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Review

Unravelling the Biological Interplay Between Genital HPV Infection and Cervicovaginal Microbiota in Sub-Saharan Africa: Implications for Cervical (Pre)cancer Prevention

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Abstract: Cervical cancer is more common in Sub-Saharan Africa (SSA) compared to developed countries, with persistent genital high-risk HPV (HR-HPV) infection identified as the cause. However, other factors contributing to this gap remain unclear. This review explores the potential role of cervicovaginal microbiota (CVM) in genital HPV infection and cervical cancer development among women in SSA. Many women of African descent, including those from SSA, lack lactobacilli dominance in their CVM, which is considered a biomarker of cervicovaginal health. Published literature has associated *Lactobacillus*-dominated CVM with a lower risk of HPV infection and cervical cancer. The converse is true for women with high diversity non-*Lactobacillus*-dominated CVM and bacterial vaginosis, the most common form of vaginal disorder. However, findings on the relationship between specific bacterial abundance and cervical disease severity are inconsistent and inconclusive due to differences in study design, study population, sampling, and potential confounders. Thus, there is a need to form consensus to advance research on CVM and HPV-associated cervical disease. Despite the exact mechanisms by which CVM influence HR-HPV persistence and cervical carcinogenesis being unknown, the backbone of the mechanisms appears to be mediated in part by the following: cervicovaginal dysbiosis, elevated vaginal pH, high ratio of L-to-D-lactic acid, cohesive biofilm formation, chronic inflammation, and immune dysregulation. Consequently, these promote cellular proliferation, genetic instability, and evasion of immune surveillance. This review calls for larger, prospective studies to unravel causal links, identify protective features, and integrate CVM interventions into HPV and cervical cancer prevention strategies in SSA.

Keywords: HPV; cervicovaginal microbiota (CVM); cervical (pre)cancer; cervical disease; Sub-Saharan Africa (SSA)

Citation: Onywera, H.; Mbulawa, Z.Z.A.; Brink, A.; Williamson, A.-L.; Mwapagha, L.M. Unravelling the Biological Interplay Between Genital HPV Infection and Cervicovaginal Microbiota in Sub-Saharan Africa: Implications for Cervical (Pre)cancer Prevention. *Venereology* **2024**, *3*, 211–231. https://doi.org/10.3390/venereology3040017

Academic Editors: Myron Christodoulides and Jacek C. Szepietowski

Received: 25 June 2024
Revised: 29 October 2024
Accepted: 1 November 2024
Published: 14 November 2024

1. Introduction

Genital HPV infection and cervical cancer cases are more widespread in Sub-Saharan Africa (SSA) than in developed countries [1] (Supplementary Table S1—list of geographic regions and in SSA). The estimated age-standardized incidence and mortality rates of cervical cancer in SSA are 33.4 and 22.6 per 100,000 women annually, respectively, two-to-three times higher than the global averages of 14.1 and 7.1 per 100,000 women [2]. Globally, HPV infection peaks in adolescents and young adults (<25 years), then declines. In Africa, a second peak occurs in older women (≥45 years) [1] for reasons not fully understood, including immunosenescence of aging (i.e., waning immunity) [3] that may lead to the reactivation of latent HPV infections acquired earlier in life [4]. As observed worldwide, HPV 16 and 18 are the most prevalent genotypes in cancer. However, HPV-35, a high-risk HPV (HR-HPV) genotype, is common among Sub-Saharan women [5,6] and has been detected in approximately 2% of invasive cervical cancers globally, and up to 10% in SSA [7]. Although numerous studies attribute the high HPV and cervical cancer burden to poor cervical screening programs [8], low HPV vaccine coverage [9,10], demographic, sociobehavioural, clinical, and host genetic factors [11–15], they do not sufficiently explain why women in SSA are more predisposed to genital HPV infection and cervical cancer compared to those in developed countries. Persistent infection with genital HR-HPV is necessary, albeit not sufficient, for the progression of cervical intraepithelial neoplasia (CIN) to cervical cancer. Other poorly understood factors, including but not limited to cervicovaginal microbiota (CVM), may at least partly account for the underlying disparities in HPV infection and cervical cancer burden in women in SSA and developed countries [14,16,17].

A large cross-sectional CVM study conducted among 396 asymptomatic reproductive-age North American women from white, black (synonymous with African ancestry), Hispanic, and Asian ethnicities observed that CVM clustered into five groups, known as community state types (CSTs): CST I to V. Of these, four were dominated by *Lactobacillus* species (CST I: *L. crispatus*, CST II: *L. gasseri*, CST III: *L. iners*, and CST V: *L. jensenii*) [18], which are considered the *sine qua non* of a healthy CVM [19], although not always [20]. The women had different prevalence of *Lactobacillus*-dominated CVM: white (89.7%), Asian (80.2%), Hispanic (61.9%), and black women (59.6%). The other CST (CST IV) lacked appreciable numbers of lactobacilli and had higher proportions of strictly anaerobic bacteria that have been associated with bacterial vaginosis (BV) [18], a polymicrobial vaginal disorder that is globally high—ranging from 23% to 29% across continents [21]. The study on North American women also found that CST IV was significantly associated with BV and was more common in black women than in white and Asian women [18]. It is important to note that, while the CVM is generally described as a distinct ecosystem, it exists along a continuum of microbial states. Studies on healthy women have observed that many CVM fall between the established CSTs [22] and can transition between different CSTs [23], indicating that the CVM operates along a continuum [22,24].

L. iners-dominated CVM, non-*Lactobacillus*-dominated CVM, and a wide range of BV-associated bacteria are often the most prevalent in women of African ancestry [18,25–27], including in SSA [25,26,28], with 38% to 83% of women in SSA found to have CVM dominated by *L. iners* [29–32], which is assumed to be the least protective among the key *Lactobacillus* spp. [19,28]. This contrasts with the observation in healthy white women (<45%), where *L. crispatus* is often the most predominant *Lactobacillus* spp. [18,25,33,34]. Non-*Lactobacillus*-dominated CVM is common among women of African ancestry, with studies on cohorts from SSA reporting a prevalence of between 23% and 64% [17,27,29,31,35]. Relatively recent published data suggest that non-*Lactobacillus*-dominated CVM (CST IV) may not be the norm in women of African ancestry since a high proportion of African American women were found to have *Lactobacillus*-dominated CVM [24]. Among women of non-African descent, particularly white women, this prevalence rarely reaches 40% [18]. While CVM varies with ethnicity [18,24,33,34], geographical location [36], and other factors [37], the CVM is not exclusive to any particular ethnic group [24]. The presence of non-*Lactobacillus*-

dominated CVM may indicate abnormal, transitioning, or variant states of health. These CVM compositions could reflect shifts in the cervicovaginal environment, potentially associated with changes in microbial diversity or imbalances that may affect health outcomes. It is believed that the high prevalence of CVM dominated by non-*Lactobacillus* spp. and *L. iners* among women of African ancestry may partly account for the high burden of BV and sexually transmitted infections (STIs) in this population [27,28,35,38].

