum epithelium cells [240]. *Tachedjian et al.* elucidated that D-lactic acid is more beneficial than L type of lactic acid. The D-type lactic acid stimulates the vaginal epithelium cells to inhibit chlamydia infection [44].

Wijgert et al. reviewed the studies on vaginal microbiota and found that more studies are reported on CST representing a higher population of either *L. iners* and *L. crispatus*. They also reported the CST of *L. crispatus* to be more stable, shifting to either *L. iners* CST or mixed *Lactobacilli* CST. On the contrary, the *L. iners* CST showed two times more prevalence to shift to a BV associated CST 4 microflora [241]. *L. crispatus* showed less chances of PTB in comparison to CST dominated by *L. iners* or *L. gasseri*. *Kindinger et al.* also showed *L. iners* to increase the chances of PTB, with *L. crispatus* showing the opposite effect. The epithelial cells of VC release pro-inflammatory cytokines under the influence of *L. iners* which increase the chances of PTB [242]. Borgdorff *et al.* showed that sexual workers with a high prevalence of *L. crispatus* in the cervical-vaginal fluid had a much lower prevalence of HIV, HPV, HSV, and bacterial STI in comparison to the women who had *L. iners* dominated CST. *L. iners* shows differential expression of its 10% genome on the basis of the microenvironment around it and is not selective about its growth condition. Thus, the production of lactic acid post fermentation by *L. iners* is sometimes interrupted by the production of short-chain fatty acids depending on the growth condition, thus increasing the vaginal pH. This is another reason why a high population of *L. iners* is seen post dysbiosis in the VC. On the other hand, *L. crispatus* is highly beneficial for its ability to produce antimicrobial compounds, lactic acid, and anti-inflammatory compounds but is fastidious in its growth pattern [243].

Lactobacillus sp. through fermentation produces a strong organic lactic acid that maintains the pH of the vaginal canal <4.5. Under infectious conditions like BV, the pH of the VC may remain less acidic >5-6 due to the production of SCFA like: succinic acid, butyric acid, propionic acid, acetic acid, and urea. According to *Tachedjian et al.* lactic acid in its protonated form is bactericidal in nature and shows higher inhibitory capacity in comparison to a solution acidified with HCl [44]. *Aldunate et al.* showed that HIV-1 virus was inactivated but not disrupted by racemic lactic acid present in CVF. However, L-type lactic acid was seventeen times more virucidal against HIV strains in comparison to D-type lactic acid [244].

Hydrogen peroxide (H_2O_2) from LAB has antimicrobial properties against microorganisms that do not produce catalase enzyme. *Eschenbach et al.* showed a higher prevalence of H_2O_2 producing LAB in normal women in comparison to women who were affected by BV [245]. *Hillier et al.* suggested a similar pattern in pregnant women with

inhibition of Bacteroides, *G. vaginalis*, *Mycoplasma* sp., *Ureaplasma* sp. *Moliluncus* sp, [246]. *Gupta et al.* showed less H_2O_2 producing *Lactobacilli* sp. were isolated from UTI patients with a higher prevalence of *E. coli* was there among infected women [247]. *Ramirez et al.* in their study showed a correlation between H_2O_2 producing *Lactobacilli* sp. and HIV virus highlighting the virucidal nature of H_2O_2 [248]. *Joo et al.* reported that vaginal H_2O_2-producing LAB inhibits the NF-κB pathway in peripheral blood mononuclear cells, monocytes, and macrophages, inhibiting transcription of TNF-α, IL-6, and IL-8 and also helps in combating *Gardnerella* sp [249].

The role of H_2O_2 has been questioned due to its inconsistent effect on vaginal pathogens. *Hanlon et al.* showed H_2O_2 to have a negative effect on *Lactobacilli* sp. at a concentration of 10mM, but organisms associated with BV were not inhibited. Moreover, in the presence of vaginal fluid, the effect of H_2O_2 was completely neutralized for all *Lactobacillus* sp. and BV organisms. However, at a low pH of 4.5 (50- 110 mM H_2O_2) completely inhibited the BV causing microbes but did not affect the *Lactobacilli* sp. This study emphasized either the single-handed role of lactic acid against BV organisms or defined a unique mechanism where the acidity increased the activity of H_2O_2 [250]. Another study by *Hanlon et al.* stated that *Lactobacilli* sp. were only able to form detectable amounts of H_2O_2 only in aerobic conditions in cervical-vaginal fluid (CVF). H_2O_2 was also able to inhibit *G. vaginalis* and *P. bivia* but in highly aerobic conditions. However, the *in-vivo* vaginal condition being hypoxic or anaerobic the production of detectable H_2O_2 is questionable [251].

