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Microbiota dysbiosis is associated with HPV-induced cervical carcinogenesis

WOJCIECH KWASNIEWSKI[1], MARIA WOLUN-CHOLEWA[2], JAN KOTARSKI[1], WOJCIECH WARCHOL[3], DOROTA KUZMA[4], ANNA KWASNIEWSKA[5] and ANNA GOZDZICKA-JOZEFIAK[4]

[1]Department of Gynaecological Oncology and Gynaecology, Medical University of Lublin, 20-081 Lublin; [2]Department of Cell Biology, Poznan University of Medical Sciences, 60-806 Poznan; [3]Department of Biophysics, Poznan University of Medical Sciences, 60-780 Poznan; [4]Department of Molecular Virology, Adam Mickiewicz University, 61-614 Poznan; [5]Department of Obstetrics and Gynaecology, Medical University of Lublin, 20-081 Lublin, Poland

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Abstract. Cervical microbial communities serve a crucial role in the persistence and development of oncogenic human papilloma virus (HPV) infections. In the present study, the authors hypothesised that disturbed heterogeneity of microbial flora was associated with HPV-induced carcinogenesis. Swabs of the cervical microbiota were collected from 250 women and the 16S ribosomal DNA was sequenced using a high throughput assay. The swabs of cervical microbiota were grouped according to the community state types (CSTs) as follows: Healthy cervical swabs; swabs taken from low-grade squamous intra-epithelial lesions (LSIL) and swabs taken from high-grade squamous intra-epithelial lesions (HSIL). Analysis of the bacterial classes revealed that the CST cervical swabs of the volunteers were characterised by *Lactobacillus crispatus*, *Lactobacillus iners* and *Lactobacillus taiwanensis,* however, *Gardnerella vaginalis* and *Lactobacillus acidophilus* were absent. In the CST of patients with LSIL the predominant type of bacteria was *Lactobacillus acidophilus* and *Lactobacillus iners,* however *Lactobacillus crispatus* was not detected. Swabs from CST women diagnosed with HSIL exhibited abundant *Gardnerella vaginalis* and *Lactobacillus acidophilus,* however, lacked *Lactobacillus taiwanensis*, *Lactobacillus iners* and *Lactobacillus crispatus*. The abundance of *Lactobacillus acidophilus* in swabs from the healthy women was compared with the swabs from the women with LSIL. The results of the present study indicated that the development of HPV-induced cancer is associated with a high diversity of vaginal microbiota, which is involved in the control of viral persistence, and is therefore indicative of disease prognosis.

Correspondence to: Dr Wojciech Kwasniewski, Department of Gynaecological Oncology and Gynaecology, Medical University of Lublin, Staszica 16, 20-081 Lublin, Poland
E-mail: wojciech.kwasniewski@umlub.pl

Dr Maria Wolun-Cholewa, Department of Cell Biology, Poznan University of Medical Sciences, Rokietnicka 5D, 60-806 Poznan, Poland
E-mail: doskon@ump.edu.pl

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Introduction

It has been reported that the female cervix maintains communities of microbial species, which have a symbiotic relationship with the host (1). It has been demonstrated that the female cervix is colonized by diverse microbiota, which serve a crucial role in cervicovaginal health (1). However, it has been indicated that the type of organisms present is dependent on the prevailing environmental conditions and host factors (1,2). Over the last decade, it has been indicated that the development and introduction of molecular-based technology has provided novel information regarding the composition of the vaginal-cervical flora, as well as the abnormal colonization of the genital tract by pathogens (2). The aforementioned findings may help to elucidate the microbiome of the genital tract in healthy women and in human papilloma virus (HPV)-dependent carcinogenesis.

The majority of previous studies have indicated that the cervical/vaginal microbial flora has a prevalence of *Lactobacillus* species, which produce lactic acids that maintain an acidic environment and may inhibit pathogenic growth (2-8). Specifically, lactic acid and other related acidic compounds have been reported to inhibit bacterial growth associated with bacterial vaginosis (BV), as well as viral infections (3). In addition, lactic acid has been recognized as a component of the immune defence system, as it has been demonstrated to potentiate the production of protective proinflammatory cytokines by vaginal epithelial cells, to promote the activation of T helper 17 lymphocytes, to stimulate dendritic cell maturation and induce interferon production (1). Over 120 *Lactobacillus* species have been identified and over 20 species have been identified in the vagina (2).

The dominant microorganisms in the vagina of a healthy woman during puberty have been reported to be from the

Lactobacillus genus, including *L. acidophilus*, *L. fermentum*, *L. plantarum*, *L. brevis*, *L. jenseni*, *L. casei, L. catenaforme, L. delbrueckii* and *L. salivarius* (1,2). It is considered that in the majority of cases, vaginal inflammation is not caused by novel microorganisms introduced from the outside, but instead by a disturbance to the proportion and number of microorganisms already existing in the vagina (4). Each bacterium has been reported as a potential etiological factor of inflammation, including *Lactobacillus* (4). It has been reported that anaerobic bacteria have a significantly increased pathogenic potential compared with aerobic bacteria (5,6). Factors, which have been reported to potentially disturb vaginal biocenosis include the following hormonal changes: Pregnancy, puberty, menopause and hormonal contraception, particularly using low doses of estrogen; vaginal sterilization following chemo- or antibiotic therapy; surgical conditions such as vaginoplasty, erosions, poor genitalia hygiene, including the vulva and the vagina, and lack of a regimented sex life, i.e. frequent, unprotected, lack of a regular sex life (4-6). Large variation in the number and composition of bacteria has been reported among women, and among time intervals for one woman (5,6). Therefore, attempting to evaluate the vaginal microbiome is extremely difficult.

A healthy cervicovaginal microenvironment has been reported to be characterised by high levels of different species of *Lactobacillus*, including the predominant *L. crispatus, L. iners*, *L. jensenii* and *L. gasseri*. Other species may occur occasionally (2,6). In rare cases, it has been reported that the cervix can be colonized by the same two or four *Lactobacillus* species (4). The aforementioned cases have been demonstrated to be dependent on a number of genetic and environmental factors, including nationality, diet and age (2,6). A number of previous studies have indicated that, in a significant proportion of healthy women, the *Lactobacillus* in the vagina may be replaced by other lactic acid-producing bacteria, including *Atopobium vaginae*, *Megaspharea* and *Leptotrichia* species (3). It has been indicated that an abnormal vaginal microenvironment may be caused by sexually transmitted infection (3). It has been reported that trichomoniasis may be caused by colonization with a microorganism not commonly identified within vaginal colonies, including *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Listeria* monocytogenes (6,7). In addition, it has been reported that an abnormal microenvironment may be caused by an invasion of an alternative organism, which is a component of the normal vaginal flora, including *Escherichia coli* (3). Bacterial vaginosis has been reported as a disorder characterised by a decrease in the quality or quantity of *Lactobacillus* and the growth of *Mycoplasma hominis*, *Gardnerella vaginalis*, *Mobiluncus* species, *Neisseria gonorrhea*, *Trichomonas vaginalis*, *Chlamydia trachomatis* and *Prevotella* species (4,5). A previous meta-analysis reported a positive association between cervical HPV infection and BV (6,7). The reverse phenomenon of HPV as a risk factor for BV invasion has also been described (8).

