Original Article

Vaginal bacteriome of Nigerian women in health and disease: A study with 16S rRNA metagenomics

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ABSTRACT

Introduction: The argument on what bacteria make up healthy vagina and bacterial vaginosis (BV) remain unresolved. Black women most often are placed in grade IV vaginal communities as lacking *Lactobacillus*-dominated microbes. We sought to determine the vaginal microbiota compositions of healthy and those with BV using 16S rRNA metagenomics methods.

Materials and Methods: Twenty-eight women provided vaginal swabs for Nugent scoring. Fifteen had BV (Nugent score 7–10), whereas 13 were normal (Nugent score 0–3). DNA was extracted and 16S rRNA V4 region amplified using custom bar-coded primers prior to sequencing with MiSeq platform. Sequence reads were imported into Illumina BaseSpace Metagenomics pipeline for 16S rRNA recognition. Distribution of taxonomic categories at different levels of resolution was done using Greengenes databases. Manhattan principal component analysis was used for similarity clustering.

Results: Non-BV subjects were colonized by 12 taxonomic phyla that represent 182 genera and 357 species. Overall, 23 phyla representing 388 genera and 805 species were identified in BV subjects. *Firmicutes* represented 95% of the sequence reads in non-BV subjects with *Lactobacillus*-dominated genera and *Lactobacillus crispatus*–dominated species, followed by *Proteobacteria* (3.78%), *Actinobacteria* (0.74%), and *Bacteriodetes* (0.05%). In BV subjects, *Firmicutes* represented 59% of the classified sequence reads, followed by *Bacteroidetes* (19%), *Actinobacteria* (15.8%), *Fusobacteria* (4.08%), *Proteobacteria* (1.48%), and *Tenericutes* (1.25%).

Conclusion: Non-BV healthy Black African, Nigerian women had *Lactobacillus* genera as the predominant microbiota, contrary to published reports. The study shows that BV subjects had varying proportions of diverse bacteria similar to studies from other parts of the world.

Key words: Metagenomics; Nigerian women; vaginal microbiome.

Introduction

In the past three decades, the study of the vaginal microbial compositions of Black African women using culture-independent methods usually emanates from the Western hemisphere. These studies, mostly carried out among African-American women, are overtly extrapolated to all women of Black African origin. Antonio *et al.*[1] reported that vaginal colonization by *Lactobacillus crispatus* or *L.jensenii* was positively associated with being "white," then one wonders whether these are persistently absent in African

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Access this article online	
Website: www.tjogonline.com	Quick Response Code
DOI: 10.4103/TJOG.TJOG_67_18	

How to cite this article: Anukam KC, Agbakoba NR, Okoli AC, Oguejiofor CB. Vaginal bacteriome of Nigerian women in health and disease: A study with 16S rRNA metagenomics. Trop J Obstet Gynaecol 2019;36:96-104.

women. Previous studies conducted with microscopy found that African-American women tend to have higher Nugent scores which are associated with bacterial vaginosis (BV) and are therefore less likely to be colonized by *Lactobacilli* than women of European ancestry.[2,3]

More recently, Fettweis *et al.*[4] reported that among healthy subjects, women of European ancestry were more likely to be colonized with *L. crispatus*, *L. jensenii*, *L. gasseri*, and *Staphylococcus*; whereas African-American women were more likely to be colonized by *Mycoplasma hominis*, *L. iners*, and *Aerococcus* and a variety of strict anaerobes, including *Anaerococcus*, BVAB1 and BVAB2, *Dialister*, *Peptoniphilus*, *Coriobacteriaceae*, *Parvimonas*, *Megasphaera*, *Sneathia*, *Prevotellaamnii*, *Atopobium*, and *Gardnerella vaginalis*.

Another contentious study concluded that vaginal bacterial communities, not dominated by species of *Lactobacillus*, are common and appear normal in Black and Hispanic women, and therefore black women were generally placed in group IV vaginal communities as lacking *Lactobacillus*-dominated communities.[5] A similar study conducted in the Netherlands showed that the most prevalent vaginal microbiome (VMB) in ethnically Dutch women was *L. crispatus*; while in African Surinamese and Ghanaian women, a polybacterial *Gardnerella vaginalis* and *L. iners*–dominated VMB were a common denominator.[6]

However, the results of studies conducted in African women (not African-Americans) in an African environment appear to be different. Our earlier study with polymerase chain reaction-denaturing gradient gel electrophoresis PCR-DGGE, followed by sequencing of the amplicons, showed that most healthy Nigerian women tested were colonized by *L. iners* as the predominant species, followed by *L. crispatus* and *L gasseri*,[7,8] similar to previous findings in Swedish women.[9] Besides, a new longitudinal cohort study conducted in three African countries (Kenya, Rwanda, and South Africa) showed that among healthy women with a normal Nugent score, a stable *Lactobacilli*-dominated microbiota was observed with prevailing *L. iners*.[10] The objectives of this study using 16S rRNA metagenomics are two-folds. First, we sought to determine the vaginal bacterial composition in healthy Nigerian women with a normal Nugent score (0–3) and second, to establish VMB composition in women with a Nugent score of 7–10, a condition associated with BV.