The role of H_2O_2 against BV pathogens remains unclear, nonetheless, some studies report the immunomodulatory effect of H_2O_2 on vaginal epithelium cells. *Mitchell et al.* reported the reduction in expression of IL-1β and increased production of SLPI by epithelial cells. Although the production of IL-6 and IL-8 was not affected [252]. *Hemalatha et al.* reported the reduction in the production of IL-6 and IL-1β on treatment with H_2O_2-producing *L. salivarius, L. plantarum,* and *L. brevis,* however simple lactic acid showed no immunomodulatory effect [139]. Alternatively, *Valor et al*. have shown how H_2O_2 negative LAB *L. gasseri* and *L. ruminis* show less adherence ability leading to their eventual loss due to sexual behaviour [253]. This highlights the importance of H_2O_2-producing LAB in maintaining vaginal health. Other than lactic acid and H_2O_2, LAB is known to produce other secondary extracellular compounds that either benefit the

epithelial cells directly, inhibit the overpopulation of pathogens or help in the overpopulation of LAB itself.

The surface-active molecules of the LAB are beneficial for their adhesion, aggregation, and co-aggregation properties and are known as para-probiotics. Whereas, the extracellular secondary metabolites released by LAB are called the postbiotics. Both lactic acid and H_2O_2 are examples of postbiotics [254]. LAB produces vitamins, short chain fatty acids (SCFA), exopolysaccharides (EPS), bacteriocins, cyclic peptides, dipeptides, teichoic acid, linoleic acid, and biosurfactants, which are examples of postbiotics [255]. Whereas, peptidoglycans, cell-wall polysaccharides, S-layer proteins, pili proteins, and aggregation promoting factor are examples of para-probiotics. All these molecules exhibit antimicrobial and antifungal activity against vaginal pathogens [256].

Nelson et al. showed LAB to produce compounds like biosurfactants that inhibit the adherence and even biofilm formation of pathogens. *Nelson et al.* showed the presence of biosurfactants: urfactin, iturin A8, octapeptin-d, plantaricin-a, lichenysin a, sakacin-a, glysperin, plusbacin and laterocin from *L. sakei*, *L. fermentum* and *L. plantarum* LAB isolated from vagina [257]. Bacteriocins are another class of molecules widely produced by gram-positive bacteria. These are made of proteins and are generally non-toxic to humans. *Thokchom et al.* divided bacteriocins into four classes (Class 1-4) [128]. The bacteriocins from LAB are heat-stable, hydrophobic, and cationic in nature [258]. According to *Howett et al.* the increase of STI at an alarming rate stirs the need of topical bacteriocins. These topical agents can successfully act as a chemical barrier against pathogens if used prior to coitus. The ability of these molecules to inactivate the pathogens in the CVF may reduce the chances of the pathogens to adhere and proliferate, thus reducing the chances of infection [259]. *Meijerink et al.* showed the production of plantaricin a potential bacteriocin produced by *L. plantarum* which helps in lower production of IL-10 and TNF-α by dendritic cells [260]. Other well know bacteriocins produced by LAB are reuterin and nisin which are widely used in the food and cosmetic industry. EPS isolated from *L. plantarum* in a study by *Zhang et al.* showed antioxidant activity by scavenging hydroxyl and 2,2-diphenylpicrylhydrazyl (DPPH) radicals [261]. Another study by *Liu et al.* reported an EPS made of xylose, glucose, and galactose from *L. plantarum* inhibiting and eradicating *E. coli* attachment to colonic cell line. Moreover,

the EPS also showed biofilm inhibition of *P. aeruginosa, S. thyphimurium,* and *S. aureus* [262].