It has been demonstrated that HPV is considered a principal factor responsible for the development of cervical cancer (8). However, it has been suggested that HPV infection alone does not cause cervical carcinogenesis and that other factors, such as prolonged oral contraceptive or smoking, may be involved in the disease progression (8). To determine whether the vaginal microflora is affected by one of these factors, the present study investigated the association between cervical and vaginal infections, and pre-cancerous lesions of the cervix.

Materials and methods

Patient samples. Women included in the present study were recruited from cervical cancer screening in the Department of Gynaecological Oncology and Gynaecology, Medical University of Lublin in Poland between February 2003 and August 2015. The study group consisted of 250 women. Patients whose molecular analysis indicated that they were HPV-positive [HPV(+)] were included within the analysed group (n=180), whereas the healthy women [HPV(-)] were included within the control group (n=70).

Cytology and HPV status were used to classify the patients into the following 3 groups: Control group [n=70); HPV(-)]; women with low-grade squamous intraepithelial lesions [LSIL; n=95; HPV(-)], and women with high-grade squamous intraepithelial lesions [HSIL; n=85; HPV(+)]. The mean ages of patients with LSIL and HSIL, and the control group, did not significantly differ: 35 years (range, 25-48) and 37 years (range, 29-48) compared with 37 years (range 21-48), respectively (P>0.05). The present study was approved by the Ethics Committee of the Medical University of Lublin (Lublin, Poland). Written informed consent was obtained from all participants in the present study and the research was performed in accordance with the Declaration of Helsinki.

The patient inclusion criteria for the present study for the research and control groups were as follows: i) Cytological diagnosis of LSIL or HSIL or patients with a normal cytological swab; ii) HPV (+) or (-) status; iii) no use of oral and/or vaginal probiotics for 30 days prior to the start of the present study; iv) absence of genital tract infection during the 30 days prior to the classification of patients in the study, and v) a maximum of one sexual partner for 30 days prior to qualification testing.

The exclusion criteria for participating in the present study included the following: i) Vaginal bleeding of unknown etiology; ii) pregnancy; iii) oral contraceptive use or hormone replacement therapy; iv) cigarette smoking; v) history of other types of cancer; vi) systemic diseases; vii) diabetes, and viii) thyroid or other endocrine diseases.

Cervical swabs were collected from patients and to rule out experimental bias or random error. pooling was performed in 3 subgroups as follows: in the control group 23, 23, 24 swabs were pooled; in the LSIL group 31, 32, 32 were pooled, and in the HSIL group 28, 28, 29 swabs were pooled. The pooled cervical swabs were stored immediately at -80˚C for a maximum 12 months. The cervical cytological findings were classified according to the Bethesda system (9).

DNA isolation from the swabs. Total DNA was extracted from cells using a DNA isolation kit (QIAamp DNA kit; cat. no. 51306; Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocols.

Identification of HPV DNA. Identification of HPV-derived DNA was performed by polymerase chain reaction (PCR)

Table I. The frequency of bacteria classes in vaginal/cervical swabs.

Class	Healthy (%)	LSIL HPV(+) (%)	HSIL HPV(+) (%)
Actinobacteria	0.21	1.0	8.10
Alphaproteobacteria	0.20	0.03	0.41
Bacilli	96.27	84.00	27.69
Bacterioidia	0.01	0.01	0.00
Betaproteobacteria	0.01	0.01	0.01
Clostridia	0.09	0.16	0.20
Deltaproteobacteria	0.00	0.00	0.01
Flavobacteriia	0.01	0.00	0.03
Gammproteobacteria	0.19	8.20	61.48
Ktedonobactetria	0.01	0.00	0.00
Methanomicrobiota	0.00	0.01	0.00
Mollicutes	0.25	0.03	0.00
Nostocophycideae	0.41	0.02	0.15
Sphingobacteria	0.00	0.01	0.01
Unclassified	2.34-2.63		

LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

amplification of HPV gene sequences. The MY09, MY11, LC1 and LC2 primers (Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland) that were complementary to the genomic sequence of the predominantly diagnosed HPV types were used, as previously described (10).

Typing of 16S ribosomal DNA (16S rRNA) by PCR-amplification and next generation sequencing. The V4 hypervariable regions of 16S rRNA in bacterial genes were detected by PCR, as previously described (11). Sequencing and sequence analysis was performed at Genomed S.A, Warszawa (Poland).

Statistical analysis. The difference in the mean age of the female patients was tested using the Kruskal-Wallis test and was statistically significant if the calculated P>0.05. Using the 16S rRNA gene sequencing data, the frequency of bacteria occurrences were calculated by multiplying the total number of present bacteria with the percentage of bacterial concentration in the cervical swabs. The statistical analysis was performed using k-means cluster analysis (k-means clustering algorithm) and Statistica 12.0 software (StatSoft Inc., Cracow, Poland).

Results

Identification and cluster analysis of bacteria in the control, LSIL HPV(+) and HSIL HPV(+) groups. 16S rRNA sequence-based methods were used to identify healthy cervical microbial colonies, as well as those associated with HPV-dependent carcinogenesis. The identified microflora were grouped into CSTs as follows: Control, HPV(-); LSIL, HPV(+), and HSIL HPV(+). A total of 6,466 bacteria species, belonging to 74 classes, were identified from the vaginal/cervical swabs of the patients. The major classes observed are presented in Table I.

Identification and cluster analysis of bacterial classes in the control, LSIL HPV(+) and HSIL HPV(+) groups. Frequency analysis of bacterial classes in the CST healthy women, as well as in patients with a diagnosis of LSIL HPV(+) or HSIL HPV(+) revealed the presence of three major clusters. The results of the bacterial classes cluster analysis are presented in Table II. The bacterial classes, which were distinct from the other classes regardless of the type of diagnosis, included *Bacilli*, *Actinobacteria* and *Gammaproteobacteria* (Table II). Analysis of the frequencies of the individual classes demonstrated that the CST composition in the healthy women, as well as the women diagnosed with LSIL was formed predominantly of the *Bacilli* bacteria. In CST patients with cancer, the *Gammaproteobacteria* class was additionally detectable. Further cluster analysis, from classes to species, was performed to determine the bacterial species making up the three selected bacteria classes.

Identification and cluster analysis of bacterial species in the control, LSIL HPV(+) and HSIL HPV(+) groups. Analysis of the bacteria species in each of the three classes of bacteria revealed no cluster formation within the *Gammaproteobacteria* class. The further analysis subsequently focussed only on the *Actinobacterium* and *Bacilli* classes of bacterial species. Regardless of the type of diagnosis, the bacterial species within the analysed classes formed two clusters. The results are presented in Tables III and IV (data repository).

The *Actinobacterium* class included *Gardnerella vaginalis* and *Propionibacterium acnes* identified in the CST healthy women; *Gardnerella vaginalis* and *Actinomyces turicensis* in the CST women diagnosed with LSIL, and *Gardnerella vaginalis, Corynebacterium glaucum, Corynebacterium matruchotii, Propionibacterium acnes* and *Propionibacterium humerusii* in women diagnosed with

Table II. Three major clusters of bacteria classes in volunteers and patients with LSIL and HSIL diagnosis.