Current knowledge suggests a potential causal role of CVM and BV in HPV-associated infections, including cervical (pre)cancer. The present discussion examines studies on genital BV, CVM, and HPV-associated cervical diseases in SSA women, comparing findings with those from other regions. It builds on our previous work [17]. In cases where SSA women are not directly studied, insights are drawn from literature involving women from other regions to speculate the scenario in SSA. The underscored knowledge may guide leveraging the translational potential of CVM in preventing HPV infection and associated diseases among women in SSA.

1.1. Antagonistic Mechanisms Used by Lactobacilli to Interfere with Pathogens

Lactobacilli, as part of the commensal CVM, employ several direct and indirect physiological mechanisms to interfere with pathogens [19,39]. Although some of these mechanisms are still poorly defined, they rely on metabolites produced by lactobacilli or lactobacilli interactions. In brief, the mechanisms relying on metabolites produced by lactobacilli include the following:

i. Production of L- and D-lactic acid, which maintains an acidic environment ($pH \leq 4$) that inhibits the growth of uropathogens, including *Escherichia coli*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis* [19,39]. Lactic acid production in the cervicovaginal milieu is species-dependent, with most *Lactobacillus* spp. able to produce both isoforms. However, *L. iners* exclusively produces the L-isomer [39]. The biocidal action of lactic acid depends on its ability, or that of its released hydrogen ions [19,39], to modify surface proteins and penetrate membranes, leading to cytosol acidification or disruption of microbial metabolism. Lactic acid can regulate host immune responses and trigger autophagy. The D-lactate isoform plays a role in maintaining tissue integrity [39].
ii. Peroxidase system: Hydrogen peroxide (H_2O_2), produced by lactobacilli and released in the cervicovaginal environment, exerts biocidal effects by crossing microbial membranes and acting as an oxidant in the cytosol. In vivo, H_2O_2 reacts with ferrous ions to generate hydroxyl radicals, which oxidize nucleic acids, proteins, and lipids, causing mutagenic and cytotoxic effects in microorganisms lacking H_2O_2-degrading enzymes like peroxidase or catalase (e.g., *Prevotella*, *Gardnerella*) [39].
iii. Bacteriocins (e.g., class II bacteriocins) and bacteriocin-like molecules produced by cervicovaginal lactobacilli can inhibit growth can inhibit the growth or biofilm formation of pathogens such as *Gardnerella vaginalis* and *Enterococcus faecalis* [19,39].
iv. Bacteriophages in *Lactobacillus* contribute to genome plasticity, modulate microbial interactions, and influence cervicovaginal microbiome homeostasis through lysogenic and lytic cycles, prophage integration, and mobile genetic elements [39].
v. Production of biosurfactants, exopolysaccharides, and extracellular vesicles, which protect against pathogens by inhibiting biofilm formation, altering cell membrane permeability, and causing cell death. Biosurfactants modulate surface chemistry to prevent pathogen attachment [39].

Mechanisms relying on lactobacilli interactions include the following:

i. Lactobacilli interfere with microbial adhesion by displaying surface structures (adhesins), which mediate interactions with the host and other microorganisms. These multifunctional proteins enable co-aggregation with pathogens, masking their surface molecules and preventing receptor recognition on host epithelium [19,39].
ii. Many lactobacilli are thought to have a higher affinity for epithelial surface receptors compared to pathogenic microorganisms and can competitively exclude pathogens

through steric hindrance. As a result, they prevent the attachment of pathogens to host epithelium, as well as colonization and invasion [39].

iii. Another competitive mechanism between lactobacilli and pathogens involves niche filtering and/or competition for growth nutrients. High glucose consumption and depletion by *L. crispatus* strains reduce *C. trachomatis* cellular infection. Glucose fermentation produces antimicrobial organic acids like lactic acid [39].

iv. Host-defense mechanisms are stimulated through species-specific modulation (immunomodulatory activity): Lactobacilli modulate immune homeostasis. They induce the production of chemokines and cytokines, which can clear and prevent infections [19,39].

1.2. Link Between BV and Genital HPV Infections and CIN

Limited research has focused on the understanding of the link between BV and HPV infections and cervical (pre)cancer. Information on the association between BV and HPV infections among women in SSA is scarce. A meta-analysis of 12 published articles involving 6372 women aged 13–69 years found that prevalent BV was linked to uterine cervical HPV infection (overall estimated odds ratio (OR), 1.43, 95% confidence interval (CI) 1.11–1.84), though none of the studies were from SSA [40]. In two separate large cohort longitudinal studies, BV was associated with persistent cervical HPV among Finnish women (OR 2.15, 95% CI 1.13–4.08) [41] and delayed clearance of HPV and HR-HPV infection among Chinese women [42]. In the HIV Epidemiology Research Study of U.S. women aged 15–56 years, preceding or current BV was associated with prevalent HPV (OR 1.14, 95% CI 1.04–1.26), incident HPV (OR 1.24, 95% CI 1.04–1.47), and delayed clearance of HPV infection (OR 0.84, 95% CI 0.72–0.97) [43]. However, a case-crossover analysis examining an alternate model for the outcome of incident BV showed that incident BV was associated with incident (OR 1.46, 95% CI 1.12–1.90) but not preceding HPV infection (OR 1.05, 95% CI 0.80–1.37) [43]. A time-lag analysis of the detectability of HPV DNA and clinical diagnosis of BV suggests that HPV infection may precede or occur simultaneously with BV [44]. BV has been associated with increased and specific HPV genotypes [6,45], such as HPV-58 (OR 2.3, 95% CI 1.0–5.2), according to a study on 616 female sex workers (FSWs) aged 18–61 years in Western Kenya [45]. Nevertheless, some investigations found no significant association of BV with HPV [45–47] and HR-HPV [6].

The association between BV and CIN remains contentious. A study involving 280 Dutch women aged 20–66 years with uterine cervix cytological abnormalities found that BV did not affect CIN severity [47]. Similarly, a South Korean cohort study found a significant correlation of BV with CIN, albeit not significant in multivariate analyses [46]. A systematic review and meta-analysis of over 20,000 women aged 13–78 years from different countries (including South Africa), representing different races/ethnicities, showed that women with BV were significantly more likely to have CIN/SIL (squamous intraepithelial lesion) than women without BV (OR 1.51, 95% CI 1.24–1.83) [48]. Interestingly, the South Korean cohort study noted that BV, with or without HPV infection, did not influence the incidence of CIN, irrespective of the severity [46]. Further research is needed to clarify the relationship between BV and HPV infections, particularly among SSA women, and its implications for cervical disease-prevention strategies.