The adherence of LAB to the vaginal epithelial cells and their close aggregation/co-aggregation with pathogens are the major mechanisms by LAB which inhibit the pathogens to thrive in the VC. *Branon et al.* elucidate how pathogenic *E. coli* adhere and infiltrate the vaginal cells and uroepithelium to cause infection [263]. *Phukan et al.* describe how protozoan pathogen *T. vaginalis* adhere to the vaginal epithelium cells with the help of Bsp-A protein, cysteine proteases, rhomboid proteases, and polysaccharides. LAB adherence is mediated by mucin binding proteins, lipoteichoic acid, aggregation promoting factors, S-layer protein, and L-layer protein [264, 265]. *Sobel et al.* illustrated both *Lactobacillus* sp. and *G. vaginalis* to have high adherence to vaginal epithelium cells. Thus, pathogens could not be differentiated from non-pathogens on the basis of adhesion, however, LAB showed the highest adherence to vaginal epithelium [266]. *Boris et al.* showed that isolates specifically isolated from humans showed higher adherence in comparison to LAB isolated from food or beverage. The LAB which was hydrophilic showed less adherence capacity, whereas hydrophobic LAB was more adherent in nature. Moreover, the vaginal *Lactobacillus* sp. was able to competitively reduce the adherence of *C. albicans* and *G. vaginalis* to vaginal epithelium cells [26]. *Kmet et al.* showed hydrophobicity of LAB had a proportional relationship with their aggregation pattern. The aggregation promoting factors increased the aggregation and co-aggregation of LAB species [267]. *Ocana et al., Boris et al.* and *Mera et al.* elucidated that aggregation of LAB is mediated by teichoic acid, lipoteichoic acids, protein, lipoproteins, glycoproteins, and carbohydrate moieties on their bacterial cell wall [268]. *Alessandro et al.* showed that >70% vaginal *Lactobacilli* sp. had high aggregation capacity, moreover, few strains showed better adherence when compared to commercial *L. rhamnosus* GG strain. This shows that the ability to adhere enhances the positive potential of vaginal LAB [269]. In a study by *Phukan et al.* they elucidate vaginal *L. gasseri* competes with *T. vaginalis* through co-aggregation. The pathogen showed better co-aggregation with *L. gasseri* from vaginal origin rather than the *L. gasseri* from gut origin. Moreover, the removal of surface protein impedes the ability of LAB to inhibit the pathogen. Thus, emphasizing the role of S-layer proteins like APF-2 in coaggregation with pathogens [264]. A study by *Malik et al.* showed that the knockout of mannose binding lectin (MBL) protein reduced the aggregation, co-aggregation, and adherence of vaginal *L. plantarum*. The MBL protein

bound effectively to the mannose moiety of HIV1 virus and *C. albicans* cell wall and showed inhibition of *E. coli*, *S. thyphimurium*, and *S. aureus* [270]. Another study by *Petrova et al.* showed Legume-type (L-type) lectin from vaginal *L. rhamnosus* reduced the adhesive property of the LAB specifically to vaginal epithelium cells only [271].

Thus, LAB in the VC inhibits the overgrowth of several pathogens like *G. vaginalis, Trichomonas vaginalis, N. gonorrhea, Chlamydia trachomatis*, *Prevotella bivia*, *P. disiens, Porphyromonas* sp, *Mobiluncus* sp, *Peptostreptococcus* sp, herpes simplex virus (HSV), human papilloma virus (HPV), human immunodeficiency virus (HIV), and *Candida albicans* and improve vaginal health or immunity. *Balakrishnan et al.* suggest the use of *Lactobacilli* rich vaginal microbiome transplant to immunocompromised women for improvement in vaginal dysbiosis [272].

2.5. Effect of Pathogens on Vaginal Health

Ling et al. discussed how BV is manifested by overgrowth of residential anaerobes like *Gardnerella vaginalis, Atopobium, Prevotella*, *Peptostreptococcus, Mobiluncus, Sneathia,* and *Leptotrichia* with reduced LAB density in the VC [66]. According to *Beigi et al.* BV pathogens in higher population tend to cause pelvic inflammatory diseases, PTB, postpartum endomyometritis, and intrauterine infection [273]. During menstruation, VC has an alkaline environment that prevails throughout the menses, this facilitates BV microorganisms to overgrow during this period as highlighted by *Srinivasan et al.* and *Onderdonk et al.* [67, 274]. BV organisms metabolize and break down amines increasing vaginal pH and causing intrauterine infections [275]. *Wiggins et al.* mentioned that sialidase, glycosidase, and proteinase enzymes from BV organisms degrade glycoproteins and mucin from mucus increasing the abundance of sialic acid in the VC [276]. As elucidated by *Gondo et al.* sialidases cause degradation of IgA and affect the cell receptors of vaginal cells. Whereas, prolidase enzyme encourages cell infiltration by the breakdown of protective mucosal barrier. Vaginolysin produced by *G. vaginalis* lyse epithelial cells and weakens the adherence of LAB. Sialidase also causes intrauterine infection amongst pregnant women, this enzyme helps BV organisms to stimulate the degradation of the epithelial barrier, ascending upwards through the VC and disrupting the integrity of foetus during pregnancy [277]. *Li et al.* and *Valore et al.* reported BV anaerobes stimulate increased level production of IL-1b, IL-2, IL-6, IL-8, IL-10, TNF-α, IFN-γ and regulated upon activation, normal T cell expressed and secreted