Materials and Methods

Sample collection and data analysis

Women of reproductive age who were attending urogenital healthcare at Nnamdi Azikiwe University Teaching Hospital (NAUTH) from August 2016 to July 2017 were enrolled in the study after ethical approval from NAUTH and informed consent. Women who are menstruating or having vaginal bleeding, using any vaginal suppository drugs, diagnosed with diabetes mellitus, previously diagnosed with human immunodeficiency virus (HIV) infection, and having visible vaginal or cervical mass suspected to be cancer were excluded. Also, women below 18 years and above 55 years of age and taking antibiotics or antifungal were excluded. A total of 350 women both symptomatic and asymptomatic provided high vaginal swab (HVS) for Nugent scoring. Fifty subjects were identified as having BV by microscopy (Nugent score 7–10) and only 15 agreed to provide additional HVS for metagenomics. Among the healthy women (Nugent score 0–3), 13 provided vaginal swab for metagenomics investigation. The vaginal swabs were collected following uBiome® (uBiome Inc., California, USA) genital sample collection protocol. The vaginal swab was agitated into a uBiome tube containing lysis and stabilization buffer that preserves the DNA for transport at ambient temperature.DNA was extracted and 16S rRNA V4 region amplified using custom bar-coded primers prior to sequencing with MiSeq platform.

Sequence analysis

The raw sequences were imported into Illumina BaseSpace Metagenomics bioinformatics pipeline for clustering, quality check (QC), and read lengths. Quantitative Insights into Microbial Ecology (QIIME) pipeline was used for preprocessing of the sequences involving QIIME-UCLUST algorithms, demultiplexing, quality filtering, and16S rRNA recognition. Operational Taxonomic Unit (OTU) picking was done at 97% identity against the Silva database and RDP, LSU, SSU, and Greengenes databases for taxonomic assignments. Microbial taxonomy was generated from the nonrarefied OTU table. Distribution of taxonomic categories at different levels of resolution was done using the ribosomal RNA. In addition, the raw paired-end sequence FASTQ reads were also imported into MG-RAST pipeline for QC and sequence features. Manhattan distance metrics principal component analysis (PCA) was used for similarity clustering.

Results

Sequence characteristics

The amalgamated datasets for the non-BV subjects, on average, generated 89,220 high-quality sequences per sample totaling a mean of 3,592,056 basepairs (bp) with an average length of 152 bp (152 ± 13 bp). Post QC produced an average bp count of 305,783 bp, post QC sequence count of 2,065, mean sequence length of 148 ± 9 bp, and mean guanine–cytosine (GC) percent of 59 ± 13%. For the BV subjects, data set on average generated 54,320 high-quality sequences per sample totaling a mean of 651,840 bp with an

average length of 123 bp (123 ± 79 bp). Post QC produced an average bp count of 20,139 bp, post QC sequence count of 520, mean sequence length of 168 ± 67 bp, and mean GC percent of 54 ± 4%. A mean of 44.94% failed to pass the QC pipeline.

The 16S rRNA metagenomics reports showed that non-BV subjects had sequence reads that represented 12 phyla, with 95% of the sequence reads categorized to Firmicutes as the most abundant [Table 1]. This was followed by Proteobacteria (3.78%), Actinobacteria (0.74%), and Bacteroidetes (0.05%). In contrast, 23 phyla were identified in BV subjects, with Firmicutes representing 59% of the classified sequence reads, followed by Bacteroidetes (19%), Actinobacteria (15.8%), Fusobacteria (4.08%), Proteobacteria (1.48%), and Tenericutes (1.25%) as shown in Table 2.

The PCA plot is represented in Figure 1, showing the taxonomic genera clustering of non-BV samples in green circle and BV samples in red circle.

At the genera taxonomic level, of 418 genera identified in both non-BV and BV subjects, 30 (7.2%) were exclusively present in non-BV women, whereas 236 (56.5%) were exclusively identified in BV women. Non-BV and BV women had 152 (36.4%) genera in common [Figure 2]. At the species level taxonomic category, of 909 species identified, 104 (11.4%) were exclusive to non-BV women, 552 (60.7%) were exclusively present in BV subjects, whereas 253 (27.8%) were found in both non-BV and BV subjects [Figure 3].