Due to the high burden of BV, HPV, and cervical cancer in SSA, investigating their potential relationship with the incidence and severity of CIN among SSA women is essential. While BV may influence HPV persistence, its role in the progression of cervical disease remains unclear and warrants further research. Conducting such a study on the SSA population is essential because of the observation that being black, although not a deterministic factor [49], is strongly associated with high vaginal pH (>4.5) [50], which has been strongly correlated with BV [18] and the detectability of HPV infection (including multiple types) at times [44,51]. Contrastingly, a previous study found no relationship between vaginal HPV detectability and vaginal pH [44]. Increased vaginal pH has been correlated with CIN severity [52].

A hallmark of BV is believed to be the presence of vaginal biofilm, an assemblage of microbial cells adhered to a surface and encapsulated in a self-produced matrix of extracellular polymeric substances [53]. There are two forms of biofilm, dispersed (planktonic) and cohesive, with the former being highly prevalent and sexually transmitted [54]. Biofilm formation has been associated with HPV and HR-HPV infections [55]. Bacterial biofilms provide a persistent and resilient milieu for opportunistic and pathogenic bacteria, thereby adeptly evading detection by the immune system and contributing to the development of chronic inflammatory diseases [56]. Thus, specific bacterial taxa in the biofilms might explain why BV is associated with increased inflammation [6]. BV may promote HPV infection by resulting in the loss of both the protective cervicovaginal lactobacilli and mucosa barrier integrity. This may, in turn, facilitate adherence, invasion, and integration of HPV oncogenes into the genome of epithelial cells of the cervical transformation zone [40]. Both BV and HR-HPV may impact the metagenome functional content of CVM [57]. It has been suggested that BV may facilitate HPV acquisition or reactivation by altering immunological balance within the cervical tissue [40]. Specific metabolic signatures such as elevated levels of the signalling eicosanoid 12-hydroxyeicosatetraenoic acid (12-HETE), a biomarker for inflammation, have been documented in women with BV [58]. Thus, understanding these interactions could elucidate mechanisms underlying HPV infection and CIN progression in SSA women.

1.3. Association of Cervicovaginal Bacteria and CSTs with Genital HPV Infections

Limited progress has been made in understanding the relationship of cervicovaginal bacteria and CSTs with genital HPV infections among women in SSA. Studies in Rwanda and Nigeria revealed associations between CSTs and HPV detection [35,59]. In Rwanda, FSWs with CST I had the lowest HPV prevalence (9.1%), including HR-HPV types (0.0%) [35], while in Nigeria, CST IV-B (characterized by the absence of *Lactobacillus* dominance and colonization with *Fannyhessea vaginae* (previously known as *Atopobium vaginae* [60]), *Prevotella* spp., and *G. vaginalis*) was linked to high HPV prevalence [35]. In cohorts from non-SSA, CST I, CST II, and, sometimes, CST III have been associated with reduced prevalent HPV and HR-HPV infection compared to CST IV [61–63]. A longitudinal study in the U.S. that comprised 50% African American women observed that women with CST IV and CST III had higher frequencies of HPV infection (71% and 72%, respectively) compared with CST I (45%) and CST II (12%) [61]. Further, there was a trend towards HPV positivity in samples from women in CST IV-B and CST III relative to CST II [61]. The study showed that there was a trend of an increased risk of transitioning to HPV positivity in women with CST IV-A (low *Lactobacillus* CVM with several anaerobes, including *Prevotella* spp., *Fannyhessea* spp., *Anaerococcus* spp., and *Streptococcus* spp.) compared to CST I [61]. In terms of rates of HPV remission (HPV positive to no detection), CST II, in particular, was associated with more rapid remission of HPV compared to CST III and CST IV-B [61]. The studies presented here suggest that CST IV and, to a lesser extent, CST III may not be protective against HPV infection. *L. iners*, assumed to have clonal variants [64], is considered the least protective of the common *Lactobacillus* spp. [19]. Other organisms, including *Candida albicans*, *C. trachomatis*, and *Ureaplasma urealyticum*, have been shown to influence the transformation of HPV lesions. Diversity increases of CVM also amplify inflammatory response and immune dysregulation through cytokine production, creating the local premises for tumour development [65].

Studies assessing the impact of HPV on microbial diversity, and vice versa, have yielded conflicting findings. A study conducted on a Nigerian cohort used weighted UniFrac distances to assess beta diversity and found that the CVM diversity differed between women with and without HR-HPV infection, but this distinction was observed only in women not infected with HIV infection [59]. HIV infection may impact CVM. While some studies have found no significant impact of HIV on alpha and beta diversity [66], others have reported contradictory results [67]. The latter observation could, therefore, be one of the reasons why the effect of HPV infection on CVM is lost when only HIV-infected

women are considered. Additionally, conflicting findings exist regarding the effect of HPV on alpha [29,30,35,67,68] and beta diversity of CVM among women in SSA [29,30,59,67,68]. These discrepancies may stem from differences in study design, study population (e.g., Ethiopian South African, Tanzanian, and Rwandan, and whether they are healthy or diseased), sampling (cervical, vaginal, and cervicovaginal lavage), 16S rRNA hypervariable region (e.g., V4, V3-V4), methods/techniques used to study CVM (Ion Torrent PGM, Illumina MiSeq, and Microarray), bioinformatic-analysis tools (e.g., QIIME, QIIME2/DADA2), and potential confounders, such as menopausal status and host genetic background. It has been shown that CVM profiles can be influenced by several technical factors, including the choice of primers and the targeted 16S rRNA gene region, the sequencing platform, sequencing quality, and bioinformatic tools used [69–71]. Controversies exist regarding whether the microbial diversity and composition across various cervicovaginal sites (vaginal introitus, mid-vagina, lateral vaginal wall, posterior fornix, ectocervix, and endocervix) are homogeneous (highly concordant) [72,73] or heterogeneous [32,74]. Despite the debate, some investigators still sample the vagina when studying infections and diseases in the cervical or cervicovaginal milieu, or the cervix when focusing on infections and diseases vaginal or cervicovaginal milieu. Together, these factors highlight the importance of methodological consistency and careful consideration of technical variables when interpreting CVM data from microbiome studies.