Control			LSIL HPV(+)			HSIL HPV(+)		
Class	Gr.	Dist.	Class	Gr.	Dist.	Class	Gr.	Dist.
Bacilli	1	0.00	*Bacilli*	1	0.00	*Bacilli*	1	1255.09
Actinobacteria	2	3.01	*Gammaproteobacteria*	1	0.00	*Gammaproteobacteria*	1	1255.09
Alphaproteobacteria	2	4.44	*Actinobacteria*	2	25.94	*Actinobacteria*	2	0.00
Gammaproteobacteria	2	5.32	*Bacteroidia*	2	33.90	*Alphaproteobacteria*	3	23.48
Mollicutes	2	0.38	*Clostridia*	2	30.74	*Bacteroidia*	3	2.23
Nostocophycideae	2	13.16	*Alphaproteobacteria*	3	20.47	*Betaproteobacteria*	3	1.69
Bacteroidia	3	0.14	*Betaproteobacteria*	3	2.14	*Chrysiogenetes*	3	2.48
Betaproteobacteria	3	0.14	*Chrysiogenetes*	3	2.82	*Clostridia*	3	11.07
Chrysiogenetes	3	0.50	*Deferribacteres*	3	2.78	*Deferribacteres*	3	2.53
Clostridia	3	7.07	*Deinococci*	3	2.82	*Deinococci*	3	2.48
Deferribacteres	3	0.50	*Deltaproteobacteria*	3	2.75	*Deltaproteobacteria*	3	2.06
Deinococci	3	0.50	*Epsilonproteobacteria*	3	3.25	*Epsilonproteobacteria*	3	2.37
Deltaproteobacteria	3	0.50	*Erysipelotrichi*	3	2.83	*Erysipelotrichi*	3	2.53
Epsilonproteobacteria	3	0.42	*Flavobacteriia*	3	2.26	*Flavobacteriia*	3	1.58
Erysipelotrichi	3	0.50	*Fusobacteria*	3	2.68	*Fusobacteria*	3	2.53
Fusobacteria	3	0.34	*Ktedonobacteria*	3	2.08	*Ktedonobacteria*	3	2.37
Ktedonobacteria	3	0.30	*Methanomicrobia*	3	2.56	*Methanomicrobia*	3	2.48
Methanomicrobia	3	0.42	*Mollicutes*	3	2.80	*Mollicutes*	3	7.58
Opitutae	3	0.50	*Nostocophycideae*	3	33.15	*Nostocophycideae*	3	11.59
Oscillatoriophycideae	3	0.26	*Opitutae*	3	2.82	*Opitutae*	3	2.37
Planctomycetia	3	0.50	*Oscillatoriophycideae*	3	2.42	*Oscillatoriophycideae*	3	2.32
Rubrobacteria	3	0.50	*Planctomycetia*	3	2.80	*Planctomycetia*	3	2.53
Sphingobacteriia	3	0.26	*Rubrobacteria*	3	2.82	*Rubrobacteria*	3	2.53
Synechococcophycideae	3	0,50	*Sphingobacteriia*	3	2.96	*Sphingobacteriia*	3	1.43
Synergistia	3	0,50	*Synechococcophycideae*	3	2.82	*Synechococcophycideae*	3	2.48
Thermotogae	3	0,50	*Synergistia*	3	2.82	*Synergistia*	3	2.53
Flavobacteriia	3	0,06	*Thermotogae*	3	2.80	*Thermotogae*	3	2.53

LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; Gr., cluster number; Dist., Euclidean.

HSIL, which formed clusters separate from the other bacterial species (Table III, data repository).

The cluster analysis of the *Bacilli* bacterial species revealed the presence of a CST subpopulation, which in the healthy women consisted of *Lactobacillus crispatus*, *Lactobacillus iners* and *Lactobacillus taiwanensis*. In women diagnosed with LSIL it included *Lactobacillus iners* and *Lactobacillus acidophilus,* and in women diagnosed with HSIL it included *Lactobacillus iners*, *Lactobacillus acidophilus* and *Lactobacillus crispatus* (Table IV, data repository). For further analysis, the levels of bacterial characteristics were determined in each of the analysed patient groups (Table V).

The analysis of the bacterial classes indicated that CST cervical swabs of the female patients were colonised by *Lactobacillus crispatus*, *Lactobacillus iners* and *Lactobacillus taiwanensis,* however, *Gardnerella vaginalis* and *Lactobacillus acidophilus* were almost not identified. In the CST patients diagnosed with LSIL, the predominant types of bacteria were *Lactobacillus acidophilus* and *Lactobacillus iners*, while *Lactobacillus crispatus* frequency was lower than in the control group. In CST patients diagnosed with HSIL, high abundance of *Gardnerella vaginalis*, *Lactobacillus acidophilus* was identified, however, *Lactobacillus taiwanensis*, *Lactobacillus iners* and *Lactobacillus crispatus* frequencies were lower than in the control group. The level of *Lactobacillus acidophilus* in the CST patients diagnosed with LSIL swabs were compared with swabs taken from women with HSIL.

Discussion

It has been indicated that the introduction of Next-Generation Sequencing (NGS) into research allowed for the identification of specific ecological niches for microorganisms within living organisms. This was possible due to the amplification and parallel sequencing of gene fragments, which were highly conserved among microorganisms, the most common being the 16S rRNA subunit, as well as RAD51 recombinase and inhibin subunit a (12). Highly conserved regions of the 16S rRNA gene (V1-V9) have been reported to allow for

Table III. The bacteria species of the class *Actinobacterium* forming clusters in volunteers and patients with L-SIL or H-SIL histopathological diagnosis. Gr.- cluster number, Dist.- Euclidean distance.