(RANTES) etc., and decreased concentration of AMPs like NGAL in the CVF [278, 279]. *A. vaginae* induces expression of chemokine (CCL20), HBD-2, IL-1β, IL-6, IL-8, and TNF-α via NF-κB pathway after being recognized by TLR-2 receptors on vaginal epithelium cells causing inflammation [280].

Group B *Streptococcus agalactiae*, *Enterococcus faecalis*, *E. coli* and *S. aureus* are causative agents of AV [281]. *Donders et al.* [282, 283] reported, AV was different from BV in relation to inflammation, redness, ulcers in the vagina, presence of leukocytes and parabasal cells in discharge, and thinning of the vaginal epithelium. *Marconi et al.* elucidated higher levels of IL-6 and 8 in women infected with AV in comparison to women infected with BV [284]. Dead GBS organisms inhibit the growth of *Lactobacillus* sp through biofilm formation. LPS from AV causing bacteria stimulate TLR which releases IL-1B, IL-6, and IL-8; these activate the NF-κB signaling pathway that recruits NK, helper, and cytotoxic T cells as well as B-lymphocytes to mount immunological response and induce inflammation as elucidated by *Privarcsi et al.* [86]. *Speigel et al.* showed high concentration of acetic, butyric, and propionic acids in women infected with both AV and BV which are less beneficial than lactic acid [285].

Vulvo-vaginal candidiasis (VVC) is majorly caused by *Candida albicans* by epithelium attachment, invasion through hypha/biofilm formation, and inflammation. The yeast attaches to the epithelial cells, however, their invasion is mediated by secreted aspartyl protease [286] and their hydrophobic moieties as elucidated by *Odds et al*. [287]. The epithelium layer is generally covered by mucus that inhibits attachment of *C. albicans* and eventual penetration to the epithelium layer. *Repentigny et al.* showed that the hyphal genes (e.g., SAP) facilitate the adherence of the yeast to the mucin proteins and eventually cause their degradation increasing their adherence and invasion ability to the epithelium layer [288]. Common VVC-caused symptoms are itching, burn, pain, redness, discharge, shedding of epithelium cells, and autoimmune cells. *Candida* infection occurs highly during the late luteal phase and pregnancy showing that hormonal fluctuation has an effect on the transition of the yeast to hyphal form. Apart from adherence and hyphal transition, the lymphocytic mononuclear cells during the late proliferative stage negatively influence the hyphal proliferation of the yeast. The activation of these immune cells depends on the high level of estrogen hormone in the blood as showed by *Kalo-klein et al.* [289].

Other than the vaginal infections caused by residential bacteria there are few vaginal pathogens that are transferred through sexual contact causing infectious diseases. The disturbed microbial flora of VC enhances the chances of acquiring STI. Generally, anti-inflammatory IFN-γ activates indoleamine 2,3-dioxygenase enzyme that cleaves tryptophan, inhibiting *Chlamydia trachomatis*. However, inflammation caused by BV and AV microbes affects the amount of IFN-γ in the VC negatively, thus enhancing the chances of chlamydia infection [290]. *Brotman et al.* have discussed the cytotoxic role of LAB on HPV tumour cells and the correlation between BV and HPV acquisition [291]. *King et al.* also elucidated how BV related microbes degrade the epithelial layer of cell, mucus, and immunoglobulins increasing the risk of acquiring HPV. However, the occurrence of VVC was not directly related to the acquisition of the virus; but mixed infection of VVC and BV or VVC with trichomoniasis increased the prevalence of HPV [292]. *Wiesenfeld et al.* showed how BV positive women were 4 times more prone to gonorrhea and 3.5 times more prone to acquiring chlamydia infection. They describe the inhibitory effect of H_2O_2 producing LAB to clear *N. gonorrhoea* in-vitro, which generally declines on the occurrence of BV. Moreover, the glycosides produced by BV organisms reduce the viscosity of vaginal fluid increasing the odds to a acquire the sexually transmitted pathogens [293]. *Bautista et al.* also discuss how women infected with BV have two times higher chances of chlamydia and 6 times higher diagnosis of gonorrhea as reported [294]. Women infected with HIV have mixed microflora with four times less prevalence of *Lactobacillus* sp. and high BV-related organisms showing strong correlation between BV and HIV. *Bayigga et al.* postulate that organism like *P. bivia* and *G. vaginalis* activate the NF-κB pathway that induces cytokine and chemokine production reducing the integrity of vaginal epithelial cells [295]. *Africa et al.* elucidate how fibrinolysin, sialidase, prolidase, and collagenase produced by *P. bivia* help in sloughing the epithelial cells in VC and increase HIV susceptibility. Hemolysins and cytolysins produced by *G. vaginalis* are cytotoxic to vaginal epithelium cells [296]. *Hearps et al.* discuss how epithelial cells on encounter with BV-associated pathogens produce IL-6, IL-8, and TNF-α that induce viral replication. Diverse vaginal microbiota during BV shows high secretion of chemokines MIP-1α and MIP-1β, which help in chemotaxis of T cells, dendritic cell, monocytes, and macrophages which act as a host for HIV virus [236].