Studies on women from SSA with and without HPV infection reveal heterogeneity in differentially abundant bacteria. Among Nigerian women with and without HIV infection, a higher relative abundance of *L. iners* and lower relative abundances of several BV-associated bacteria, including *Prevotella* spp., *Sneathia* spp., and *Dialister* spp., were strongly associated with HPV infection [59]. BV-associated bacteria have been correlated with prevalent HPV among Ethiopian adults [68] and asymptomatic South African adolescents [6]. Among Tanzanian women with and without HIV infection, higher relative abundances (at least 10-fold increase) of Bacteriodetes and Fusobacteria and lower relative abundance of Actinobacteria were associated with HPV infection [67]. The latter finding is in disagreement with a study on a South African cohort study [30]. However, the association of Fusobacteria with HPV infection aligns with previous reports [30,63]. In the South African study, the CVM of women with HR-HPV infection was enriched with *G. vaginalis*, *Sneathia* sp., *F. vaginae*, *Aerococcus*, and *Pseudomonas veronii* [30]. Notably, the observation of *Sneathia* sp. is consistent with findings on Italian [75], South Korean [63], Nigerian [59], and Chinese cohorts [76]. It is worth pointing out that higher relative abundances of genus *Fannyhessea* were strongly linked with HPV persistence [75]. These findings underscore the complexity of CVM composition in the natural history of HPV infection and highlight potential biomarkers for HPV infection.

Several studies involving women from non-SAA have linked higher abundances of specific or all common cervicovaginal lactobacilli with lower prevalent HPV infection [62,63,77,78], including HPV-16 [79]. A study on a North American cohort prospectively assessing the relationship between CVM and HPV infection, while controlling for immune status, found that among the *Lactobacillus* spp. (*L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*), only the relative abundance of *L. crispatus* was significantly associated with decreased HPV or oncogenic HPV detection after adjustment for multiple covariates, including vaginal pH [62]. Furthermore, this study on the North American cohort found no association of relative abundance of *Lactobacillus* spp. as a group with prevalent HPV [62], as opposed to a South Korean twin cohort cross-sectional study [63]. Interestingly, a prospective study on 59 African/Caribbean women in Canada associated both low bacterial load and abundances of *L. gasseri* with prevalent HPV [77]. Additionally, an abundance of *L. iners* has been associated with the clearance of HR-HPV infection among Costa Rican women [80].

The role of CVM in the natural history of HPV infection, including among women from SSA, remains unclear. However, several mechanisms have been speculated [81]. Cervicovaginal lactobacilli may play a protective role against HPV infection via antagonistic mechanisms, primarily by maintaining a low acidic milieu attributed to lactic acid

production [82]. Relative abundances of *Lactobacillus* spp., including *L. crispatus*, have been inversely correlated with vaginal pH [62], which may also be important in HPV pathogenesis. Reduced risk of HPV detection has been associated with a vaginal pH of <5.0 [51], due to the susceptibility of the HPVE5 oncoprotein to acidic pH [83] as proposed in a previous review [81]. This reduces and/or obliterates the viral propagation in the host epithelia. Among the individual *Lactobacillus* spp., the relative abundance of *L. crispatus* has been found to exhibit the highest correlation with acidic pH, followed by *L. jensenii*, *L. gasseri*, and *L. iners* [62]. Also, *Lactobacillus* spp. vary in their ability to produce H_2O_2, with *L. iners* and *L. gasseri* typically being very low H_2O_2 producers compared to *L. crispatus* and *L. jensenii* [84–86]. Lactobacilli-derived H_2O_2 may be protective against BV [84,85,87]. While H_2O_2-producing lactobacilli may exhibit antibacterial, antiviral, and antitumoral properties [88–90], their presence may not consistently affect cervical neoplasia or HR-HPV positivity [91]. Differences in *Lactobacillus* spp. may, therefore, explain the differing effects on the natural history of HPV infections among women with *Lactobacillus*-dominated CVM [61]. Nonetheless, the lack of CVMs with *Lactobacillus* dominance among women in SSA may partly clarify why these women have an increased risk for HPV infection.

In addition to low vaginal pH, lactobacilli such as *L. crispatus* may use other biological mechanisms, such as the isoform of lactic acid to foil the detection of HPV infection [62]. D- and L-isoforms of lactic acid possess antiviral activity [92,93]. However, high ratios of L-to-D-lactic acid, as evident in *L. iners*-dominated CVM [92,94], may predispose women to BV and cause loss of cervical integrity [94], which can allow HPV particles to enter and infect the basal cells [95]. Differences in prevalence, persistence, and remission of HPV may further be pointed to the immunological responses [77], temporal changes of the cervicovaginal milieus, stability of the CSTs [61], and altered metabolic pathways of the CVM [79]. Specific inflammatory mediators (chemokines, cytokines, and growth factors) have been correlated with HPV and HR-HPV infection [96], although this association may be lost after adjusting for BV status [6]. Elevated levels of interferon gamma-induced protein 10 (IP-10) and monokine induced by gamma interferon (MIG) have been associated with prevalent HPV, while increased cervical Langerhans cells, involved in HIV acquisition, have been linked to HPV clearance [77]. Women with HPV-16 have shown depleted metabolic pathways, such as replication and repair [79].

1.4. Association of Cervicovaginal Bacteria and CSTs with HPV-Associated Cervical (Pre)cancer

There is a scarcity of studies on the relationship between CVM composition and diversity and HPV-associated cervical (pre)cancer in women in SSA. A small cohort prospective study performed by Sims et al. [66] that included 21 and 10 Batswana women with cervical dysplasia (CIN2+) and cervical cancer, correspondingly, with and without HIV infection, noted that the prevalence of *Lactobacillus*-dominated CVM was significantly lower in women with cervical cancer (10%) compared to those with CIN2+ (29%). Similarly, a study by Klein et al. [67] involving 144 Tanzanian women (with an average age of 37 years) with precancerous lesions found that high-grade squamous intraepithelial lesion (HSIL) samples considerably lacked *Lactobacillus* dominance observed in samples negative for intraepithelial lesions or malignancy (NILM). Moreover, a lower relative abundance of *Lactobacillus* spp. was recently noted among 120 adult Ethiopian women with adenocarcinoma (cervical cancer) compared to women with and without premalignant dysplasia (atypical squamous cells with undetermined significance [ASCUS]/NILM, low-grade squamous intraepithelial lesion [LSIL]/CIN1/2+, and HSIL/CIN3) [68].

The aforementioned findings indicate an inverse relationship between lactobacilli dominance and HPV pathogenesis. Moreover, this may be true since the findings of a cross-sectional study on 169 UK women (aged 18–45 years) observed a decreasing prevalence of CST I with increasing disease severity (CST I prevalence: 50% in normal, 42% in LSIL, 40% in HSIL, and 20% in invasive cervical cancer), while CST IV prevalence increased significantly in women with LSIL (21%), HSIL (27%), and invasive cervical cancer (40%) compared to disease-free healthy controls [97]. Although a study on 151 Nigerian women

(median age: 52 years) found a null association between CST and cervical pathology, it associated HR-HPV infection in CST III and CST IV with HSIL and cervical cancer [16]. Likewise, a large study of HR-HPV-positive North American women aged 19–50 years associated CVM dominated by *L. iners* and an unclassified *Lactobacillus* with CIN2+ [98]. However, caution should be taken when interpreting this result since there was no association between CIN2+ and *L. iners* sequence reads when examined independently [98]. A small cohort study on Ethiopian women found that *L. iners* was enriched in women with dysplasia (CIN1/2/3) [68]. Despite this, it may be acknowledged that accumulating literature suggests that loss of abundances of lactobacilli or shift of *Lactobacillus*-dominated CVM (particularly CST I) to non-*Lactobacillus*-dominated CVM (such as CST VI) could, therefore, be a cofactor in the development of cervical disease.