The most abundant genera that occurred in high proportion in all the non-BV women was *Lactobacillus*, which accounted for 94.86% of the total sequence reads [Figure 4]. The next in abundance was *Escherichia* (2.3%) but appeared in 6 of 13 women (6/13), *Enterococcus* (0.26%; 5/13), *Pediococcus* (0.16%; 12/13), *Staphylococcus* (0.28%; 8/13), *Streptococcus* (0.17%; 9/13), and others.

The genera *Lactobacillus* accounted for 25.58% of the total sequence reads and was identified in all BV

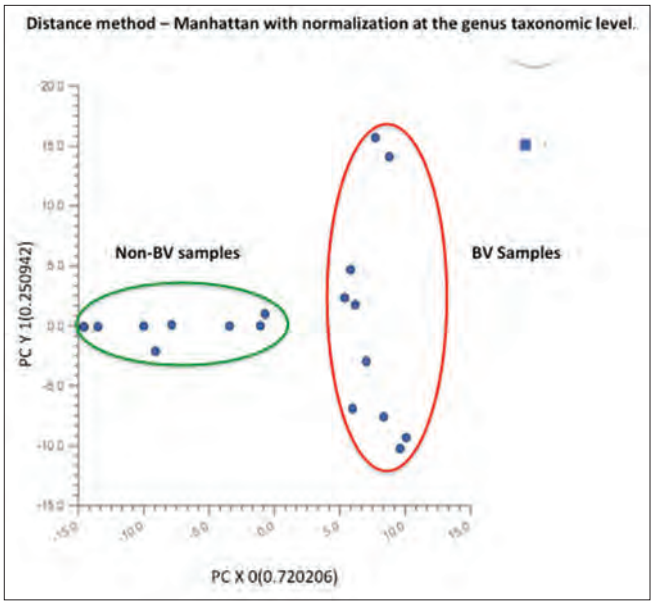

Figure 1: Principal component analysis (PCA) with Manhattan distance method showing the clustering of the genera in non-BV and BV subjects

Table 1: Phyla identified and the corresponding sequence reads and categories of taxonomic levels identified in non-BV subjects

Phylum	NV1	NV2	NV3	NV4	NV5	NV6	NV7	NV8	NV9	NV10	NV11	NV12	NV13	Total	Percentage
Firmicutes	252803	5598	1075	451	11999	33177	21698	7349	8710	4222	12350	4363	21164	384959	95.00%
Proteobacteria	1308	1032	6138	4529	67	49	48	79	13	15	38	127	112	13555	3.78%
Actinobacteria	283	201	257	24	1674	11	2	146	16	31	15	78	21	2759	0.74%
Bacteroidetes	56	4	28	1	1	77	3	13	2	12	6	16	23	242	0.05%
Tenericutes	26	1	1	0	1	8	0	0	10	0	0	6	2	55	0.01%
Spirochaetes	5	0	0	0	0	0	2	0	0	0	0	0	0	7	0.01%
Cyanobacteria	4	4	0	0	2	2	2	0	0	0	2	1	4	21	
Chlorobi	0	0	0	0	0	2	0	0	0	0	0	0	0	2	
Chloroflexi	3	0	0	0	0	0	0	1	0	0	0	0	0	4	
Thermi	0	0	0	1	0	0	1	0	0	0	0	0	0	2	
Fusobacteria	1	0	0	0	0	0	0	0	0	0	0	0	0	1	
Verrucomicrobia	0	0	0	0	0	0	0	0	3	0	0	1	0	4	
Unclassified	141263	395055	528774	441453	156720	387472	218371	21803	326222	481584	304215	59827	263474	3726233	87.73%

Non-BV subjects	NV1	NV2	NV3	NV4	NV5	NV6	NV7	NV8	NV9	NV10	NV11	NV12	NV13
Total phylum-level identified	9	6	5	3	6	7	7	5	6	4	5	7	6
Total class-level identified	15	12	10	10	11	13	12	10	9	10	13	14	12
Total order-level identified	26	17	18	19	17	21	16	15	12	16	16	25	17
Total family-level identified	50	32	35	27	29	40	25	29	21	28	24	43	32
Total genus-level identified	81	44	55	35	36	56	30	34	22	38	29	61	42
Total species-level identified	169	66	76	37	43	62	31	65	23	57	31	91	73

BV, Bacterial vaginosis; NV, Normal vagina

Table 2: Phyla identified and the corresponding sequence reads and categories of taxonomic levels identified in BV subjects