2.6. Probiotics Against Vaginal Pathogen

Hawes et al. stated the absence of H_2O_2-producing *Lactobacilli* strains from CST 1, 2, and 5 shows overgrowth of *Bacteroides* sp, *Fusobacterium* sp, and *Prevotella* sp which cause BV [297]. On the contrary, *Eschenbach et al.* suggested that the absence of H_2O_2 positive or negative strain does not have any correlation with BV [245]. Organisms from CST1: *L. crispatus* inhibits vaginolysin production by *G. vaginalis* according to *Castro et al.* [298]. Growth and penetration of GBS in vaginal epithelium cells is inhibited by exudates produced by *Lactobacillus reuteri* 6475 [299] according to *Shiroda et al*. *L. reuteri* MT051601 inhibited *E. faecalis* and reduced the production of IL by upregulation of anti-inflammatory Fox-p3 and INF-γ in mice model as shown by *Shazada et al.* [300]. *L. rhamnosus* HN001 and *L. acidophilus* GLA-14 inhibit the growth of E. *coli, S. aureus, G. vaginalis,* and *A. vaginae* the causative organism of BV and AV as discussed by *Bertuccini et al.* [301]. *Lactobacillus delbrueckii* 45E in a study by *Bnfaga et al.* showed inhibition of enteric genital microbes and reduced the production of inflammatory IL-17 cytokines from HeLa cells showing the positive effect of LAB on vaginal health [302]. H_2O_2 and lactic acid cannot completely inhibit the growth of *C. albicans* but a high population of *Lactobacilli* sp. and their extracellular metabolites helps in maintaining *Candida* sp. in yeast form inhibiting expression of hyphal genes as shown by *Mc Alpine et al.* [303]. *Babu et al*. demonstrated the relationship between infertility and the population of LAB. Only 3.5% of women harboured *Lactobacillus* sp. in the infertile group in comparison to 27.8% of women in the healthy group**.** Women with issues of infertility showed a higher abundance i.e. 27.6% of enteric and fungal pathogens with signs of asymptomatic vaginosis, whereas the healthy group had a 7% abundance of enteric and fungal pathogens [304].

Brotman et al. showed women with CST 1, 2, and 5 *Lactobacilli* sp showed a lesser prevalence of TV infection, whereas women with CST 3 and 4 with *L. iners, Sneathia, Mycoplasma, Parvimonas,* and other anaerobic microbes were infected with TV [305]. D-lactic acid produced by these CST's responsible for the inhibition of TV. *Parolin et al.* showed *L. crispatus, L. gasseri,* and *L. vaginalis* inactivate the reticulate body (RB) form of the pathogen by membrane disruption. Lactic acid and metabolites produced by LAB inhibit chlamydial growth [306]. The presence of lactic acid reduces the pH of VC to 4.5 killing gonococci cells mediated by hydrogen ions in the acid by loosening the integrity