Sims et al. [66] further noted significant compositional and diversity disparities among Batswana women with cervical dysplasia and cancer, with higher alpha diversity (measured using the Shannon diversity index) in women with cancer relative to those with CIN2+. This observation augments a UK study that associated increasing CVM diversity with CIN severity, irrespective of HPV status [97]. Klein et al. [67] observed a higher alpha diversity in the CVM of HIV-positive women with HSIL versus those without cervical lesions (NILM). Similarly, increased diversity has been associated with progression to CIN2+ among Costa Rican women [80]. A pilot study on a Mexican cohort found higher microbial diversity among HPV-positive women with SIL and cervical cancer compared to HPV-negative women without lesions [99]. Sims et al. [66] further noted that the beta diversity of Batswana women with cervical dysplasia was significantly different from that of women with cervical cancer. Similar findings of alpha and beta diversities have been reported in other African cohorts with and without cervical cancer [68]. Nevertheless, Klein et al. [67] reported CVM differences based on cervical cytology among HIV-positive Tanzanian women. There are still conflicting reports on the association between CVM and CIN severity, with null association being observed by other investigators [98]. In as much as inconsistencies and conflicting results could be attributed to differences in study methodologies and a lack of adjusting for some confounding factors, such results could be an indication that other factors are involved in the aetiology of cervical disease.

Machine-learning algorithms have been instrumental in identifying differentially abundant bacteria in Sub-Saharan women with cervical (pre)cancer. Serving as examples, genus *Lachnospira* (in class Clostridia) and several taxa in phylum Proteobacteria (*Betaproteobacteria*, *Gammaproteobacteria*, and *Burkholderiaceae*) have been found to be enriched in Batswana women with cervical dysplasia and cervical cancer, respectively [66]. High relative abundances of *Porphyromonas somerae*, *Porphyromonas asaccharolytica*, *Prevotella timonensis*, and an uncultured bacterium have been associated with cervical cancer among Ethiopian women [68]. In the study by Klein et al. [67] on the Tanzanian cohort, the average relative abundance of Firmicutes was found to be lower in HIV-positive women compared to HIV-negative women (4.2% versus 44.4%) and varied by cervical cytology, though with no obvious pattern [67]. However, in HIV-positive women, the average relative abundance of Firmicutes decreased in women with cervical lesions (from 55.8% to between 35.2% and 43.8%) [67]. Tenericutes, including order *Mycoplasmatales* (genera *Mycoplasma* and *Ureaplasma*), exhibited a clear increase in the relative abundance with increasing severity of lesions in both HIV-negative and positive women [67]. *Mycoplasmatales*, *Mollicutes*, and Tenericutes were found to be more abundant in HSIL versus NILM cervices, including in age-matched HIV-positive women [67]. Since abundances *Mycoplasma* displayed the strongest positive linear relationship with the severity of lesions in both HIV-negative and positive women, Klein et al. [67] posited that *Mycoplasma* could result in a CVM type that promotes HPV-associated cervical lesions.

Studies on women from various regions globally, akin to those on SSA women, have yielded varied results on the association between the abundance of bacteria and different stages of HPV-associated cervical (pre)cancer. In a South Korean case-control study, women with a predominance of *G. vaginalis* and *F. vaginae*, in tandem with a high *L. iners*/*L. crispatus*

ratio, were more likely to have CIN [78]. Higher relative abundances *G. vaginalis* have been strongly correlated with progression to CIN2+ [80]. A synergistic effect between BV-associated bacteria, particularly *F. vaginae*, and HR-HPV has been associated with an increased risk of CIN [78]. In agreement with this, high abundances of *G. vaginalis* and *F. vaginae* have been linked to SIL outcomes among Dutch women [100]. Among women from the UK with HSIL, lower relative abundances of *L. jensenii* and *L. coleohominis* and higher relative abundances of *Sneathia sanguinegens*, *Anaerococcus tetradius*, and *Peptostreptococcus anaerobius* have been observed relative to women with LSIL [97]. Conversely, Mexican women with normal cervical cytology have been found to have higher relative abundances of *L. crispatus* and *L. iners* than women with SIL and cervical cancer, respectively [99]. Further, *Fusobacterium* sp. was significantly more abundant in late-stage cervical cancer compared to early stages (HPV-negative or non-cervical lesions), whereas *Fusobacterium necrophorum* was exclusive to women with cervical cancer [99]. The rarity of lactobacilli dominance in the CVM of women of African ancestry might explain their less distinct presence in the CVM of African women with HPV-associated cervical (pre)cancer compared to their non-African counterparts.

1.5. Potential Mechanisms Protecting Against and Promoting HPV-Induced Cervical Carcinogenesis

Cervicovaginal lactobacilli may exert influence over inflammation and immunity [101], potentially impacting cancer development through complex mechanisms. The observation of diminished abundance of *Lactobacillus* in women with cervical (pre)cancer is becoming a common finding in CVM studies. This suggests a consensus that lactobacilli may confer protection against HPV infection, thus reducing the risk of HPV-induced cervical disease. Evidence supporting the protective role of *Lactobacillus* against HPV-induced precancerous lesions was demonstrated in a small-scale 6-month longitudinal controlled pilot study investigating the impact of oral probiotic *Lactobacillus casei* on the clearance of HPV-induced lesions [102]. Although the difference was not statistically significant, probiotic users had a higher chance of clearing HPV infections compared to the control group [102]. Lactic acid from lactobacilli exhibits antiviral properties [103]. It can inhibit a virus by direct virus–probiotic interactions aided by adsorption or a trapping mechanism. Alternatively, lactic acid bacteria can evoke the host's immune antiviral responses and/or produce nonproteinaceous metabolites with inhibitory capabilities [103]. Lactic acid also acidifies the cervicovaginal environment, hence resulting in low pH [62,82], which is a key mechanism for controlling the composition of CVM and preventing colonization of the microenvironment with pathogens [82,104]. Lactic acid can suppress HPVE6 and E7 oncogene expression [105]. Elevated vaginal pH has been associated with the detection of HR-HPV [51] and the severity of intraepithelial lesions [51,52]. HR-HPV types are known to be common in women, including those from SSA, with cervical neoplasia [5,15,16]. Lactobacilli appear to have other mechanisms used to curtail viral infections. Motevaseli and colleagues (2013) [106] showed using culture and bioassay experiments that *L. crispatus* and *L. gasseri* have cytotoxic activity on tumours independent of pH and lactic acid. It has been suggested that CVM homeostasis is necessary to maintain a certain level of inflammatory surveillance that is required for clearing HPV and preventing re-infections (maintaining an HPV-negative state). This thought is supported by a recent study that investigated the CVM and its associated inflammatory milieu during HPV 16 pre-acquisition, persistence, and clearance states. The study found that 9 of 13 inflammatory cytokines levels—interleukin (IL-4, -5, -10, -12, and -13), interferon (IFN-γ and IFN-α2), macrophage inflammatory protein-1-alpha (MIP-1α), and tumour necrosis factor-alpha (TNF-α)—were significantly increased in the immediate post-clearance visit compared to the pre-acquisition or infection visits [107]. Anti-inflammatory nucleotides, adenosine, and cytosine have been positively correlated with *Lactobacillus* abundance and negatively correlated with genital inflammation, suggesting that such nucleotides could be molecules associated with maintaining cervicovaginal health [108].