Phylum	BV1	BV2	BV3	BV4	BV5	BV6	BV7	BV8	BV9	BV10	BV11	BV12	BV13	BV14	BV15	Total	Percentage
Firmicutes	172295	290561	18767	39006	5952	193920	4625	202118	25043	9853	264789	281114	5155	46148	1227	1560573	59%
Bacteroidetes	145704	169081	12917	68787	9336	10215	1342	57881	4646	7280	369	10	479	2448	2250	492745	19%
Actinobacteria	85270	88515	13512	8519	8742	51085	4957	60878	7837	10161	29393	38194	7188	5426	240	419917	15.81%
Fusobacteria	45106	1580	1321	42502	0	408	978	8159	3962	1375	2	3	890	2280	0	108566	4.08%
Proteobacteria	1330	1318	264	739	765	2299	75	1295	584	1979	26751	1362	25	415	116	39317	1.48%
Acidobacteria	187	27	0	9	0	0	0	2	0	0	0	0	0	0	0	225	
Thermi	24	17	3	14	0	1	0	7	5	0	1	0	1	0	0	73	
Spirochaetes	18	31	7	3	2	32	0	2	4	0	23	36	0	11	0	169	
Chloroflexi	13	13	0	1	0	2	0	6	5	0	5	1	0	0	0	46	
Tenericutes	9	5396	220	1009	19	14314	65	7718	633	1585	1669	289	1	271	0	33198	1.25%
Cyanobacteria	3	0	0	0	0	10	0	10	0	0	11	8	3	0	3	48	
Fibrobacteres	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Deferribacteres	0	3	1	1	0	0	0	0	0	0	0	0	0	0	0	5	
Thermodesulfobacteria	6	3	0	2	0	6	0	0	1	0	0	0	0	0	0	18	
Thermotogae	5	2	0	1	0	2	0	2	0	0	0	0	0	0	0	12	
Chrysiogenetes	0	7	0	1	0	0	0	4	0	0	0	0	0	0	0	12	
Nitrospirae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	
Chlorobi	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	3	
Crenarchaeota	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Verrucomicrobia	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	2	
Armatimonadetes	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3	
Chlamydia	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	
Planctomycetes	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	
Unclassified reads	2786	1945	18361	10892	3452	5589	11431	262876	240330	205198	93568	91351	117	6357	378325	2654937	

BV subjects	BV1	BV2	BV3	BV4	BV5	BV6	BV7	BV8	BV9	BV10	BV11	BV12	BV13	BV14	BV15
Total phylum-level identified	14	16	9	14	6	16	6	14	10	6	11	10	8	7	5
Total class-level identified	29	31	20	24	14	30	12	29	19	14	24	20	15	16	15
Total order-level identified	54	51	37	46	21	57	23	46	34	25	55	38	22	32	23
Total family-level identified	109	101	75	79	58	110	44	89	70	52	115	78	40	67	43
Total genus-level identified	189	177	122	120	96	201	74	147	119	81	213	130	55	114	59
Total species-level identified	242	239	140	141	116	270	89	195	145	128	352	192	48	201	76

BV, Bacterial vaginosis

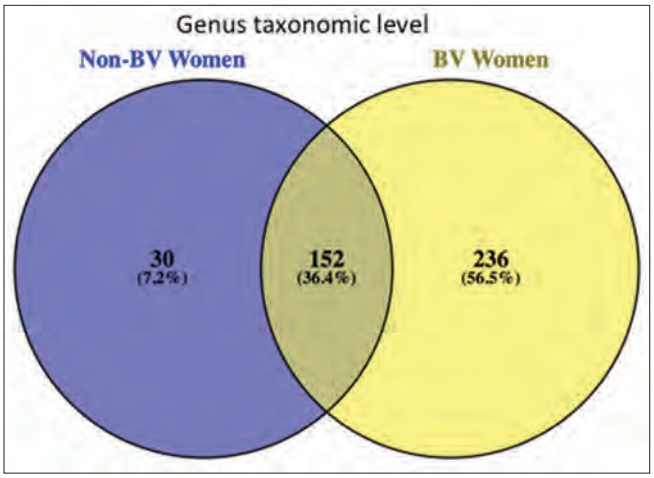

Figure 2: Venn diagram showing the number of genera-level exclusive and shared between non-BV and BV subjects