Control			L-SIL			H-SIL		
Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.
Gardnerella vaginalis	0,00	1	*Actinomyces turicensis*	0,00	1	*Gardnerella vaginalis*	0,00	1
Propionibacterium acnes	0,00	1	*Gardnerella vaginalis*	0,00	1	*Corynebacterium glaucum*	0,08	1
Actinomyces georgiae	0,06	2	*Actinomyces georgiae*	0,04	2	*Corynebacterium matruchotii*	0,08	1
Actinomyces neuii	0,06	2	*Actinomyces neuii*	0,20	2	*Propionibacterium acnes*	0,32	1
Actinomyces odontolyticus	0,06	2	*Actinomyces odontolyticus*	0,05	2	*Propionibacterium humerusii*	0,15	1
Actinomyces turicensis	0,06	2	*Corynebacterium amycolatum*	0,04	2	*Actinomyces georgiae*	0,02	2
Corynebacterium amycolatum	0,02	2	*Corynebacterium aurimucosum*	0,08	2	*Actinomyces neuii*	0,02	2
Corynebacterium aurimucosum	0,02	2	*Corynebacterium coyleae*	0,06	2	*Actinomyces odontolyticus*	0,06	2
Corynebacterium coyleae	0,09	2	*Corynebacterium diphtheriae*	0,04	2	*Actinomyces turicensis*	0,02	2
Corynebacterium diphtheriae	0,02	2	*Corynebacterium glaucum*	0,05	2	*Corynebacterium amycolatum*	0,02	2
Corynebacterium glaucum	0,02	2	*Corynebacterium glucuronolyticum*	0,04	2	*Corynebacterium aurimucosum*	0,02	2
Corynebacterium glucuronolyticum	0,06	2	*Corynebacterium jeikeium*	0,04	2	*Corynebacterium coyleae*	0,07	2
Corynebacterium jeikeium	0,06	2	*Corynebacterium kroppenstedtii*	0,04	2	*Corynebacterium diphtheriae*	0,02	2
Corynebacterium kroppenstedtii	0,09	2	*Corynebacterium kutscheri*	0,05	2	*Corynebacterium glucuronolyticum*	0,02	2
Corynebacterium kutscheri	*0,25*	*2*	*Corynebacterium lipophiloflavum*	*0,05*	*2*	*Corynebacterium jeikeium*	0,020883	2
Corynebacterium lipophiloflavum	*0,02*	*2*	*Corynebacterium matruchotii*	*0,13*	*2*	*Corynebacterium kroppenstedtii*	0,02	2
Corynebacterium matruchotii	*0,06*	*2*	*Corynebacterium mucifaciens*	*0,05*	*2*	*Corynebacterium kutscheri*	0,07	2
Corynebacterium mucifaciens	*0,02*	*2*	*Corynebacterium pseudogenitalium*	*0,06*	*2*	*Corynebacterium lipophiloflavum*	0,16	2
Corynebacterium pseudogenitalium	*0,06*	*2*	*Corynebacterium riegelii*	*0,05*	*2*	*Corynebacterium mucifaciens*	0,02	2
Corynebacterium riegelii	*0,02*	*2*	*Corynebacterium sundsvallense*	*0,05*	*2*	*Corynebacterium pseudogenitalium*	0,02	2
Corynebacterium sundsvallense	*0,06*	*2*	*Corynebacterium tuberculostearicum*	*0,12*	*2*	*Corynebacterium riegelii*	0,02	2
Corynebacterium tuberculostearicum	*0,09*	*2*	*Corynebacterium ureicelerivorans*	*0,06*	*2*	*Corynebacterium sundsvallense*	0,06	2
Corynebacterium ureicelerivorans	*0,06*	*2*	*Corynebacterium variabile*	*0,06*	*2*	*Corynebacterium tuberculostearicum*	0,02	2
Corynebacterium variabile	*0,06*	*2*	*Propionibacterium acnes*	*0,62*	*2*	*Corynebacterium ureicelerivorans*	0,02	2
Propionibacterium avidum	*0,06*	*2*	*Propionibacterium avidum*	*0,15*	*2*	*Corynebacterium variabile*	0,02	2
Propionibacterium granulosum	*0,02*	*2*	*Propionibacterium granulosum*	*0,05*	*2*	*Propionibacterium avidum*	0,02	2
Propionibacterium humerusii	*0,09*	*2*	*Propionibacterium humerusii*	*0,08*	*2*	*Propionibacterium granulosum*	0,02	2
Propionibacterium microaerophilum	*0,02*	*2*	*Propionibacterium microaerophilum*	*0,05*	*2*	*Propionibacterium microaerophilum*	0,09	2
Streptomyces lazureus	*0,06*	*2*	*Streptomyces lazureus*	*0,02*	*2*	*Streptomyces lazureus*	0,02	2

Table IV. The bacteria species of the class Bacilli forming clusters in volunteers and patients with L-SIL or H-SIL histopathological diagnosis. Gr.- cluster number, Dist.- Euclidean distance.

Control			L-SIL			H-SIL		
Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.
Lactobacillus crispatus	633,17	1	*Lactobacillus iners*	0,00	1	*Lactobacillus iners*	0,00	1
Lactobacillus iners	633,17	1	*Lactobacillus acidophilus*	0,00	1	*Lactobacillus acidophilus*	0,00	1
Lactobacillus taiwanensis	0,00	1	*Actinobacillus parahaemolyticus*	8,19	2	*Lactobacillus crispatus*	0,00	1
Actinobacillus parahaemolyticus	1,43	2	*Actinobacillus porcinus*	8,17	2	*Actinobacillus parahaemolyticus*	3,84	2
Actinobacillus porcinus	1,35	2	*Actinobacillus rossii*	8,17	2	*Actinobacillus porcinus*	3,88	2
Actinobacillus rossii	1,43	2	*Alkalibacillus haloalkaliphilus*	8,07	2	*Actinobacillus rossii*	3,88	2
Alkalibacillus haloalkaliphilus	1,19	2	*Alkalibacillus salilacus*	7,30	2	*Alkalibacillus haloalkaliphilus*	3,86	2
Alkalibacillus salilacus	0,47	2	*Bacillus alcaliinulinus*	8,06	2	*Alkalibacillus salilacus*	3,01	2
Bacillus alcaliinulinus	1,11	2	*Bacillus anthracis*	8,19	2	*Bacillus alcaliinulinus*	3,88	2
Bacillus anthracis	1,43	2	*Bacillus arbutinivorans*	8,20	2	*Bacillus anthracis*	3,88	2
Bacillus arbutinivorans	1,43	2	*Bacillus aryabhattai*	8,19	2	*Bacillus arbutinivorans*	3,80	2
Bacillus aryabhattai	1,35	2	*Bacillus axarquiensis*	8,20	2	*Bacillus aryabhattai*	3,78	2
Bacillus axarquiensis	1,35	2	*Bacillus azotoformans*	8,16	2	*Bacillus axarquiensis*	3,88	2
Bacillus azotoformans	1,43	2	*Bacillus benzoevorans*	8,14	2	*Bacillus azotoformans*	3,88	2
Bacillus benzoevorans	1,43	2	*Bacillus cereus*	8,15	2	*Bacillus benzoevorans*	3,88	2
Bacillus cereus	1,433	2	*Bacillus flexus*	8,18	2	*Bacillus cereus*	3,84	2
Bacillus flexus	1,43	2	*Bacillus foraminis*	8,20	2	*Bacillus flexus*	3,88	2
Bacillus foraminis	1,43	2	*Bacillus fordii*	8,19	2	*Bacillus foraminis*	3,86	2
Bacillus fordii	1,43	2	*Bacillus fortis*	8,19	2	*Bacillus fordii*	3,88	2
Bacillus fortis	1,43	2	*Bacillus ginsenggisoli*	8,16	2	*Bacillus fortis*	3,88	2
Bacillus ginsenggisoli	1,35	2	*Bacillus hackensackii*	8,20	2	*Bacillus ginsenggisoli*	3,88	2
Bacillus hackensackii	1,43	2	*Bacillus herbersteinensis*	8,19	2	*Bacillus hackensackii*	3,86	2
Bacillus herbersteinensis	1,35	2	*Bacillus horneckiae*	8,07	2	*Bacillus herbersteinensis*	3,88	2
Bacillus horneckiae	1,43	2	*Bacillus isabeliae*	8,13	2	*Bacillus horneckiae*	3,86	2
Bacillus isabeliae	1,43	2	*Bacillus koreensis*	8,19	2	*Bacillus isabeliae*	3,88	2
Bacillus koreensis	1,43	2	*Bacillus litoralis*	8,17	2	*Bacillus koreensis*	3,88	2
Bacillus litoralis	1,43	2	*Bacillus mucilaginosus*	8,17	2	*Bacillus litoralis*	3,88	2
Bacillus mucilaginosus	1,27	2	*Bacillus oleronius*	8,17	2	*Bacillus mucilaginosus*	3,78	2
Bacillus oleronius	1,43	2	*Bacillus olivae*	8,20	2	*Bacillus oleronius*	3,88	2
Bacillus olivae	1,27	2	*Bacillus oryzae*	8,15	2	*Bacillus olivae*	3,86	2
Bacillus oryzae	1,43	2	*Bacillus pseudomegaterium*	8,19	2	*Bacillus oryzae*	3,88	2
Bacillus pseudomegaterium	1,35	2	*Bacillus sonorensis*	8,15	2	*Bacillus pseudomegaterium*	3,86	2

Table IV. Continued.

