Defence Life Science Journal, Vol. 9, No. 3, July 2024, pp. 215-223, DOI : 10.14429/dlsj.9.19257
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Impact of Vaginal Microbiota on Cervical Intraepithelial Neoplasia, Human Papillomavirus Infection, and Cervical Cancer Prevention

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ABSTRACT

Cervical cancer ranks as the fourth most prevalent cancer among women globally, with human papilloma virus (HPV) infection and other vaginal infections playing crucial roles in cervical lesion progression. While the body's immune system can often combat HPV infections, conventional cervical cancer treatments may have cytotoxic effects. Therefore, prioritizing strategies to either decrease HPV infection rates or mitigate existing cervical cancer severity is vital. Certain Lactobacillus strains, prominent in the vaginal microbial community, contribute to vaginal epithelium protection by inhibiting pathogen colonization and producing antibacterial substances like bacteriocins. This review explores how cervicovaginal microbiota, particularly dominated by Lactobacillus species, can lower HPV infection risk, and impede cervical cancer progression. Emphasizing the probiotic impact, the article delves into the potential of vaginal lactobacilli and bacteriocin-producing strains against cervical intraepithelial neoplasia (CIN) and cervical cancer, highlighting underlying mechanisms. A healthy vaginal microbiota emerges as pivotal in averting diverse genital tract infections, preventing cervical lesions, and ultimately reducing the risk of cervical cancer.

Keywords: Cervical cancer; Cervical intraepithelial neoplasia; Human papilloma virus; Cervicovaginal microbiota; Lactobacillus species

NOMENCLATURE

CC	: Cervical Cancer
CFS	: Cell-Free Supernatants
CFSF	: Crude Filtrate Supernatant Fluid
CIN	: Cervical Intraepithelial Neoplasia
HPV	: Human Papilloma Virus
NCL	: Non-Cervical Lesions
SIL	: Squamous Intraepithelial Lesions
RF	: Random Forest
VIA	: Visual Inspection Acetic Acid
WHO	: World Health Organisation

1. INTRODUCTION

1.1 Cervical Cancer

One of the most common gynaecologic cancers worldwide is cervical cancer. According to global statistics data, cervical cancer was the fourth most common cancer in women and ranked fourteenth overall. Most cases (more than 75 % of cases) of cervical cancer are typically brought on by Human Papilloma Virus (HPV), which is the primary cause of the disease. Majority of the HPV infections automatically eliminated clear spontaneously within years without any adverse effect[1,2]. HIV-infected women are more prone (nearly 6 times higher) to develop cervical cancer than non-infected women (WHO, 2020). HPV infection is most prevalent during the young adult stage (before 25 years) and the mortality rate of cervical cancer is optimal in middle age around 40 to 50 years[1-2]. According to WHO, 57,0000 females have been detected with cervical cancer globally, and nearly 311,000 females expired this in 2018[3]. India ranked first for the highest mortality among worldwide which was 60 000 in numbers. Approximately 35 % of worldwide cervical cancer cases and deaths are in India and China only[4].

Most death cases (nearly 90 %) related to cervical cancer were from only lower-middle-income countries. Cervical cancer can be curable through primary prevention methods such as HPV vaccination, secondary prevention methods like proper pre-cancerous lesions screening and treatment, and tertiary prevention methods such as invasive cervical cancer diagnosis and treatment and palliative care[3] (Fig. 1). To reduce the prevalence and mortality rate of cervical cancer first step is initial prevention and screening procedure. Initial screening and diagnosis including pap smear and HPV testing help in the early detection of high-risk Human Papilloma Virus (HPV) lesions[1].

Received : 27 June 2023, Revised : 18 September 2023
Accepted : 25 September 2023, Online published : 21 August 2024

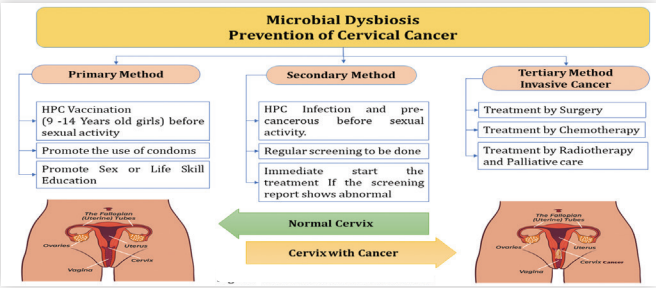

Figure 1. Prevention of cervical cancer.