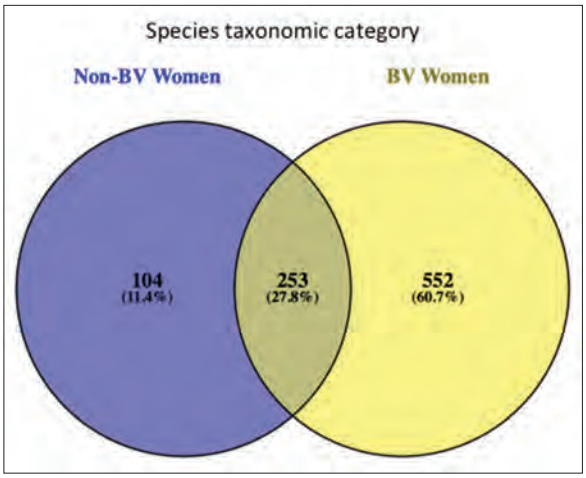

Figure 3: Venn diagram showing the number of species-level exclusive and shared between non-BV and BV subjects

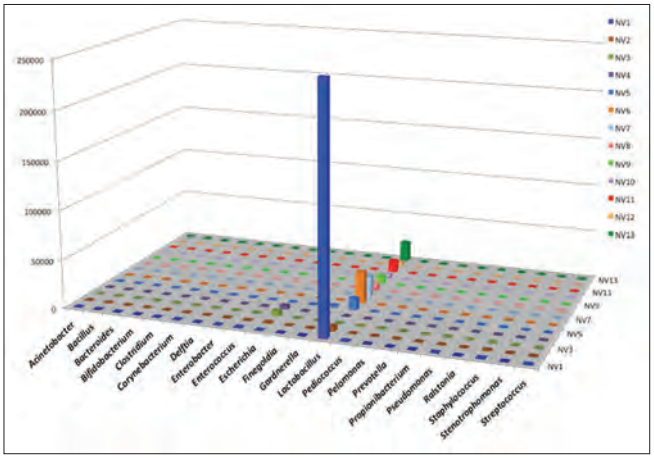

Figure 4: 3D bar chart for some genera found in healthy non-BV subjects

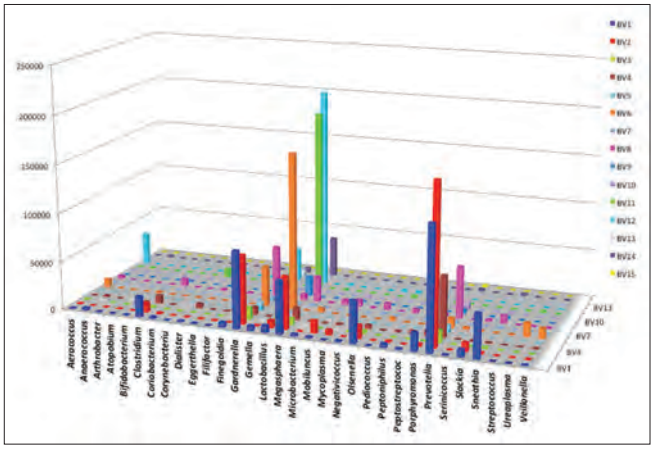

Figure 5: 3D bar chart for some genera found in BV subjects

subjects [Figure 5]. This was followed by *Prevotella* (17.77%; 15/15), *Gardnerella* (13.91%; 14/15), *Megasphaera* (6.18%; 13/15), *Olsenella* (3.02%; 13/15), *Staphylococcus* (3.82%; 7/15), *Sneathia* (2.53%; 12/15), *Clostridium* (2.02%; 15/15), *Aerococcus* (1.94%; 14/15), *Porphyromonas* (1.17%; 12/15), *Slackia* (0.85%; 14/15), *Mobiluncus* (0.76%; 7/15), *Dialister* (0.74%; 15/15), *Ureaplasma* (0.67%; 12/15), *Mycoplasma* (0.62%; 12/15), *Veillonella* (0.56%; 12/15), *Peptoniphilus* (0.47%; 14/15), *Microbacterium* (0.42%; 13/15), *Corynebacterium* (0.41%; 14/15), *Gemella* (0.38%; 11/15), *Peptostreptococcus* (0.33%; 7/15), *Pediococcus* (0.28%; 12/15), *Anaerococcus* (0.27%; 10/15), *Finegoldia* (0.24%; 9/15), *Coriobacterium* (0.14%; 13/15), *Serinicoccus* (0.12%; 12/15), *Bifidobacterium* (0.09%; 13/15), *Negativicoccus* (0.07%; 13/15), *Bacillus* (0.07%; 13/15), *Arthrobacter* (0.06%; 13/15), *Eggerthella* (0.03%; 13/15), *Anaerobranca* (0.03%; 10/15), *Streptococcus* (0.02%; 12/15), *Shewanella* (0.02%; 10/15), *Atopobium* (0.02%; 10/15), *Bacteriodes* (0.02%; 12/15), and surprisingly *Shuttleworthia*, which although found in 4 of 15 BV women accounted for 11.76% of the total sequence reads.