Control			L-SIL			H-SIL		
Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.
Bacillus sonorensis	0,79	2	*Bacillus thermoamylovorans*	8,20	2	*Bacillus sonorensis*	3,88	2
Bacillus thermoamylovorans	1,43	2	*Brevibacillus brevis*	8,20	2	*Bacillus thermoamylovorans*	3,84	2
Brevibacillus brevis	1,35	2	*Brevibacillus centrosporus*	8,14	2	*Brevibacillus brevis*	3,80	2
Brevibacillus centrosporus	1,43	2	*Brevibacillus choshinensis*	8,02	2	*Brevibacillus centrosporus*	3,86	2
Brevibacillus choshinensis	1,43	2	*Brevibacillus formosus*	7,81	2	*Brevibacillus choshinensis*	3,78	2
Brevibacillus formosus	1,27	2	*Brevibacillus ginsengisoli*	7,78	2	*Brevibacillus formosus*	3,50	2
Brevibacillus ginsengisoli	0,95	2	*Brevibacillus invocatus*	8,18	2	*Brevibacillus ginsengisoli*	3,67	2
Brevibacillus invocatus	1,43	2	*Brevibacillus limnophilus*	8,12	2	*Brevibacillus invocatus*	3,86	2
Brevibacillus limnophilus	1,35	2	*Brevibacillus panacihumi*	8,07	2	*Brevibacillus limnophilus*	3,78	2
Brevibacillus panacihumi	0,71	2	*Brevibacillus reuszeri*	8,17	2	*Brevibacillus panacihumi*	3,68	2
Brevibacillus reuszeri	1,43	2	*Geobacillus thermoglucosidans*	8,18	2	*Brevibacillus reuszeri*	3,88	2
Geobacillus thermoglucosidans	1,35	2	*Lactobacillus acidifarinae*	8,17	2	*Geobacillus thermoglucosidans*	3,56	2
Lactobacillus acidifarinae	1,43	2	*Lactobacillus amylolyticus*	8,20	2	*Lactobacillus acidifarinae*	3,88	2
Lactobacillus acidophilus	19,47	2	*Lactobacillus antri*	19,75	2	*Lactobacillus amylolyticus*	3,86	2
Lactobacillus amylolyticus	1,35	2	*Lactobacillus apis*	7,11	2	*Lactobacillus antri*	3,54	2
Lactobacillus antri	1,43	2	*Lactobacillus brantae*	8,17	2	*Lactobacillus apis*	2,78	2
Lactobacillus apis	118,51	2	*Lactobacillus camelliae*	8,12	2	*Lactobacillus brantae*	3,69	2
Lactobacillus brantae	0,71	2	*Lactobacillus casei*	8,18	2	*Lactobacillus camelliae*	3,74	2
Lactobacillus camelliae	0,47	2	*Lactobacillus coleohominis*	8,20	2	*Lactobacillus casei*	3,88	2
Lactobacillus casei	1,43	2	*Lactobacillus crispatus*	721,14	2	*Lactobacillus coleohominis*	1,50	2
Lactobacillus coleohominis	1,43	2	*Lactobacillus diolivorans*	8,20	2	*Lactobacillus diolivorans*	3,86	2
Lactobacillus diolivorans	1,43	2	*Lactobacillus equi*	8,18	2	*Lactobacillus equi*	3,71	2
Lactobacillus equi	1,43	2	*Lactobacillus equicursoris*	8,13	2	*Lactobacillus equicursoris*	3,84	2
Lactobacillus equicursoris	1,43	2	*Lactobacillus fabifermentans*	7,99	2	*Lactobacillus fabifermentans*	3,88	2
Lactobacillus fabifermentans	1,43	2	*Lactobacillus faeni*	7,06	2	*Lactobacillus faeni*	3,27	2
Lactobacillus faeni	0,23	2	*Lactobacillus farraginis*	8,20	2	*Lactobacillus farraginis*	3,86	2
Lactobacillus farraginis	1,43	2	*Lactobacillus fermentum*	8,17	2	*Lactobacillus fermentum*	3,49	2
Lactobacillus fermentum	1,35	2	*Lactobacillus frumenti*	5,83	2	*Lactobacillus frumenti*	3,80	2
Lactobacillus frumenti	1,43	2	*Lactobacillus gallinarum*	22,36	2	*Lactobacillus gallinarum*	16,20	2
Lactobacillus gallinarum	2,23	2	*Lactobacillus gasseri*	5,54	2	*Lactobacillus gasseri*	3,57	2
Lactobacillus gasseri	1,43	2	*Lactobacillus gastricus*	8,20	2	*Lactobacillus gastricus*	3,88	2
Lactobacillus gastricus	1,35	2	*Lactobacillus gigeriorum*	6,92	2	*Lactobacillus gigeriorum*	2,30	2
Lactobacillus gigeriorum	0,55	2	*Lactobacillus guizhouensis*	8,20	2	*Lactobacillus guizhouensis*	3,88	2

Table IV. Continued.