Several mechanisms have been propagated to elucidate how CVM promotes the development of cervical (pre)cancer, notwithstanding the ethnic variations in CVM. One such mechanism assumes that HR-HPV infection causes the CVM to shift from *L. crispatus* to *L. iners* predominance [99]. As the HPV-infected cells continue to transform to SIL, CVM diversity increases alongside pathobionts, mainly *Sneathia* and *Fusobacterium* spp. *Fusobacterium necrophorum* is present when cervical cancer finally ensues. This shift is mediated by the immunosuppressive microenvironment evoked by HPV infection, maintaining cytokine–CVM cross-talk [99]. *L. iners* in tumours has been found to cause lactate-induced metabolic, conferring chemotherapy and radiation resistance in cervical cancer cells and leading to poor patient survival [109]. Another model, termed the "diversity model for HPV progression", assumes that *G. vaginalis* drives expansion in CVM diversity, serving as a risk factor for the progression of HR-HPV infection to CIN2+ [80]. Prolonged *Lactobacillus* depletion, high microbial diversity, and increased abundance of bacteria associated with CST IV (*G. vaginalis* and *F. vaginae*) are associated with the development of SIL 6 months post-HR-HPV diagnosis [100]. *G. vaginalis* is linked to the stability of *Lactobacillus*-depleted communities in women with HR-HPV infections [110]. This may result in delayed HPV clearance, hence persistence, which can lead to the emergence of transitional communities, e.g., *Megasphaera genomosp type 1*-dominated CVM [110]. Thus, bacterial species such as *G. vaginalis*, and *F. vaginae* could serve as biomarkers for cervical carcinogenesis. Apart from the presence of specific BV-associated bacteria, HPV persistence and cervical carcinogenesis are mediated by dysbiosis-induced mucus breakdown by bacterial sialidases (leading to loss of the epithelial integrity) and subsequent production of biogenic amines (putrescines and nitrosamines) associated with oxidative stress-induced DNA damage, which is a crucial step in carcinogenesis [81]. Links between HPV-induced cervical carcinogenesis and CVM, cellular, cervicovaginal metabolomes, and cytokine markers of inflammation have been underscored [108,111].

Accumulating published research suggests that the backbone of HPV carcinogenesis encompasses cervicovaginal dysbiosis (increased CVM diversity), elevated vaginal pH, high ratio of L-to-D-lactic acid (due to BV), biofilm formation, chronic inflammation, and immune dysregulation, including waning immunity (Figure 1). Certain *Lactobacillus* spp., like *L. iners*, with low production of D-lactic acid and H_2O_2, may not effectively maintain a healthy CVM, potentially predisposing women to STIs. Inflammation induced by CVM and BV is a key driver of genital HPV infection and cervical precancerous and cancer changes. Significant alterations, including aberrant metagenome functions, cohesive biofilm formation, and chronic inflammation, promote an environment conducive to HR-HPV persistence, contraction of the diversity of HR-HPV, and progression to cervical cancer. HR-HPV diversity reduces during cervical carcinogenesis [15], possibly due to other factors such as microbial (metabolic) interactions, selection pressure, and viral competition. Also, proliferating cancerous cells may not actively support HPV replication as effectively as the precancerous or normal cells [112]. HPV has been detected in bacterial biofilms [113]. Biofilms can cause mucous depletion and chronic mucosal inflammation, facilitating cellular transformation and cancer progression. Bacteria in biofilms can also promote cancer via other mechanisms, such as altering host metabolisms, producing toxins functioning as carcinogens, and oncogene activation [114]. CVM dominated by *G. vaginalis*, which is thought to drive biofilm formation, has been linked to elevated immune activation, disruption of epithelial integrity, and impaired wound healing [115].