Of the 357 taxonomic species identified in non-BV subjects, 36 *Lactobacillus* species accounted for 96.95% of the total sequence reads. *Lactobacillus crispatus* was the most abundant species identified in 9 of 13 women and accounted for 36.13% of the sequence reads [Figure 6]. This was followed by *L. iners* (10/13; 21.25%), *L. jensenii* (8/13; 16.37%), *L. acidophilus* (6/13; 15.28%), *L. gallinarum* (7/13; 2.13%), *L. ultunensis* (7/13; 1.44%), *L. taiwanensis* (12/13; 0.82%), *L. equicursoris* (2/13; 0.54%), *L. kitasatonis* (5/13; 0.15%), and others. Eleven *Enterococcus* and four *Enterobacter* species were present in over two-third of the non-BV subjects, with *Enterococcus lactis*, *Enterococcus durans*, *Enterococcus gilvus*, and *Enterobacter amnigenus* contributing 0.28% of the sequence reads.

In contrast, among the 805 species identified in BV subjects, 42 *Lactobacillus* species accounted for 12.35% of the sequence reads. *Lactobacillus iners* (15/15; 10.45%) was the most abundant *Lactobacillus* species found in all the BV subjects [Figure 7] followed by *Lactobacillus*

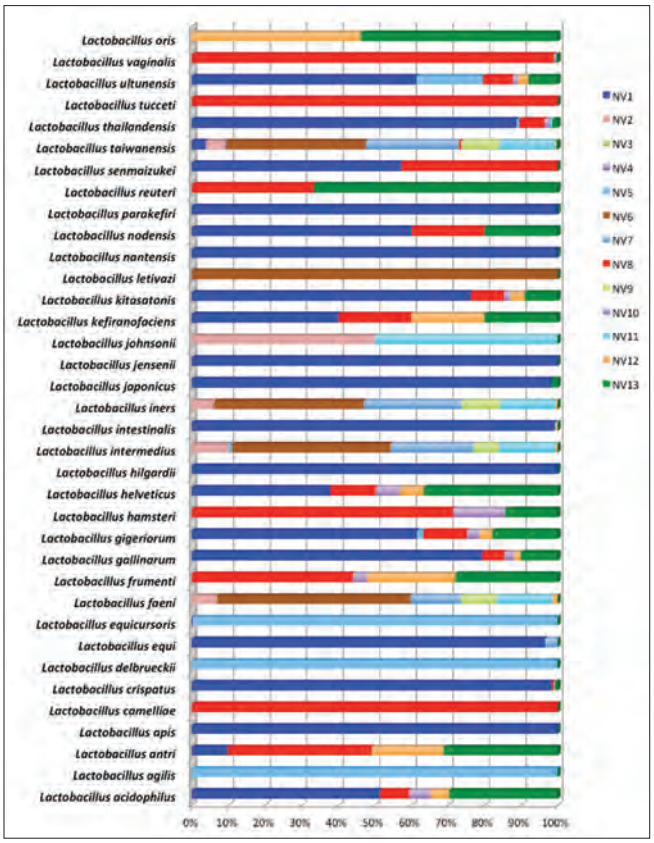

Figure 6: The relative abundances of the 36 *Lactobacillus* species (100% stacked 3D bar chart) that are found in non-BV subjects