of the pathogen cell membrane. *L. crispatus* produces biosurfactants, that also show inhibition of gonococcal cells according to *Foschi et al.* [307]. *Ribelles et al.* and *Cortes-Perez. et al* discussed how genetically modified LAB with E7 antigen [308], and L1 capsid gene genetically engineered in LAB are used as a potent vaccine for the HPV virus [309]. HSV virion is inhibited by H_2O_2 and lactic acid produced by LAB, the metabolites are virucidal in nature according to *Conti et al.* Whereas, the live *L. brevis, L. salivarius, L. plantarum* inhibited the adhesion of the HSV virion to the vaginal epithelium and also reduce the escalating multiplication of the virus [310]. *Mastromarino et al.* suggested the inhibition of HSV virus by *L. brevis* S layer proteins [311]. Vaginal *L. gasseri, L. acidophilus,* and *L. plantarum* showed inhibition of the HSV virus by recognizing molecules on the virus membrane/envelope and binding to it as shown by *Kassaa et al.* [312]. *Lactobacilli* attached to epithelium cells downregulate inflammatory cytokine, whereas IL-1RA an anti-inflammatory cytokine is produced by vaginal cells when treated with lactic acid as elucidated by *Hearps et al.* [236]. *Kumar et al.* suggested the use of genetically engineered protein-based microbicide producing LAB as a protective mucosal agent. These engineered probiotics could prevent sexually transmitted viral infections by topical application [313]. Thus, HIV AIDS acquisition is higher if the natural vaginal microflora is disturbed.

2.7. Alternative Medications Against Vaginal Pathogens

Brown et al. state that AMP aids in tissue repair and cellular development, influences innate and adaptive immune response and thus has great potential as a therapeutic agent [314]. The use of gramicidin for surface wounds and oral-nasal infection, polymyxins for eye infection, and daptomycin for skin infection are few FDA approved AMPs used for treatment [315]. *Russo et al.* have elucidated that the use of bovine lactoferrin with probiotic LAB showed less recurrence of VVC, BV, less vaginal irritation, and itching in comparison to the placebo group [316, 317]. Synthetic AMPs and natural AMPs extracted from various organisms showed inhibitory effects on a wide range of vaginal pathogens. *Tanphichitr et al.* predict AMPs to show potent capacity as antibacterial, spermicidal, and antiviral components if they do not cause inflammatory immune response [318]. Fuzeon or enfuvirtide, an artificial AMP, has been FDA approved against HIV virus. Epi-1, a marine synthetic AMP, can inhibit *T. vaginalis* as reported by *Huan et al.* [319]. Cathelicidin-PG1 from porcine inhibits *Treponema pallidum* [320]. Penaeidin-3 from

Indian shrimp inhibits HSV virus [321]. Diptericin from drosophila increases the permeability of the outer and iner membranes of *E. coli* [320]. *Ballweber et al.* showed cecropin (D2A21/D4E1) from butterfly inhibits *C. trachomatis, C. albicans* and suppresses viral transcription by forming trans-bilayer pores in the cell membrane [322]. Similarly, *Sambri et al.* showed SMAP 29 and CAP-18 from sheep inhibit *T. pallidum* and *C. albicans,* respectively [320, 323]. Melittin from bee venom inhibits HIV-1 mRNA transcription and decreases production of Gag antigen as shown by *Wachinger et al*. [324]. Polyphemusin from horseshoe crabs prevents entry of the virus into the host cell [325]. *S. epidermidis* is inhibited by ranalexin from bull frogs and buforins1 from Asian toads [326]. Magainin-2 and Ranatuerin-1T from frog skin inhibit *E. coli, S. aureus,* and *C. albicans* [320, 327, 328]. Pep-1, an artificial AMP, stops the growth of *Trichomonas* sp inside host cells and inhibits chlamydia as elucidated by *Park et al.* [329]. Dermaseptin-S1 and S4 are AMPs from frogs that inhibit HSV, C. *albicans, S. aureus,* and *E. coli* [330-333]. *Hancock et al.* showed that mycoprex, a synthetic peptide, inhibited *Candida* sp in mice models [334]. *Lin et al*. showed that siamycin-I and II from *Streptomyces* [335] have potential against HIV envelope protein. Similarly, *Algburi et al.* showed subtilosin A from *B. subtilis* stops *G. vaginalis* biofilm formation [336]. Thus, the vaginal lactic acid bacteria could be studied for mapping the AMPs produced by them and their action against the pathogens and also their effect on humans could be studied more prominently.