1.2 Healthy Vaginal Microbiome

Microbiota present in the vagina plays important role in women's reproductive health and wellness. Vaginal microbial communities in the human body act as the first-line defense mechanism against harmful pathogenic infections. The vaginal microbiota is composed of different kinds of many aerobic and anaerobic microorganisms including *Lactobacilli* species. *Lactobacillus* spp. is the most abandoned microbes present in a healthy vagina[5]. *Lactobacillus* can prevent the growth of wide spectrum pathogenic microbes by producing lactic acid, many by-products after the fermentation process, enhancing host immune response, and some antimicrobial substances like bacteriocins and hydrogen peroxides. *Lactobacillus-produced lactic acid maintains a vaginal acidic environment for healthy vaginal microbiota, and* bactericidal proteinaceous bacteriocins can destroy the pathogenic microbes by pore formation on the target cell membrane, and hydrogen peroxide. *Gardnerella vaginalis, Sneathia, Brucella* dominated. *Lactobacillus* also stimulates host innate immunity by exhibiting IL-23 level which further stimulates the host Th-17 pathway and reduces HPV infection prognosis[6-7].

Mechanisms of *Lactobacillus-derived* bacteriocin to destroy pathogens are very much interesting. Bacteriocin promotes cell death of the pathogens by attaching with lipid II and inhibits the synthesis of the cell wall. Another mechanism is bacteriocin accelerates target cell membrane insertion and membrane pore formation process by using lipid II as a docking molecule which ultimately results in the death of the specific target cell[8]. Bacteriocins are effective against both phylogenetically different and similar host bacterial strains. Bacteriocins also have a protective role against plant pathogens, viruses, and cancer[9].

2. A BRIEF SUMMARY OF CERVICAL CANCER

Cancer occurs in the cervix part of the body known as *cervical cancer* when cells of the cervix multiply and grow out of control and accumulate those cells to form a tumor mass. Initially, abnormal cervix cells start growing within tissues of the cervix which is termed dysplasia. Abnormal cells may convert to cancer cells and spread all over the cervix and surrounding area simultaneously. Not only cervix HPV can also contribute to cancer in the anus, vagina, vulva, oropharynx, and penis. Though more than 130 types of HPV have been identified to date only 20 types of HPV are cancerous in nature. Most dangerous HPV infections happen due to Human Papilloma Virus (HPV) type 18 and 16. HPV can be sexually transmitted that transmitted from person to person during sexual intercourse. Although most sexually active persons have HPV in their bodies only a few females will develop cervical cancer. Women population is more prone to get cervical cancer and the mortality rate of cervical cancer is also high. Women without screening (for the last 5 years) and regular follow-up after the detection of the precancerous lesion are at higher risk[1-2]. Two of the most common forms of cervical cancer are squamous cell carcinoma and adenocarcinoma.

Most cervical cancer cases (approximately 75 %) caused due to squamous cell carcinoma. Squamous cell carcinoma mainly occurs at the transformation zone of the ectocervix. Another type of adenocarcinoma is responsible for approximately 25 % of all cervical cancer cases and generally starts in the endocervix at the site of the glandular columnar layer. Commonly detected risk factors of cervical cancer are marriage at a young age, too early (before 15-16 years) first sexual intercourse, polygamy, having many, intercourse partners, through HPV infected partner, sexual activity without or infrequent usage of protection like condoms, gels, diaphragms, use of immunosuppressant, prone towards infection due to the poor immune system, past record of sexually transmitted infections, poor knowledge about cervical cancer, poor accessibility, and availability of proper screening facilities to detect cervical cancer (Fig. 02). HPV type-specific three different vaccines such as quadrivalent HPV vaccine (Gardasil®), 9-valent vaccine (Gardasil 9®), and bivalent vaccine (Cervarix ®) are currently present to prevent HPV infection.

World Health Organisation specifies the targeted age group of girls that is 9 to 14 years old for HPV vaccination because generally, girls in this age group are not sexually active and respond more actively towards the vaccine compared to late teen ages[10]. Commonly visible signs and symptoms of cervical cancer are unusual vaginal bleeding (mainly after sexual intercourse) other than normal menstrual bleeding, pain in the pelvic area, pain at the time of sexual activity, and abnormal vaginal discharge.

Primary cervix examination (Physical exam), pelvic examination, pap test, Human Papilloma Virus (HPV) test, endocervical curettage, colposcopy, and biopsy are the next steps in the HPW diagnosis process. Based on the severity of the HPV infection doctor prescribes appropriate treatment procedures including surgery (conization, cold-knife conization can be done), Pelvic exenteration, radical hysterectomy, total hysterectomy, radical trachelectomy, modified radical hysterectomy, bilateral salpingo-oophorectomy, radiation therapy, Immunotherapy, and chemotherapy[12]. Generally, HPV testing is recommended for women above 30 years of age. Some useful tools for precancerous lesions

screening and treatment in developing nations are visual inspection with acetic acid (VIA), HPV DNA testing screening technique, ablative techniques (including cold coagulation and cryotherapy), excisional techniques (including loop electrocautery excision procedure) and cone biopsy[11].