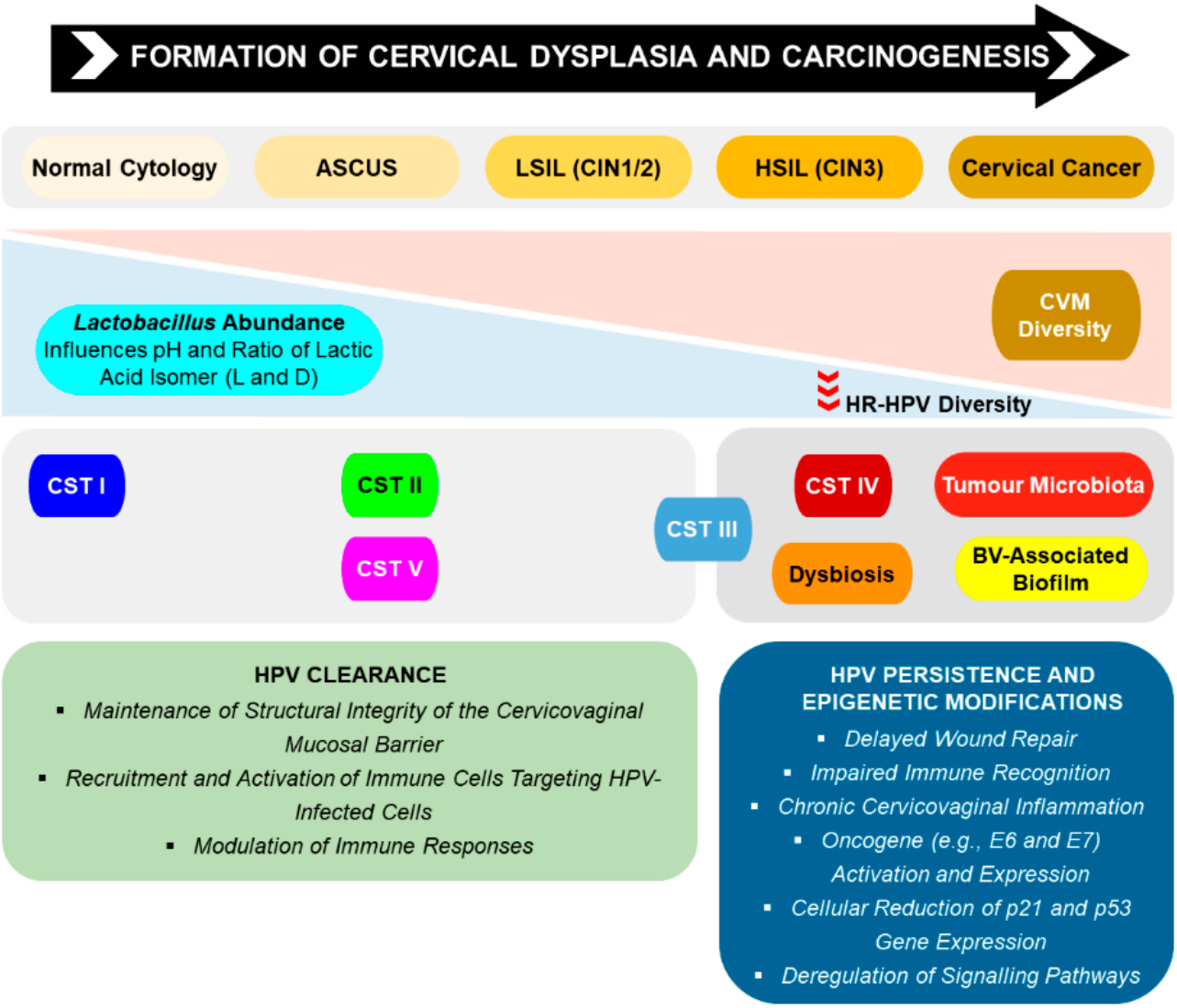

Figure 1. Possible immunobiological mechanism that CVM contributes to HPV-induced cervical carcinogenesis. Depletion of protective commensal bacteria (notably *Lactobacillus* spp.) in the CVM can lead to proliferation of opportunistic bacteria and classical pathogens, thereby altering the CVM structure, function, and diversity. CVM dysbiosis increases the risk of HPV infection and persistence. HPV can exploit biofilms and its oncogenes (e.g., E6 and E7) to dampen or evade host immune and adaptive responses. BV- or biofilm-induced inflammatory responses, cervicovaginal metabolic changes, and molecular and cellular changes induced by HPV infection collectively contribute to architectural damage in the cervical cells, leading to precancerous lesions, which can then develop into cervical cancer. The interaction of HPV with opportunistic and pathogenic cervicovaginal bacteria may result in oncogene activation, contraction of HR-HPV diversity (favouring aggression of specific HPV types), and emergence of a tumour microbiota. The contraction of HR-HPV diversity could also be due to selection pressure, immune responses, and viral competition. Tumour microenvironment can facilitate oncogene expression and cancer progression through barrier dysfunction, bacterial translocation, and persistent inflammation in the cervicovaginal milieu. HPV- and CVM-induced epigenetic changes are crucial events in cervical carcinogenesis. Protective commensal bacteria may facilitate HPV clearance and induce regression of cervical dysplasia or slow cervical tumorigenesis.

Microabrasions in the cervicovaginal tract enable HPV access to basal epithelial cells of the cervical transformation zone [95]. E6 oncogene may trigger cellular transformation through cellular reduction of p21 and p53 gene expression and deregulation of signalling pathways (e.g., PI3K/AKT) involved in DNA damage repair, cell survival, and cell proliferation [116]. E5 oncoprotein results in decreased cytotoxic activity of natural killer cells and a lack of cytotoxic T lymphocyte response against HPV, while E6 and E7 oncoproteins help HPV to evade host immune responses by inhibiting the recruitment of macrophages and other immune cells into the site of infection [111]. E7 oncoprotein can mechanistically interact with several core proteins (NEMO, CK1, and β-TrCP) involved in both NF-kB- and Wnt/β-catenin-signaling pathways, leading to significant down-regulation of host-defense (mucosal innate) peptides used by the predominant *Lactobacillus* spp. (*L. crispatus*, *L. jensenii*, and *L. iners*) as amino acid sources for growth and survival. This impairment leads to reduced *Lactobacillus* abundance and creates a cervicovaginal milieu conducive to dysbiosis. The resulting microbial imbalance may contribute to the persistence and subsequent immune evasion of HPV infections, further promoting the development of pre-neoplastic or neoplastic lesions within the cervicovaginal milieu [117]. Certain epigenetic changes (e.g., methylation of the L1 and L2 late viral genes) during productive HPV infection in both virus and host cell genomes play an essential role in hosting specific microbial communities (e.g., tumour microbiota), viral persistence, and cervical carcinogenesis [118–120].

2. Issues and Challenges in the Current Literature and Way Forward

In this subsection, we outline key issues, challenges, and supporting data in the current literature regarding CVM and HPV-associated cervical disease in women in SSA, alongside proposed future directions for research and interventions to improve cervicovaginal health outcomes (Table 1).

Table 1. Current issues, challenges, and future directions for interventions on CVM and HPV-associated cervical disease in women in SSA.

Issue/Challenge	Description and/or Existing Data	Future Direction
Question of whether CVM is distinct or exists as a continuum and how this impacts cervicovaginal health	• The proposition that CSTs are not entirely distinct but exist along a continuum [22] challenges the one-size-fits-all approaches for diagnostic and treatment	• Researchers to standardize nomenclature and considerations for CVM research • CST framework needs to be continuously updated; CST classification needs to be expanded to understand their dynamics and improve diagnostic and treatment strategies for infections and diseases
Limited data on the relationship of CVM and HPV-associated cervical disease in SSA, which limit cross-study comparisons and generalizability	• Existing studies are predominantly cross-sectional with inconsistent results due to differences in design, population characteristics (sometimes underpowered), methodologies (sampling strategy, hypervariable regions, sequencing technologies, and bioinformatics tools), and bacterial clonal variants • Lack of adjustment for potential confounders that might impact the relationship of HPV-associated cervical disease • Second peak of HPV infection in older women may be partly due to immune waning with age [3,4]	• Conduct region-specific, longitudinal studies with larger cohorts, appropriate controls, and standardized culture-independent approaches to investigate the relationship between CVM and HPV-associated cervical disease • Investigate how waning immunity in older women impacts CVM and HPV; study the impact of aging on CVM and reactivation of a latent HPV infection
Understudied role of non-*Lactobacillus* spp. and non-*Lactobacillus*-dominated CVM in HPV progression to CIN	• Whether certain non-*Lactobacillus* spp. (e.g., *Veillonella* spp.) and non-*Lactobacillus*-dominated CVM are pathogenic or have protective attributes against HPV infection and persistence, as well as CIN severity [30,59,121] • Uncertain role of pathogenic roles of certain non-*Lactobacillus* spp.	• Investigate whether cervicovaginal milieus with non-*Lactobacillus* spp. or dominance may offer protection against CIN severity in women in SSA women
Unexplored impact of antimicrobial properties of lactobacilli on HR-HPV and cervical neoplasia	• Limited understanding of the role of antagonistic mechanisms of lactobacilli (e.g., H_2O_2 production) in the natural history of HR-HPV infection and cervical neoplasia [91]	• Study the antimicrobial properties of lactobacilli in the context of HR-HPV and cervical neoplasia