AMPs (natural or synthetic) exhibit microbicidal activity, although in high concentration, they are mildly cytotoxic in nature [337]. AMPs are designated to kill specific pathogens; however, on the contrary, they may also eradicate other commensal or beneficial bacteria. This may induce a disturbance in microbial population density by causing overgrowth of other potential pathogens, leading to a more serious alteration of natural microbiota. Moreover, natural human peptides share structural similarities to artificial peptides; thus, AMPs run the risk of negatively triggering human immune cells [338]. Prolonged use of artificial peptide pexiganan on *S. aureus* resulted in resistance towards the AMP showing their negative effect on overuse as highlighted by *Habets et al.* [339]. Natural AMPs lack structural stability and synthetic AMPs have a high cost of manufacturing [340]. Because of these limitations, the wide scaled usage of AMPs is not yet prevalent in the medical industry.

2.8. Prospects in Improvement of Probiotics for use in Vaginal Health

Despite the exorbitant usage of probiotics in the pharmaceutical industry, many clinical trials have cited their drawbacks as well as their ineffectiveness according to *Abbasi et al.* [341]. *Marcotte et al.* showed that singular use of antibiotic treatment on BV patients showed a similar recovery pattern with additive dosage of probiotics *L. rhamnosus* DSM 14870 and *L. gasseri* DSM 14869 [342]. On the contrary, *Larson et al.* showed immediate recovery of BV was reported amongst groups supplemented with or without probiotics, although the rate of infection recurrence was lesser in groups using probiotics [343]. This elucidates the long-term effect of probiotics. However, probiotic administration following an antibiotic course sometimes may prolong the time taken by the natural vaginal fora to repopulate, this may be due to competitive exclusion posed by the probiotic strains. Then sudden discontinuation of probiotics may result in the sudden escalation of fast-growing pathogens, causing the reoccurrence of infections manifesting no long-term positive impacts. *Pendharkar et al.* showed usage of azole drugs with or without probiotics showed insignificant difference for recovery of VVC also [155]; only probiotics treatment proved to be least potent for the treatment of VVC [344]. The ineffectiveness of probiotics in the above-mentioned scenarios may be reasoned by the fact that probiotic colonization depends upon multiple factors like physiological growth conditions in infected VC, microbiome competitiveness, and poor host immunity.

Probiotic's effect on healthy vaginal microbiota has been studied scarcely. *Khalesi et al.* suggest in-depth long-term investigations to determine if probiotic consumption builds on the stability of a healthy vagina or unnecessarily promotes disruption of existing natural fora [345]. The sparse population of LAB at various stages of the menstrual cycle exhibits no infectious symptoms, manifesting vaginal acclimatization to a lower population of these beneficial inhabitants. Thus, according to *Buggio et al.* use of probiotic treatment may just be an extravagance [346]. Personalized metagenomic study can improve the right choice of probiotic strain for patients, rather than treating available probiotics as a single "countermeasure" to all kinds of vaginal infections as suggested by *Donders et al.* [347].

A systemic review by *Bafta et al* of randomized clinical trials (RCT) [348] pointed out the lack of harms-related information provided in (~ 87% of abstracts, ~ 97% of methods, ~ 94% of results, and ~ 29% of discussions) on use of the probiotics and

prebiotics. Adverse events (AE) and serious adverse events (SAE) associated with probiotic usage are mentioned inadequately/partially in RCTs, raising their "risk to benefit ratio" and indicating the ambiguity of their benefits. *Van et al.* also suggest the need to effectuate clinical trials with properly designed filters without market bias [349]. According to *Singhal et al.* most commercially available probiotics are rarely identified to their species level and thus may create confusion among consumers [350]. The following parameters: participants from varied geographical backgrounds; larger sample size; the long-term impact of probiotics on vaginal health; microbiome study at the genetic level; length, time, and mode of probiotic dosage; and placebo group study should be kept in mind to assure the benefits of these products according to *Suez et al.* [351].