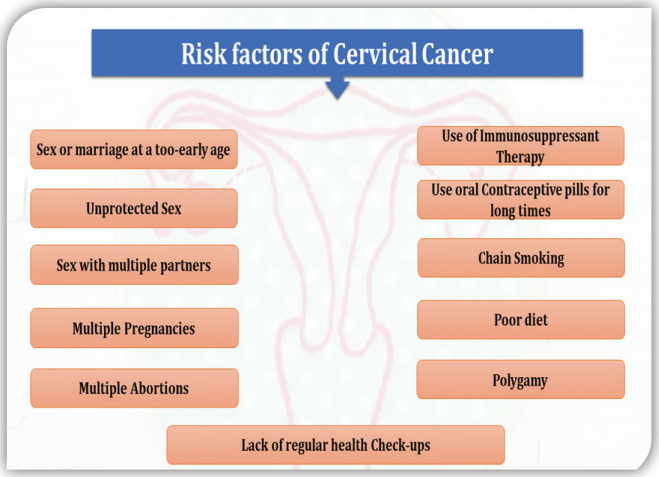

Figure 2. Risk factors of cervical cancer.

3. A BRIEF SUMMARY OF VAGINAL LACTOBACILLUS SPECIES

Lactobacillus spp. is an anaerobic, Gram-positive, non-sporulating, microaerophilic, acid-tolerant, lactic acid-producing bacteria. Produced lactic acid in the vaginal lumen from *Lactobacillus* spp. keeps the vaginal pH acidic (nearly pH 3.5–4.5). A vaginal acidic environment gives full or partial protection from pathogenic microorganisms and inhibits their growth also.

Some antimicrobial compounds like bacteriocins and hydrogen peroxide (H_2O_2) from *Lactobacillus* spp. also have a protective role in the human body against pathogens. Imbalance or microbial dysbiosis may occur due to low *Lactobacillus* spp. concentration or presence of a higher amount of facultative anaerobic microorganisms in the vagina (Fig. 3). Healthy vaginal microbiota diversity depends on many factors including sexual activity, diet, menstruation cycle, douching practices, antibiotics usage, hormone levels, and hygiene practices[5]. Alteration in the composition of vaginal microbiota may happen *during* different stages of life including infancy, puberty stage, gestational stage, and after menopause. Some commonly visible *Lactobacillus* strains in healthy vaginal microbiota are *Lactobacillus gasseri, Lactobacillus crispatus, Lactobacillus jensenii, Lactobacillus iners,* and *Lactobacillus vaginalis. Among those strains, Lactobacillus crispatus enriched vaginal microbiota is mostly associated with* healthy vagina and *vaginal microbiota* dominated with *Lactobacillus iners* consequently leading to vaginal dysbiosis. Experimental studies confirmed the potentiality of *Lactobacillus acidophilus* KS400 to reduce the growth rate of various urogenital pathogenic microbes by producing the antibacterial substance bacteriocin[6-7].

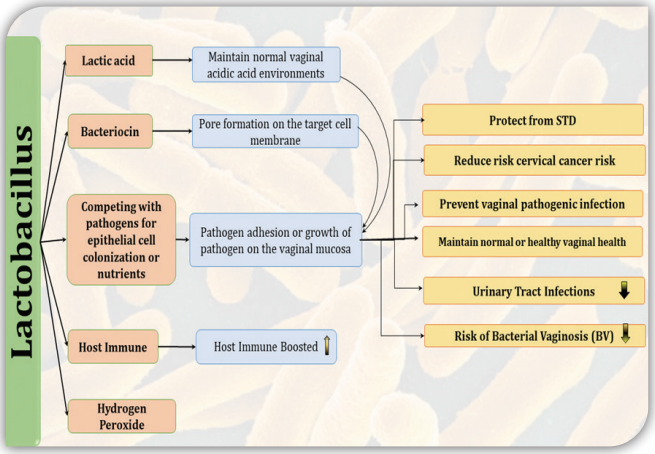

Figure 3. Diagram representing the role of Lactobacillus against vaginal microbiota dysbalance.