Table 1. *Cont.*

Issue/Challenge	Description and/or Existing Data	Future Direction
Need to underscore if association means causation during CVM alterations	• Need to evaluate whether the alterations in the CVM could be a consequence of HPV infection and/or precancerous cervical lesions rather than a factor influencing the course of HPV infection (persistence/clearance)	• Conduct prospective studies to establish causal links between CVM alterations and HPV persistence or carcinogenesis
Need for deeper insight into the mechanistic role of CVM and host changes in HPV persistence and carcinogenesis	• Need to chart the biological interplay between different HPV genotypes and CVM and an association between CVM and epigenome (of the virus and host), plus the functional capacity of CVM in healthy and diseased women • Conflicting data on HPV and cytokine profiles [111]	• Examine the biological and epigenetic interactions/interplay between HPV genotypes, CVM, and the host immune system
Limited understanding of the role of bacterial biofilms in the natural history of HPV infection and HPV-induced cancer	• HPV has been observed in biofilms [113] • Biofilms have been associated with HR-HPV and HPV infections, and their formation may serve as an indirect marker for atypical cytological changes [55]	• Establish the role of biofilm as a reservoir for HPV • Research to establish a definitive link between biofilms and HPV carcinogenesis and CVM alterations in tumour-containing tissues • Explore biofilm-targeted interventions
Unexplored impact of HPV vaccines on CVM diversity and composition	• Certain bacteria (e.g., *Prevotella*, phyla Caldithrix, and Nitrospirae) may dampen the immunological effect of therapeutic vaccines (e.g., PepCan) [122] • Current evidence indicates a null effect of HPV vaccines on the composition and diversity [122], yet CVM is impacted by race/ethnicity [18]	• Repeat research with a larger sample size to obtain additional data for confirmatory purposes • Conduct further research to understand the relationship between HPV vaccination and CVM • Explore the possibility of harnessing protective cervicovaginal features (including immunomodulatory properties of specific bacteria) that may be used alongside HPV vaccines for cervical dysplasia and cancer prevention

Table 1. *Cont.*

Issue/Challenge	Description and/or Existing Data	Future Direction
Low uptake and coverage of HPV vaccines and late diagnosis of cervical cancer	• High HPV and cervical cancer burden in SSA is due to inequalities in the distribution of the HPV vaccine [10], as well as both low uptake (some as low as 4%) and coverage of HPV vaccines [9] • In many parts of Africa, cervical cancer is often not diagnosed early, but rather at advanced stages, which are associated with poor outcomes [123]	• Accelerate efforts to expand HPV vaccination coverage • Intensify campaigns to improve uptake of HPV vaccines in SSA • Develop more comprehensive, accurate, and accessible screening methods • Enhance early detection of cervical cancer
Underexplored role of oestrogen in CVM and cancer risk	• Variations in oestrogen levels and DNA adducts linked to cancer risk have been noted across different racial and ethnic groups [124,125], thereby suggesting varied oestrogen metabolism and cancer risks across populations [126]	• Investigate the impact of oestrogen metabolism on CVM and cervical carcinogenesis in SSA populations
Debate on CVM homogeneity versus heterogeneity and definition of a healthy versus diseased CVM in asymptomatic women of African descent	• Ongoing debates highlight the complexities of CVM composition [32,72–74], which is not fully understood if certain non-*Lactobacillus*-dominated CVM represent abnormal, intermediate, or variant states of health	• Ensure methodological consistency and careful consideration of technical variables when interpreting data from microbiome studies • Clarify definitions of healthy versus diseased CVM in women of African descent to inform HPV prevention and cancer progression
Existence of genomic diversity and reclassification of certain bacterial species	• Certain bacteria such as *G. vaginalis* and *L. iners* exhibit genomic heterogeneity [127,128] and clonal variants [64] • Some bacteria (e.g., *A. vaginae* to *F. vaginae* [60]) have recently undergone reclassification. However, some investigators are still using the former taxonomic names	• Conduct genomic and functional investigations using more accurate approaches that have detailed resolution, which will improve understanding of the role of these bacteria in HPV infection and cervical cancer • Investigators should adopt the updated taxonomic classifications of bacteria to ensure consistency and accuracy in scientific communication
Limited translatable research in CVM	• There is limited CVM research that is effectively translated into practical applications	• Ensure constant communication between microbiota research and translational science to improve genital health [129]

3. Conclusions

Next-generation sequencing technologies have revealed insights into the interplay between genital HPV infection and CVM. The available evidence, albeit scarce, on women from SSA indicates that CVM with lactobacilli dominance, which has been negatively associated with HPV infection and severity of CIN, is uncommon among women of African descent. Associations of CVM and specific bacterial taxa with HPV and cervical disease are hypothesis-generating, warranting further investigations. Current research suggests that disruptions in the CVM, marked by reduced key *Lactobacillus* spp. and overgrowth of BV-associated bacteria, may heighten HPV persistence and cervical abnormalities. More evidence is needed to link specific bacteria in CVM with HPV and cervical disease, underscoring the importance of understanding the interplay between genital HPV and CVM for the prevention and control of cervical disease in SSA. The relationship between CVM, HPV, and HPV-induced carcinogenesis highlights the need to integrate CVM manipulation with prevention programs for STI and cervical cancer.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/venereology3040017/s1, Table S1: List of Sustainable Development Goal (SDG) geographic regions in Sub-Saharan Africa.

Author Contributions: Conceptualization, H.O.; Writing—original draft preparation, H.O.; Writing—review and editing, H.O., Z.Z.A.M., A.B., A.-L.W. and L.M.M.; Visualization—Figures and captions, H.O.; Supervision, H.O.; Project administration, H.O.; Funding acquisition, none. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no funding.

Acknowledgments: The authors thank Tracy L. Meiring for partly providing intellectual guidance on the initial draft, partly captured in Harris Onywera's doctoral thesis (http://hdl.handle.net/11427/36399, accessed on 21 May 2021). Harris Onywera acknowledges the late Bethwel Kiplagat Tanui, whose intellectual advice hitherto is nostalgic and profound.

Conflicts of Interest: Author Harris Onywera was employed by the company Tunacare Services Health Providers Limited. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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