Control			L-SIL			H-SIL		
Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.
Lactobacillus guizhouensis	1,27	2	*Lactobacillus hamsteri*	7,78	2	*Lactobacillus hamsteri*	3,72	2
Lactobacillus hamsteri	0,07	2	*Lactobacillus helveticus*	12,5	2	*Lactobacillus helveticus*	8,65	2
Lactobacillus helveticus	2,38	2	*Lactobacillus hilgardii*	8,20	2	*Lactobacillus hilgardii*	3,70	2
Lactobacillus hilgardii	1,43	2	*Lactobacillus ingluviei*	8,06	2	*Lactobacillus ingluviei*	3,88	2
Lactobacillus ingluviei	1,43	2	*Lactobacillus intermedius*	22,70	2	*Lactobacillus intermedius*	1,78	2
Lactobacillus intermedius	7,40	2	*Lactobacillus intestinalis*	8,02	2	*Lactobacillus intestinalis*	3,73	2
Lactobacillus intestinalis	1,27	2	*Lactobacillus japonicus*	6,42	2	*Lactobacillus japonicus*	3,82	2
Lactobacillus japonicus	1,59	2	*Lactobacillus jensenii*	268,38	2	*Lactobacillus jensenii*	39,95	2
Lactobacillus jensenii	1,43	2	*Lactobacillus johnsonii*	113,22	2	*Lactobacillus johnsonii*	10,03	2
Lactobacillus johnsonii	0,87	2	*Lactobacillus kalixensis*	8,16	2	*Lactobacillus kalixensis*	3,88	2
Lactobacillus kalixensis	1,43	2	*Lactobacillus kisonensis*	8,20	2	*Lactobacillus kisonensis*	3,86	2
Lactobacillus kisonensis	1,43	2	*Lactobacillus kitasatonis*	5,94	2	*Lactobacillus kitasatonis*	0,56	2
Lactobacillus kitasatonis	0,00	2	*Lactobacillus letivazi*	7,74	2	*Lactobacillus letivazi*	3,65	2
Lactobacillus letivazi	1,11	2	*Lactobacillus manihotivorans*	8,20	2	*Lactobacillus manihotivorans*	3,84	2
Lactobacillus manihotivorans	1,43	2	*Lactobacillus mucosae*	8,19	2	*Lactobacillus mucosae*	3,81	2
Lactobacillus mucosae	1,43	2	*Lactobacillus oris*	6,08	2	*Lactobacillus oris*	3,73	2
Lactobacillus oris	1,43	2	*Lactobacillus paracasei*	8,19	2	*Lactobacillus paracasei*	3,88	2
Lactobacillus paracasei	1,35	2	*Lactobacillus parafarraginis*	8,20	2	*Lactobacillus parafarraginis*	3,86	2
Lactobacillus parafarraginis	1,43	2	*Lactobacillus parakefiri*	8,19	2	*Lactobacillus parakefiri*	3,76	2
Lactobacillus parakefiri	1,43	2	*Lactobacillus paraplantarum*	7,97	2	*Lactobacillus paraplantarum*	3,88	2
Lactobacillus paraplantarum	1,43	2	*Lactobacillus pentosus*	8,89	2	*Lactobacillus pentosus*	3,66	2
Lactobacillus pentosus	1,03	2	*Lactobacillus plantarum*	7,78	2	*Lactobacillus plantarum*	3,69	2
Lactobacillus plantarum	1,03	2	*Lactobacillus reuteri*	7,26	2	*Lactobacillus reuteri*	3,88	2
Lactobacillus reuteri	1,43	2	*Lactobacillus rhamnosus*	8,04	2	*Lactobacillus rhamnosus*	3,63	2
Lactobacillus rhamnosus	0,00	2	*Lactobacillus ruminis*	8,20	2	*Lactobacillus ruminis*	3,86	2
Lactobacillus ruminis	1,43	2	*Lactobacillus salivarius*	8,20	2	*Lactobacillus salivarius*	3,77	2
Lactobacillus salivarius	1,43	2	*Lactobacillus senmaizukei*	7,43	2	*Lactobacillus senmaizukei*	3,27	2
Lactobacillus senmaizukei	0,79	2	*Lactobacillus siliginis*	8,11	2	*Lactobacillus siliginis*	3,86	2
Lactobacillus siliginis	1,35	2	*Lactobacillus similis*	8,20	2	*Lactobacillus similis*	3,76	2
Lactobacillus similis	1,43	2	*Lactobacillus suebicus*	8,18	2	*Lactobacillus suebicus*	3,84	2
Lactobacillus suebicus	1,43	2	*Lactobacillus taiwanensis*	477,97	2	*Lactobacillus taiwanensis*	156,77	2
Lactobacillus thailandensis	1,35	2	*Lactobacillus thailandensis*	8,15	2	*Lactobacillus thailandensis*	3,80	2
Lactobacillus tucceti	0,87	2	*Lactobacillus tucceti*	7,80	2	*Lactobacillus tucceti*	3,30	2
Lactobacillus ultunensis	48,75	2	*Lactobacillus ultunensis*	48,09	2	*Lactobacillus ultunensis*	45,05	2

Table IV. Continued.

Control			L-SIL			H-SIL		
Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.
Lactobacillus vaginalis	1,43	2	*Lactobacillus vaginalis*	6,75	2	*Lactobacillus vaginalis*	3,73	2
Lactobacillus versmoldensis	1,35	2	*Lactobacillus versmoldensis*	8,18	2	*Lactobacillus versmoldensis*	3,88	2
Lactobacillus zeae	0,79	2	*Lactobacillus zeae*	8,20	2	*Lactobacillus zeae*	3,86	2
Lentibacillus kapialis	0,47	2	*Lentibacillus kapialis*	7,82	2	*Lentibacillus kapialis*	3,48	2
Lentibacillus salinarum	1,43	2	*Lentibacillus salinarum*	8,15	2	*Lentibacillus salinarum*	3,81	2
Lysinibacillus boronitolerans	1,43	2	*Lysinibacillus boronitolerans*	8,17	2	*Lysinibacillus boronitolerans*	3,88	2
Lysinibacillus cresolivorans	0,87	2	*Lysinibacillus cresolivorans*	8,12	2	*Lysinibacillus cresolivorans*	3,66	2
Lysinibacillus fusiformis	1,35	2	*Lysinibacillus fusiformis*	8,20	2	*Lysinibacillus fusiformis*	3,88	2
Lysinibacillus parviboronicapiens	1,43	2	*Lysinibacillus parviboronicapiens*	8,12	2	*Lysinibacillus parviboronicapiens*	3,78	2
Lysinibacillus xylanilyticus	1,19	2	*Lysinibacillus xylanilyticus*	8,19	2	*Lysinibacillus xylanilyticus*	3,68	2
Paenibacillus caespitis	1,43	2	*Paenibacillus caespitis*	8,20	2	*Paenibacillus caespitis*	3,84	2
Paenibacillus castaneae	1,43	2	*Paenibacillus castaneae*	8,21	2	*Paenibacillus castaneae*	3,84	2
Paenibacillus cellulosilyticus	1,43	2	*Paenibacillus cellulosilyticus*	8,17	2	*Paenibacillus cellulosilyticus*	3,88	2
Paenibacillus cellulositrophicus	1,35	2	*Paenibacillus cellulositrophicus*	8,185	2	*Paenibacillus cellulositrophicus*	3,86	2
Paenibacillus cookii	1,43	2	*Paenibacillus cookii*	8,17	2	*Paenibacillus cookii*	3,88	2
Paenibacillus ehimensis	1,35	2	*Paenibacillus ehimensis*	8,16	2	*Paenibacillus ehimensis*	3,86	2
Paenibacillus elgii	1,43	2	*Paenibacillus elgii*	8,20	2	*Paenibacillus elgii*	3,86	2
Paenibacillus filicis	1,35	2	*Paenibacillus filicis*	8,21	2	*Paenibacillus filicis*	3,88	2
Paenibacillus forsythiae	1,43	2	*Paenibacillus forsythiae*	8,21	2	*Paenibacillus forsythiae*	3,86	2
Paenibacillus gansuensis	1,43	2	*Paenibacillus gansuensis*	8,21	2	*Paenibacillus gansuensis*	3,86	2
Paenibacillus ginsengagri	1,43	2	*Paenibacillus ginsengagri*	8,17	2	*Paenibacillus ginsengagri*	3,81	2
Paenibacillus jamilae	1,43	2	*Paenibacillus jamilae*	8,21	2	*Paenibacillus jamilae*	3,80	2
Paenibacillus lactis	1,43	2	*Paenibacillus lactis*	8,21	2	*Paenibacillus lactis*	3,86	2
Paenibacillus macerans	1,43	2	*Paenibacillus macerans*	8,17	2	*Paenibacillus macerans*	3,88	2
Paenibacillus mendelii	1,43	2	*Paenibacillus mendelii*	8,21	2	*Paenibacillus mendelii*	3,86	2
Paenibacillus motobuensis	1,43	2	*Paenibacillus motobuensis*	8,14	2	*Paenibacillus motobuensis*	3,88	2
Paenibacillus naphthalenovorans	1,43	2	*Paenibacillus naphthalenovorans*	8,18	2	*Paenibacillus naphthalenovorans*	3,88	2
Paenibacillus ourofinensis	1,03	2	*Paenibacillus ourofinensis*	8,07	2	*Paenibacillus ourofinensis*	3,84	2
Paenibacillus panacisoli	1,43	2	*Paenibacillus panacisoli*	8,17	2	*Paenibacillus panacisoli*	3,81	2
Paenibacillus pini	1,35	2	*Paenibacillus pini*	8,20	2	*Paenibacillus pini*	3,88	2
Paenibacillus pocheonensis	1,43	2	*Paenibacillus pocheonensis*	8,20	2	*Paenibacillus pocheonensis*	3,86	2
Paenibacillus polymyxa	1,43	2	*Paenibacillus polymyxa*	8,20	2	*Paenibacillus polymyxa*	3,80	2
Paenibacillus residui	1,43	2	*Paenibacillus residui*	8,18	2	*Paenibacillus residui*	3,88	2
Paenibacillus vortex	1,43	2	*Paenibacillus vortex*	8,17	2	*Paenibacillus vortex*	3,88	2

Table IV. Continued.