4. ASSOCIATION BETWEEN CERVICAL CANCER AND VAGINAL LACTOBACILLUS STRAINS

Vaginal *Lactobacillus* strains can produce lactic acid from glycogen that maintains the acidic pH of the vaginal environment and controls pathogenic bacterial growth inside the vagina. Acidic pH inside the vagina also helps to retain the antimicrobial activities of bacteriocin and hydrogen peroxide. *Lactobacillus* excreted several metabolites including phosphorylated polysaccharides, peptidoglycans, and exopolysaccharides, which further reduce tumors cell multiplication and growth. Lactobacillus-derived antimicrobial bacteriocin can fight against malignant tumor-causing pathogens and other components and reduce the growth of those pathogens. Lactobacillus-produced hydrogen peroxide can destroy harmful pathogens directly by using a peroxidase-hydrogen peroxide-halide bactericidal system[13].

4.1 Relation Between Lactobacillus & Human Papilloma Virus

Wang[14] determined the correlation between cervicovaginal *Lactobacillus* strains and high-risk Human papillomavirus infection chance. Studies detected that high-risk Human papillomavirus infection, cervical intraepithelial neoplasia, and cervical cancer rates are less among female patients with an abundant amount of cervicovaginal lactobacillus strains. Pooled data analysis revealed that *Lactobacillus crispatus* dominated community state type I significantly associated with a lower number of high-risk Human Papilloma Virus infections and cervical intraepithelial neoplasia cases. Interestingly *Lactobacillus iners* were not at all associated with high-risk Human papillomavirus infection as per this study analysis.

4.2 Relation Among Lactobacillus, Human Papilloma Virus & Cancer

Wang[15] concluded that vaginal Lactobacillus derived Cell-free supernatants can reduce cervical cancer cells viability by controlling HPV oncogenes expression and cytotoxicity of *Lactobacillus* supernatant is not dependent on their acidity at concentrations within 20 and 40 %.

4.3 Direct or Indirect Role of Cervical Microbiota in Cancer

Gardnerella was frequently present in the cervical microbiota, according to a study[16] on the direct and indirect relationships between cervical intraepithelial neoplasia (CIN) severity and microbiota.

Pathogenic microbes including *Streptococcus agalactiae*, *Pseudomonas stutzeri, Bacteroides fragilis, Peptostreptococcus anaerobius* were more frequently detected among HPV-positive cases of higher amounts of Lactobacillus delbrueckii strain was detected among HPV- negative cases (P <0.05). The study also showed women with normal cytology were less prone (10.9 %) to HPV infection. At last, the study concluded that along with the HPV infection, a dysbalance of the cervical microbiota could also contribute to cervical carcinogenesis.

4.4 Role of Vaginal Microbiota in the Acceleration of Regression Rate of CIN2 Lesions

One more experimental study by Mitra[17]chooses a total of 87 adolescents and young females who had confirmed and untreated cervical intraepithelial neoplasia 2 (CIN2) lesions. The goal of this study was to find out the ability of vaginal microbiota to accelerate the regression rate of CIN2 lesions within 24 months' time period. The study results recommended that we can determine the disease state of women according to the composition of the vaginal microbiota and may use it as preventive and curative treatment options. The study confirmed that Lactobacillus predominant vaginal microbiota at baseline ensures a regressive disease state within 12 months. Women with a deficit state of vaginal Lactobacillus spp. and an abundance of various anaerobes (*Prevotella timonensis*, *Gardnerella vaginalisare*, *Megasphaera*) may reduce the chance of regression from the CIN2 state.

4.5 Experiment on Three Groups of Women

Audirac-Chalifour[18] took a total of 32 female candidates for their experimental study cases for this study. The study divided the total population into three groups, The first one was females who had non-cervical lesions (NCL) including 10 HPV-negative and 10 HPV-positive cases. Four HPV-positive women with squamous intraepithelial lesions (SIL) came under the second group and eight HPV-positive women with cervical cancer (CC) were listed under the third group. The cervical microbiota of these women was analyzed by 16S rDNA amplicons. Significantly different vaginal microbiota diversity was observed between the three groups. Statistically significant phylogenetic diversity was observed in both cases, between SIL and HPV negative NCL (p: 0.006) and between CC and HPV negative NCL (p: 0.036).

Lactobacillus crispatus and *Lactobacillus iners* were the most frequently observed strains within the vaginal microbiota of women with normal cervical cytology. *Sneathia* spp. and *Fusobacterium* spp. were abundantly present among women with SIL and CC respectively. Women with HPV-negative and NCL had also enough *Gardnerella vaginalis* within their microbiota and like *Lactobacillus* strains, the quantity of *Gardnerella vaginalis* was also reduced in case of HPV infection, SIL, and CC.