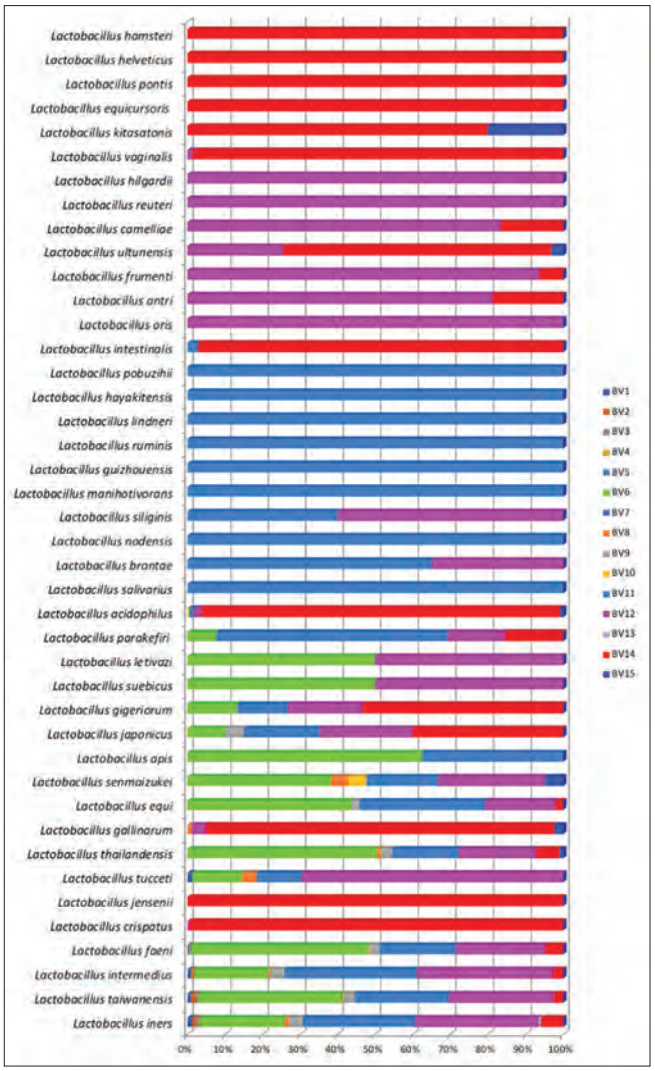

Figure 7: The relative abundances of the 42 *Lactobacillus* species (100% stacked 3D bar chart) that are found in BV subjects

taiwanensis (1.61%). Seven *Megasphaera* species were identified with *Megasphaera* sp. UPII 199-6 (12.16%) found in six subjects: *M. hominis* (0.09%; 10/15), *M. elsdenii* (0.01%; 3/15), *M. geminatus* (<0.01%; 2/15), *M.* sp. UPII 135-E (2.47%; 3/15), *M.* sp. BV3C16-1 (0.02%; 2/15), and *M.* sp. NP3 (0.02%; 2/15). There were two species of *Gardnerella*, notably *Gardnerella vaginalis* found in 14 of 15 BV subjects accounting for 5.95% of the sequence reads and *Gardnerella* sp. S3PF20 found in 8 of 15 accounting for 24.41% of the sequence reads.

Thirty-two *Prevotella* species accounted for 9.4% of the sequence reads, with *P. timonensis* (3.39%) as the most abundant species found in 13 of 15 BV subjects [Figure 8]. *Prevotella buccalis* was found in 13 of 15 subjects accounting for 2.18% of the sequence reads, followed by *P. amnii* (12/15; 1.25%), and *P. bivia* (7/15; 0.49%).

Other interesting species found in BV subject include but not limited to *Aerococcus christensenii* (14/15; 0.97%). Five *Anaerococcus* species were identified in the BV cohort, which include *A. lactolyticus* (7/15; 0.03%), *A. tetradius* (10/15; 0.06%), *A. hydrogenalis* (7/15; 0.01%), *A. vaginalis*, *A. prevotii*, and *A. octavius*. Other species in most of the BV subjects were *Atopobium vaginae* (10/15; 1.19%), *Atopobium* sp. S3MV24 (7/15; 12.16%), *Atopobium* sp. S3PFAA1-4 (7/15; 0.74%), *Bifidobacterium cuniculi* (14/15; 0.04%), *Clostridium cadaveris* (9/15; 0.09%), *Clostridium frigoris* (15/15; 0.04%), and *Coriobacterium glomerans* (13/15; 0.07%). Forty-three *Corynebacterium* species were identified in the BV subjects and accounted for less than 0.02% with *C. genitalium and C. amycolatum* occurring in most of the subjects. Six *Dialister* species accounted for 4.40% of the sequence reads with *D. micraerophilus* (15/15; 0.6%) identified in all the BV subjects. This was followed by *D. succinatiphilus* (9/15; 2.54%), *D. invisus* (8/15; 0.03%), *D. propionicifaciens* (7/15; 1.13%), *Dialister* sp. S4-23 (7/15; 0.10%), and *Dialister* sp. S7MSR5 (1/15; <0.01%). Only *Eggerthella sinensis* (0.01%) was identified in 13 of 15 BV subjects. *Finegoldia magna* (0.12%) was found in 10 of 15 subjects, while *Finegoldia* sp. S3MVA9 (0.72%) was identified in 4 of 15 subjects.