Control			L-SIL			H-SIL		
Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.	Bacteria species	Dist.	Gr.
Paenibacillus woosongensis	1,11	2	*Paenibacillus woosongensis*	8,07	2	*Paenibacillus woosongensis*	3,71	2
Paenibacillus xinjiangensis	1,43	2	*Paenibacillus xinjiangensis*	8,19	2	*Paenibacillus xinjiangensis*	3,80	2
Pontibacillus halophilus	1,27	2	*Pontibacillus halophilus*	8,02	2	*Pontibacillus halophilus*	3,68	2
Pontibacillus marinus	1,35	2	*Pontibacillus marinus*	8,19	2	*Pontibacillus marinus*	3,88	2
Streptococcus agalactiae	1,11	2	*Streptococcus agalactiae*	97,05	2	*Streptococcus agalactiae*	3,65	2
Streptococcus alactolyticus	1,43	2	*Streptococcus alactolyticus*	8,18	2	*Streptococcus alactolyticus*	3,88	2
Streptococcus anginosus	1,27	2	*Streptococcus anginosus*	10,85	2	*Streptococcus anginosus*	25,55	2
Streptococcus australis	1,43	2	*Streptococcus australis*	8,16	2	*Streptococcus australis*	3,88	2
Streptococcus bovis	1,27	2	*Streptococcus bovis*	7,46	2	*Streptococcus bovis*	3,84	2
Streptococcus cristatus	1,43	2	*Streptococcus cristatus*	8,17	2	*Streptococcus cristatus*	3,8186	2
Streptococcus fryi	1,35	2	*Streptococcus fryi*	8,07	2	*Streptococcus fryi*	3,80	2
Streptococcus gallinaceus	1,43	2	*Streptococcus gallinaceus*	8,20	2	*Streptococcus gallinaceus*	3,80	2
Streptococcus gordonii	1,43	2	*Streptococcus gordonii*	8,19	2	*Streptococcus gordonii*	3,88	2
Streptococcus infantis	1,43	2	*Streptococcus infantis*	7,96	2	*Streptococcus infantis*	3,80	2
Streptococcus intermedius	1,43	2	*Streptococcus intermedius*	8,15	2	*Streptococcus intermedius*	3,88	2
Streptococcus macedonicus	1,43	2	*Streptococcus macedonicus*	7,97	2	*Streptococcus macedonicus*	3,88	2
Streptococcus milleri	1,35	2	*Streptococcus milleri*	8,08	2	*Streptococcus milleri*	19,66	2
Streptococcus mutans	1,43	2	*Streptococcus mutans*	7,98	2	*Streptococcus mutans*	3,88	2
Streptococcus oligofermentans	1,43	2	*Streptococcus oligofermentans*	8,13	2	*Streptococcus oligofermentans*	3,88	2
Streptococcus oralis	1,43	2	*Streptococcus oralis*	8,09	2	*Streptococcus oralis*	3,80	2
Streptococcus orisratti	1,43	2	*Streptococcus orisratti*	10,78	2	*Streptococcus orisratti*	3,84	2
Streptococcus parasanguinis	1,43	2	*Streptococcus parasanguinis*	8,18	2	*Streptococcus parasanguinis*	3,88	2
Streptococcus pasteuri	1,43	2	*Streptococcus pasteuri*	8,18	2	*Streptococcus pasteuri*	3,88	2
Streptococcus pseudopneumoniae	1,27	2	*Streptococcus pseudopneumoniae*	7,73	2	*Streptococcus pseudopneumoniae*	3,88	2
Streptococcus sanguinis	1,43	2	*Streptococcus sanguinis*	8,19	2	*Streptococcus sanguinis*	3,86	2
Streptococcus thermophilus	1,35	2	*Streptococcus thermophilus*	8,20	2	*Streptococcus thermophilus*	3,88	2
Streptococcus tigurinus	1,35	2	*Streptococcus tigurinus*	7,82	2	*Streptococcus tigurinus*	3,78	2
Streptococcus vestibularis	1,27	2	*Streptococcus vestibularis*	8,03	2	*Streptococcus vestibularis*	3,47	2
Ureibacillus thermophilus	1,43	2	*Ureibacillus thermophilus*	8,19	2	*Ureibacillus thermophilus*	3,86	2
Virgibacillus byunsanensis	1,43	2	*Virgibacillus byunsanensis*	8,20	2	*Virgibacillus byunsanensis*	3,86	2
Virgibacillus salexigens	1,43	2	*Virgibacillus salexigens*	8,20	2	*Virgibacillus salexigens*	3,84	2
Viridibacillus arvi	0,87	2	*Viridibacillus arvi*	8,18	2	*Viridibacillus arvi*	3,67	2
Viridibacillus neidei	1,35	2	*Viridibacillus neidei*	8,18	2	*Viridibacillus neidei*	3,88	2

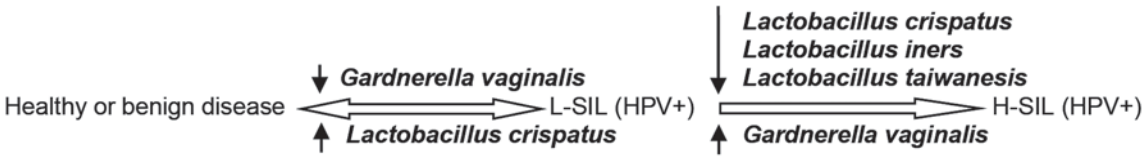

Figure 1. Proposed model for the analysis of the association between occurrence of the bacterial species and histopathological diagnosis.

Table V. Analysis of the selected bacteria species frequency in control, LSIL and HSIL diagnosed patients. Frequencies >10 have been highlighted.

Species	Control	LSIL	HSIL
Actinomyces turicensis	0	0.63	0
Corynebacterium glaucum	0.08	0	0.19
Corynebacterium matruchotti	0	0.07	0.19
Gardnerella vaginalis	11.47	46.93	304.49
Lactobacillus acidophilus	20.87	1273.62	1193.89
Lactobacillus crispatus	2506.01	405.03	245.87
Lactobacillus iners	3772.35	3183.67	1217.21
Lactobacillus taiwanensis	320.69	410.57	116.49
Propionibacterium acnes	0.88	0.42	0.48
Propionibacterium humerusii	0.16	0.13	0.14

phylogenetic and taxonomic characterisation of the analysed microbial communities (12). In the present study, the V4 hypervariable regions of 16S rRNA were used, which allowed for the identification of 3 CSTs of HSIL HPV(+), LSIL HPV(+) and the healthy group HPV(-).