4.6 Importance of Healthy Vaginal Microbiota in Decreasing Cervical Cancer

So,[19] selected 50 (including high-risk HPV positive and 10 high-risk HPV negative women) women between the age group of 20 and 50 for cervicovaginal sample collection. Increased vaginal microbial diversity, anaerobic pathogenic bacteria like *Gardnerella vaginalis*, *Porphyromonas uenonis,* and *Peptostreptococcus anaerobius,* and a lower amount of *Lactobacillus crispatus* strain were commonly identified within the vaginal microbiota of HIV-infected women with CIN and cervical cancer compared to normal women. Among all vaginal anaerobic bacterial strains, *Gardnerella vaginalis* mostly encouraged CIN2/ CIN3 and cervical cancer development and other microbes like *Atopobium vaginae, Finegoldia magna*, *Dialister invisus*, *Prevotella buccalis Prevotella timonensis*were also enhanced the CIN2/ CIN3 and cervical cancer risks. At the end of the study, experts advised that vaginal microbiota may decrease cervical cancer risks by managing healthy vaginal microbial balance and reducing HPV infection prognosis.

4.7 Impact of the Number of Healthy Vaginal Microbiota Over Cervical Intraepithelial Neoplasia

Mitra[20] wanted to test the effect of increasing vaginal microbiome diversity on the severity of cervical intraepithelial neoplasia. The study ensured that *Peptostreptococcus anaerobius* (p<0·05), *Anaerococcus tetradius* (p<0·05), and *Lactobacillus jensenii* (p<0·01) were abundantly present among women with high-grade squamous intraepithelial lesions than the women with low-grade squamous intraepithelial lesions. The severity of cervical intraepithelial neoplasia is associated with high levels of microbiome diversity and decreased quantity of *Lactobacillus* strains in vaginal microbiota and the association is not dependent on HPV status.

4.8 RelationAmong *Gardnerella Vaginalis, HPV, Lactobacillus Gasseri & Lactobacillus Iners*

McKee[21] reported a highly diverse vaginal microbial community and abundance of *Gardnerella vaginalis* in the women who had high-risk human papillomavirus infection or abnormal cervical cytology compared to women with normal cytology. Less amount of *Lactobacillus gasseri* and a high amount of *Lactobacillus iners*were also noticed among these female individuals. The study group observed *Lactobacillus* spp. predominant vaginal microbiota among women who had normal cervical cytology.

4.9 Biomarker and Prediction of Cervical Intraepithelial Neoplasia 1 (CIN1)

An experimental study conducted by Lee[22] confirmed the presence of various *Streptococcus* strains such as Streptococcus agalactiae**,** *Streptococcus anginosus***,** *Streptococcus canis*, *Streptococcus massilensis*, *Streptococcus*

vestibularis, and *Lactobacillus iners* were present within the vaginal microbiota of the women with CIN. A Random Forest (RF) model was formed to identify the biomarkers/ strains that enhance the severity of cervical intraepithelial neoplasia among women. Within 33 bacterial strains *Lactobacillus iners* was recognised as the most impactful biomarker to predict the severity of CIN. Some other predictor strains were *Streptococcus agalactiae*, *Lactobacillus johnsonii*, *Streptococcus canis*, *Streptococcus anginosus*.

4.10 Role of *Lactobacillus Plantarum* on Cancer Cell

Nami[23] wanted to investigate the beneficial roles of vaginal microbiota The study results clearly indicated the effect of *Lactobacillus plantarum* on cancer cells. The isolated strain had probiotic properties including resistance towards high bile salt and low pH, antimicrobial activity against harmful microbes, and antibiotic susceptibility. The study also analyzed the anticancer properties of the *Lactobacillus plantarum* strain by using various cell lines such as HeLa cells for cervical cancer, AGS for gastric cancer, MCF-7 for breast cancer, and HT-29 for colon cancer. Anticancer activities were confirmed in this study ($P \leq 0.05$). The study also proved that *Lactobacillus plantarum* had no cytotoxic effect ($P \leq 0.05$) on HUVEC normal cells with more than 93 % normal growth.

4.11 Association Between Cervical Intraepithelial Neoplasia and Cervical Microbiota

A Korean study, performed by Oh[24] analysed the relationship between cervical intraepithelial neoplasia and cervical microbiota and concluded that *Gardnerella vaginalis*, *Atopobium vaginae, Lactobacillus iners, and deficiency of Lactobacillus crispatus inside the cervical microbiota* enhanced CIN risk significantly. Among those pathogens abundance of *Atopobium vaginae within the cervical microbial environment contributed major risk of CIN. The findings of the experiment cleared that a combination of* microbial dysbiosis within the cervix and oncogenic HPV can be an impactful risk factor for cervical neoplasia.