2.9. Vaginitis/ Vaginal Infection in India

The prevalence of vaginal infection among Indian women population from various states has been studied. The occurrence of BV and VVC infection has been reported in women of rural/urban populations, pregnant/ non-pregnant women, and diabetic/ non-diabetic women. *Narayankhedkar et al.* in a study from Navi-Mumbai, Maharashtra, reports 17.3% prevalence of BV, 1.8% prevalence of TV infection, and 30% prevalence of VVC [352]. Similarly, *Pramanick et al.* and *Dharmik et al.* from the various locations of Nagpur and Mumbai report 18.4% and 20% prevalence of VVC [353, 354]. A study by *Khan et al.* from Delhi, reported 20% prevalence of BV and 26.2% of VVC, with fluconazole resistance observed in *C. albicans* species [355]. Whereas, another study by *Bhalla et al* reports 32% prevalence of BV [356]. *Goswami et al.* performed a comparative study in New Delhi, showing 46% prevalence of VVC among diabetic women and non-diabetic women having 23% occurrence [357]. A study from Bhopal, Madhya Pradesh by *Siddiqi et al.* showed highest percentage of *C. albicans* (34.6%) followed by *C. tropicalis* (23.6%) and *C. glabrata* [358]. Among these species, ~82% had lipolytic activity. Studies in Chennai, Tamil Nadu by *Ahmad et al.* showed ~42%women harboured BV and ~19% harboured VVC among their study population [359]. *Anirudh et al.* reported ~49% of patients with viral STI, ~30% with fungal STI, and ~22% with bacterial STI in a study from Devangere, Karnataka [239]. *Swaminathan et al.* reported a study from the rural area of Karnataka with 37.3% prevalence of VVC with 60% caused by *C. albicans.* Among this population ~30% were pregnant and ~5% were diabetic. A prominent study by *Ahmad et al.* in Aligarh, Uttar Pradesh with a

population size of >1000. They reported ~20% overall prevalence of VVC, with only 10% VVC infection caused by *C. albicans* [359]. Another study in the rural area of Bhojipura, Uttar Pradesh by *Agarwal et al.* reported 39% prevalence of BV, 23% prevalence of VVC, and 6% prevalence of TV infection among married women [360]. A study by *Kalia et al.* from Amritsar, Punjab reported 45.5% prevalence of BV and 31% prevalence of VVC, whereas 20.5% prevalence of mixed vaginal infection among married women [361]. A study on pregnant women by *Bamniya et al.* reported ~22% of patients had vaginal infection, among which 31% had VVC and 9% had BV in the tertiary care unit of Ahmedabad, Gujrat [362]. *Arora et al.* undertook a comparative study in Haryana among urban and rural populations for VVC reporting 4.2% occurrence in rural areas and 0.6% occurrence in urban areas. Overall rural areas had 28 % prevalence of vaginal infection in comparison to 16% in urban cities [363]. A study by *Lavanya et al.* in Andhra Pradesh showed 28% prevalence of VVC with *C. albicans* and *C. krusei* being the leading causative organism [364]. Studies regarding the occurrence of AV in the Indian population have rarely been reported. A tertiary care Unit from Bihar reports 15% prevalence of AV among 430 women. VVC had the highest prevalence of 37.5%, whereas BV 8.55% and UTI 10 % were less prevalent [365].

Northeast Indian states have few reports published on the occurrences of vaginal infections among their women population. A study in Jorhat Medical College, Assam by *Borgohain et al.* with an enrolment of 366 women, showed 33% vaginitis among the age group of 29-38 years. Among this 30% of women suffered from BV and 13% from VVC [366]. A study from Tripura by *Mullick et al.* reported 25 % of women had VVC, with 63% prevalence of VVC caused by non-albican species [367]. Another study by the same group reported 63% of women to have STIs. 20 % of women had VVC, 9% reported BV and 5% reported Trichomoniasis. Whereas ~10% of women had a mixed population infection [368]. A study from Meghalaya reported ~72% of women suffer from VVC with ~64% showing RVVC [369].

2.10. Rationale Behind the Study

The role of LAB in maintaining the vaginal health has been clearly elucidated. However, the isolation of probiotics from the vagina has been stringent in comparison to the large-scale isolation of beneficial bacteria from fermented food products. Moreover, the taboos regarding sexual health in the country have inhibited the study to a greater extent. Thus,

samples were collected to isolate potential LAB and potential aerobic pathogens prevalent in reproductive women. The inhibitory effect of the LAB was studied, and the probiotic potential of the LAB was measured partially. Although, probiotics are widely studied their use at the commercial level is compromised. On the basis of the above mentioned information the following objectives were taken up in the present investigation. This study, elaborately discloses the promising use of the LAB and their extracellular metabolites in the maintenance of vaginal health. The antimicrobial metabolites produced by the LAB were identified which may be further studied for their prospects in vaginal health.

2.11. Objectives of the Study

I. To assess the microbial flora from vaginal swabs of healthy reproductive-aged women (21-45 y).
II. To characterize the isolated microbes and study the interaction between *Lactobacillus* sp. and potential pathogens.
III. To exploit *Lactobacillus* sp. and their culture free supernatant for future industrial usage.