In addition, >70 classes of bacteria were identified and the analysis of their frequencies demonstrated that cervical swabs of flora obtained from the volunteers and women diagnosed with LSIL HPV(+) were predominantly composed of the *Bacilli* class. The presence of *Gammaproteobacteria* and *Actinobacterium* classes in patients with HSIL HPV(+) were also detected. Further analysis revealed no cluster formation within the *Gammaproteobacteria* class and, therefore, this class was excluded from the present study.

Further analysis focussed only on the *Actinobacterium* and *Bacilli* classes of bacterial species. Regardless of the type of diagnosis, the bacterial species within the analysed classes only formed two clusters. It was observed that swabs from healthy women were characterised by an increased level of *Lactobacillus crispatus*, *Lactobacillus iners* and *Lactobacillus taiwanensis*. However, *Gardnerella vaginalis* and *Lactobacillus acidophilus* were absent. In the CST patients diagnosed with LSIL HPV(+) the predominant types of bacteria were *Lactobacillus acidophilus* and *Lactobacillus iners,* and there was no presence of *Lactobacillus crispatus* detected. The CST cervical swabs obtained from women with HSIL HPV(+) were rich in *Gardnerella vaginalis* and *Lactobacillus acidophilus*, while *Lactobacillus taiwanensis*, *Lactobacillus iners* and *Lactobacillus crispatus* were not detected. However, similar levels of *Lactobacillus acidophilus* were identified in the control and LSIL HPV(+) groups.

In the majority of healthy women in the present study, *Lactobacillus crispatus*, *Lactobacillus iners* and *Lactobacillus taiwanensis* were the dominant cervical microbiota. According to previous studies, these species were also reported to be dominant in the vaginal microbiota of Asian women (6,13). The women with the highest risk of HSIL HPV(+) indicated low levels of *Lactobacillus crispatus, Lactobacillus iners* and *Lactobacillus taiwanensis*. It has been reported that hydrogen peroxide-producing Lactobacilli are present in 96% of women with a normal vaginal bacterial community (4). In addition, species in the *Lactobacillus* genus have been reported to maintain a low pH by producing lactic acid (4). It has been reported that *Lactobacillus crispatus* was associated with a low vaginal pH compared with *Lactobacillus iners*, suggesting that these two species differ in ecological function (14). The cervical swabs obtained from healthy women consisted mainly of *Lactobacillus* bacteria. Due to their presence, the physiological pH value (3.6-4.5) was achieved by fermentation of epithelial glycogen. In addition, it has been reported that *Lactobacillus* bacteria have a high adherence potential, which allows them to adhere tightly to the vaginal epithelium and cover its surface, protecting the vagina from colonisation by pathogenic microorganisms (15-17). A number of *Lactobacillus* species can form biofilms and produce bacteriocins and hydrogen peroxide, which together have been reported to inhibit the development of undesirable anaerobes in the vagina (15,16). *Lactobacillus iners* has been previously reported to become a predominant part of the microbial community in the vaginal microbiota transition between abnormal and normal states (17). HPV infection can alter the mucosal metabolism and host immunity, and can induce changes in the vaginal microbiota (18).

It has been reported that microbe-induced inflammation can contribute to cervical cancer by stimulating the production of specific cytokines and chemokines, which promote proliferation and/or inhibit apoptosis (18,19). Microbiota have been demonstrated to increase, as well as decrease susceptibility to HPV infection (18,19). An association between vaginal microbiota and HPV infection was previously described by Gao *et al* (19). They observed a significant presence of *Lactobacillus* species, including *Lactobacillus gallinarum*, *Lactobacillus iners* and *Lactobacillus gasseri* in all women as well as *Lactobacillus gasseri* and *Gardnerella vaginalis* in HPV(+) women. The reduced population of the *Lactobacillus species* and the presence of *Fusobacteria* species in HPV(+) patients was also observed by Lee *et al* (6). The present study demonstrated that bacterial communities in the cervix are more complex than previously thought. The analyses suggest an association between HPV infection and decreased abundance of *Lactobacillus* species and increased abundance of *Gammaproteobacteria* anaerobes.

The aforementioned results are similar to those reported by Lee *et al* (6) and Dareng *et al* (8). The changes to the 'core microbiome' may be associated with changes in human health and risk of exposure to HPV infection and cervical cancer development.

In summary, analysis of the association between the occurrence of bacterial species and the histopathological diagnosis in the analysed population revealed that the vaginal microbiota may be clustered into two groups. Cluster 1 was predominantly affected by *Lactobacillus crispatus*, *Lactobacillus iners* and *Lactobacillus taiwanensis* and Cluster 2 predominated by *Gardnerella vaginalis*. The frequency of *Lactobacillus acidophilus* was identical in the clustered groups. The proposed mechanism of a cervical cancer formation may start with a sexually transmitted carcinogenic HPV infection (Fig. 1).

The fastest rate of HPV clearance may occur in women with Cluster 1 bacteria as the dominant microbiota as >50% of all infections were cleared within a year. The lowest clearance rate may occur in women classified with Cluster 2 dominant microbiota. In the aforementioned patients the chance of HSIL(+) development gradually increases, representing the growth of a clonal high-grade lesion up to a number of years following HPV infection. It was recently demonstrated that *Gardnerella vaginalis*, a dominant Cluster 2 bacterium, was able to adhere to and displace precoated protective *Lactobacilli* from vaginal epithelial cells, while other BV-associated anaerobes, including *Atopobium vaginae,* were less virulent (20).

The findings of the present study demonstrated a possible interaction between bacterial flora and HPV infection, as well as an association between this interaction and clinical cervical neoplasia. It was observed that bacterial dysbiosis, characterized by a predominance of *Gardnerella vaginalis* and a concomitant paucity of *Lactobacillus crispatus*, *Lactobacillus iners* and *Lactobacillus taiwanensis*, may be an HPV-dependent cofactor for cervical neoplasia development. However, without continuous observation it is difficult to confirm that microbiota dysbiosis contributes to HPV infection and carcinogenesis. Future researches are required to confirm the results of 16S rRNA sequencing and determine that microflora dysbiosis may be associated with HPV-induced cervical carcinogenicity.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request. The datasets presented in Tables III and IV can be found online at the following webpages: http://www.katbiolkom.ump.edu.pl/wp-content/uploads/2018/07/table_III.docx and http://www.katbiolkom.ump.edu.pl/wp-content/uploads/2018/07/table_IV.doc x (The repository of the University of Medical Sciences, Poznan, Poland).

Authors' contributions

WK conceived the study, collected samples, wrote the materials and methods and edited the manuscript. MWC analyzed the data and wrote the results and discussion sections. JK conceived the study, collected samples and contributed resources. DK performed laboratory assays. WW conceived the study, prepared figures and wrote background information. AK conceived the study, collected samples, contributed resources and approved the final draft. AGJ conceived the study, contributed resources, collected samples and approved the final draft.

Ethics approval and consent to participate

The investigations were approved by the Ethics Committee of the Medical University of Lublin (Lublin, Poland; Resolution of the Bioethics Committee; approval no: 0254/30/2002. Written informed consent was obtained from all individuals, and the research was performed by the principles of the Helsinki Declaration.

Patient consent for publication

Study participants provided written informed consent for the publication of any data and associated images.

Competing interests

The authors declare that they have no competing interests.

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