4.12 Comparison of Cervical Cancer Cells' Reactions to Vaginal Lactobacilli in Normal and Malignant Cells

Another study performed by Motevaseli[25] showed the cytotoxic effect on cervical tumor cells but not on normal cells and the cytotoxic effect of vaginal *Lactobacillus* strains was not dependent on pH and lactate. The study also detected Lactobacilli culture supernatants were more successfully decreased tumour cell growth compared to lactate and pH-adjusted controls but cell apoptosis responses were decreased (because cell apoptosis hindered by hCG) by *Lactobacilli* culture supernatants with consistently high levels of HCG 332 β expression.

4.13 Impact of a Higher Number of *Gardnerella Vaginalis on* Vaginal *Lactobacillus* Strains

Another study by Kovachev[26] included 32 female cervical cancer (FIGO I stage) patients (between 38–55 years) for their study to check the present condition of their vaginal microbiota. Out of 32 patients, 23 patients (71.9 %) had disturbed vaginal microbiota. Obligate anaerobes (46.9%), *Streptococcus* spp., *Candida albicans* (3.1 %), *Trichomonas vaginalis* (3.1 %), were mostly responsible for the disturbed vaginal microbiota. This study also explained the fact that microbial dysbiosis due to the presence of unwanted anaerobic bacteria, reduced concentration vaginal *Lactobacillus* strains, aerobic vaginitis, and several sexually transmitted infections can increase the rapidness of cervical neoplasia and cervical cancer development among women with HPV.

4.14 Through the Overexpression of E-cadherin, Lactobacilli Prevent Cervical Cancer Cell Migration *in Vitro* and Lessen Tumour Burden *in Vivo*.

Li[27] proved the ability of *Lactobacillus* to reduce cervical cancer cell migration by up-regulating E-cadherin expression in U14 and HeLa cells ($p<0.05$). On the other hand, inactivated lactobacilli were not able to alter E-cadherin expression in HeLa and U14 cells. *Lactobacillus* treatment in a mouse model showed that it could be more effective in reducing the volume and weight of the existing tumors ($p<0.01$) than the PBS-treated group, at the same time, inactivated *Lactobacilli* had no effect on mice model with U14 tumor ($p>0.05$).

5. EFFECT OF VAGINAL *LACTOBACILLI* PRODUCED BACTERIOCINS ON CERVICAL CANCER

Bacteriocins are bacteria that produce small cationic peptide molecules that contain approximately 30–60 amino acids and show stability in high temperatures[28]. During the primary growth phase, wide varieties of bacterial strains produce antimicrobial bacteriocins by ribosomal synthesis. Bacteriocins show antimicrobial activity at higher concentrations compared to natural concentrations. Bacteriocins exhibit antimicrobial potentiality by pore formation, damaging the membrane function and cell division mechanism[9]. In vivo, the study confirmed that *Lactobaccilusplantarum* NCIMB8826 produced bacteriocin, plantaricin EF (class IIb bacteriocin) can exhibit IL-6 and TNF-α levels in the intestine of mouse[8]. *Lactobaccilus* use specific immunity proteins to defend themselves from their secreted bacteriocins[28]. Bacteriocins effectively prevent various plant pathogens and protect plants from harmful microbial attacks. Bacteriocins exert anticancer properties by targeting and interacting with host cell membranes.

Bacteriocins have a cationic charge that attracts and attaches to the targeted host cell membrane due to the negative charge on the cell membrane. Bacteriocins can stop the replication cycle of a virus particle by blocking the receptor sites of the virus particle[29].

5.1 Bacteriocin-like Compound Generated by *Lactobacillus Fermentum* Found and Partially Characterised

Sabia[30] isolated different *Lactobacillus* species from vaginal swab samples and finally selected *Lactobacillus fermentum* CS57 for Crude filtrate supernatant fluid (CFSF). After 4 hours of incubation at pH 6, bacteriocin-like substances (BLS) production (40 AU/ml) was started from *Lactobacillus fermentum* CS57. After 16 h of incubation, at pH 5 *Lactobacillus fermentum* CS57(concentration was 6.210^9cfu/ ml) produced the maximum amount of BLS which was 320 AU/ ml. Crude filtrate supernatant fluid (CFSF) of *Lactobacillus fermentum* CS57 had a broad spectrum of inhibitory activity including against *Streptococcus agalactiae* (most powerful inhibition activity), *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes,* and *Candida albicans* but did not show any effect against Gram-negative microbes. The bactericidal property of this bacteriocin-like substance was destroyed after adding proteinase K and trypsin into it, which confirmed the proteinaceous character of the bacteriocin-like substance. The bactericidal activity of the bacteriocin-like substance was not hindered by urease and catalase. Heat treatment for 30 min at 100 ^{0}C temperature affected the antimicrobial activity of the bacteriocin-like substance but after autoclaving, the activity was destroyed. This bacteriocin-like substance could resist the pH from 3.0 to 7.0.

The study group put this bacteriocin-like substance into class III bacteriocins. This study suggested that vaginal lactobacillus-produced bacteriocin/ bacteriocin-like substance can be an effective antimicrobial treatment against various vaginal opportunistic microbes and pathogens when combined with a vaginal acidic environment and great redox potentiality. Bacteriocin/ bacteriocin-like substances can also prevent lower concentrations of vaginal Lactobacillus-associated vaginal infections.

5.2 The Biological Characteristics and Bacteriocin-like Inhibitory Compounds Produced by Human Vaginal Lactobacillus sp. Strains

In one study, Fuochi[31]selected ten *Lactobacilli* cell-free supernatants due to their higher bactericidal capacity with minimal inhibitory concentrations. All chosen *Lactobacillus* strains had the potential to inhibit both Gram-negative and positive pathogenic bacteria. Experts also mentioned the cell-free supernatants of those selected lactobacillus as bacteriocin-like substances.

Another experimental study isolated *Lactobacillus* species (*Lactobacillus crispatus*, *Lactobacillus gasseri*) from vaginal samples and proved that growth of *Klebsiella* strain, *Staphylococcus aureus* strain, *Escherichia coli* strain, *Enterococus faecalis* strain, *Candida parapsilosis* were effectively decreased by *Lactobacillus* strains. Further well-diffusion test was performed with the (CFS) of effective isolates. Cell-free supernatants (CFS) had also minimal activity as an antimicrobial agent. PCR sequencing confirmed that the L. gasseri G7 strain had genes encoding the bacteriocin, gassericin T and bacteriocin, acidocin LF221A encoded genes were present in *Lactobacillus gasseri*G3, *Lactobacillus crispatus* G4, and *Lactobacillus gasseri* G7 strain. Further, *Lactobacillus gasseri* G3, *Lactobacillus crispatus* G4, and *Lactobacillus gasseri* G7 strains were taken for partial characterization of their antimicrobial substance and results of the experiment detected that autoclave treatment destroyed the antimicrobial activity of those three mentioned lactobacilli CFS.

As per the results, experts confirmed the existence of bacteriocin-like substances within those Lactobacillus strains. Sequencing of the bacteriocin production associated genes of *Lactobacillus gasseri* G3, *Lactobacillus crispatus* G4, and *Lactobacillus gasseri* G7 strain proved the presence of gassericin A gene in the *Lactobacillus crispatus* strains genome for the very first time by Stoyancheva[32].*Lactobacillus gasseri* can destroy pathogenic bacteria directly by releasing some antimicrobial compounds including bacteriocins.

Experts isolated *Lactobacillus gasseri* EV1461 from the vaginal sample of a healthy female and showed that the L. gasseri EV1461 strain could produce a new bacteriocin, called gassericin E (GasE). A study performed by Maldonado used the new bacteriocin gassericin E (GasE) for their further experiments. The findings of the study confirmed the wide spectrum of inhibitory activity of this new bacteriocin. Bacteriocin gassericin E (GasE) targets all the strains, related as well as not related to the producing strain. *Lactobacillus gasseri* EV1461 itself reduced bacterial vaginosis causing pathogenic microorganisms by producing antimicrobial compound**,** bacteriocin, gassericin E (GasE). Another important finding of this in vitro study was that *Lactobacillus gasseri* EV1461 only produced bacteriocin, gassericin E (GasE) when present at higher concentration in the broth medium, and at lower concentration of *Lactobacillus gasseri* EV1461 in the broth stopped further gassericin E (GasE) production[33]. Anti-cancerous role of Flavonoids, Catechin, β-sitosterol, and Lignin Glycosides from Saraca *asoca* (Ashoka) have been discussed with special reference to the female reproductive system[34]. Researchers from many nations studied the antineoplastic effects of sulforaphane on HeLa cells by altering signalling pathways and epigenetic pathways, and they demonstrated how chemical interventions[35] can reduce the risk of cancer. Nanotechnology can completely alter cancer treatment by providing cutting-edge options for medication delivery, diagnostics, and imaging, among other problems. While nanotechnology cannot be used to cure cancer directly through food, it can be used to improve the efficacy of cancer treatment when added to medicinal formulations or medical equipment[36] .

6. AIM AND SIGNIFICANCE

The Impact of Vaginal Microbiota on Cervical Intraepithelial Neoplasia, Human Papilloma Virus (HPV) Infection, and Cervical Cancer Prevention" aims to investigate the relationship between the composition of vaginal microbiota and the development of Cervical Intraepithelial Neoplasia (CIN), HPV infection, and its potential implications for cervical cancer prevention. Let's break down the significance and objectives of this study:

6.1 Understanding Vaginal Microbiota

The study seeks to understand the complex microbial communities that inhabit the vaginal environment. Vaginal microbiota plays a crucial role in maintaining vaginal health and overall well-being. The composition of these microbiota can vary among individuals, and understanding these variations is vital.

6.2 Cervical Intraepithelial Neoplasia (CIN)

CIN refers to abnormal changes in the cells on the cervix, which can be a precursor to cervical cancer. Investigating the impact of vaginal microbiota on the development of CIN is significant because it can help identify potential risk factors and preventive measures.

6.3 Human Papillomavirus (HPV) Infection

HPV is a well-known risk factor for cervical cancer. This study aims to explore the relationship between the composition of vaginal microbiota and the risk of HPV infection. Identifying how vaginal microbiota influence HPV infection can provide insights into cervical cancer prevention strategies.

6.4 Cervical Cancer Prevention

Ultimately, the study aims to contribute to the prevention of cervical cancer. By understanding how vaginal microbiota may impact CIN and HPV infection, researchers can potentially identify interventions or treatments that could reduce the risk of cervical cancer. This is particularly important because cervical cancer is a major cause of morbidity and mortality among women worldwide.

6.5 Personalized Medicine

The study may also lead to personalized approaches for cervical cancer prevention and management. If specific patterns of vaginal microbiota are associated with increased or decreased risk, healthcare providers could tailor screening and preventive strategies for individual patients.

6.6 Public Health Impact

Cervical cancer is a significant public health concern. Understanding the role of vaginal microbiota in its development could have far-reaching implications for healthcare policies, screening programs, and vaccination strategies (e.g., HPV vaccines).

7. MECHANISM OF ACTION OF BACTERIOCINS

Bacteriocins are small proteinaceous molecules produced by certain bacteria to inhibit or kill closely related bacterial strains. They serve as a natural defense mechanism, allowing the producing bacterium to compete for resources in a particular environment. In the context of vaginal microbiota and cervical health, bacteriocins play a role in maintaining a balanced microbial community. Here's how they exert their antibacterial effects:

7.1 Targeted Action

Bacteriocins are highly specific in their action. They typically target bacteria that are closely related to the producing strain. This specificity is essential in the vaginal environment, where maintaining a specific balance of beneficial bacteria is crucial for health.

7.2 Receptor Recognition

Bacteriocins recognize specific receptors on the surface of the target bacteria. These receptors are often components of the bacterial cell envelope, such as cell wall proteins or membrane receptors. This recognition ensures that bacteriocins bind selectively to the target bacteria.

7.3 Disruption of Membrane Integrity

Once bound to the target bacterium, bacteriocins can disrupt the integrity of the bacterial cell membrane. They may form pores or channels in the membrane, leading to the leakage of cellular components and ions. This disruption compromises the target bacterium's ability to maintain its structural and functional integrity.

7.4 Cell Death

The disruption of the cell membrane can lead to cell lysis, ultimately causing the death of the target bacterium. This is a direct antibacterial effect that helps control the population of potentially harmful bacteria in the vaginal microbiota.

8. CONCLUSION

In conclusion, this review indicated that high microbial diversity within vaginal microbiota is a key factor for persistent Human Papilloma Virus (HPV) infection and related consequences including cervical cancer in women. In addition, the elimination of Lactobacilli strains inside the vaginal microbiome is significantly associated with HPV infection, Cervical Intraepithelial Neoplasia (CIN), and, cervical cancer development. Some unique char characteristics of antibacterial activity due to the production of organic acids, hydrogen peroxides (H_2O_2), and bacteriocins, modulate host immunity, and direct interaction with pathogens followed by cell lysis are present behind the pathogens-reducing properties of vaginal Lactobacillus strains. Lactobacillus strains in the predominant vaginal microbial environment consequently enhance health and further reduce colonization, and vaginal dysbiosis, blocking HPV infection and cervical lesions progression into severe cervical cancer stage.

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