Five species of *Gemella* were identified. Notably, *G. asaccharolytica* (9/15; 3.88%) predominated, followed

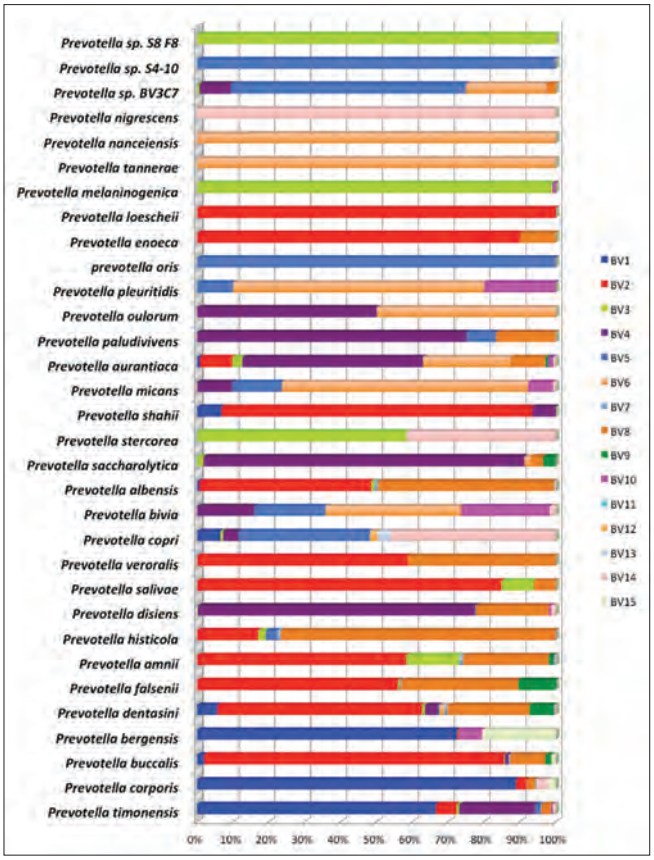

Figure 8: The relative abundances of the *Prevotella* species (100% stacked 3D bar chart) that are found in BV subjects

by *G. bergeri* (9/15; 0.15%), and *G. cunicula* (11/15; 0.01%). Among the *Mobiluncus* species, only two were found in BV subjects – *M. curtisii* (2/15; <0.01%) and *M. mulieris* (4/15; 0.30%).

Eleven species of *Mycoplasma* that included *M. edwardii* (7/15; 0.11%), *M. equirhinis* (5/15; <0.01%), *M. hominis* (8/15;0.19%), *M. insons* (4/15; <0.01%), *M. lipophilum* (7/15; <0.01%), *M. timone* (8/15; <0.01%), *M. canadense* (3/15; <0.01%), *M. haemominutum* (1/15; <0.01%), *M. lagogenitalium* (1/15; <0.01%), *M. iguana* (3/15; <0.01%), and *M. verecundum* (1/15; <0.01%) were detected in BV subjects.

BV subjects had 14 species of *Peptoniphilus*, which accounted for 1.07% of the sequence reads. Thirteen subjects had *P. asaccharolyticus* (13/15; 0.07%), *P. harei* (2/15; <0.01%), *P. coxii* (8/15; 0.02%), *P. lacrimalis* (10/15; 0.09%), *P. indolicus* (8/15; <0.01%), *P. methioninivorax* (8/15; <0.01%), *Peptoniphilus* sp. 2002-38328 (8/15; 0.44%), *Peptoniphilus* sp. BV3AC2 (5/15; 0.03%), *Peptoniphilus* sp. DNF00192 (2/15; <0.01%), *Peptoniphilus* sp.gpac018A (2/15; 0.17%), *Peptoniphilus* sp. JCM 8143 (1/15; <0.01%), *Peptoniphilus* sp. oral taxon 375 (4/15; 0.02%), *Peptoniphilus* sp. S4-A10 (3/15; 0.04%), and *Peptoniphilus* sp. S9 PR-13 (2/15; 0.14%). *Peptostreptococcus anaerobius* (9/15; 0.16%) and *Peptostreptococcus stomatis* (7/15; <0.01%) were found to colonize half of the BV subjects.

Only *Sneathia sanguinegens* (13/15; 0.50%) was found in the BV cohort, while 24 *Staphylococcus* species were found in 7 of 15 subjects accounting for 0.39% of the sequence reads. Twenty *Streptococcus* species were found in 10 of 15 BV subjects and accounted for less than 0.01% of the sequence reads, with *Streptococcus agalactiae* and *S. pseudopneumoniae* identified in 2 of 15 and 4 of 15 subjects, respectively.

Seven *Ureaplasma* species accounted for 1.62% of the sequence reads in BV subjects. *U. urealyticum* (11/15; 1.3%) and *U. parvum* (13/15; 0.24%) were high in abundance, while *U. gallorale* (7/15; 0.02%), *U. felinum* (6/15; <0.01%), *U. cati* (4/15; <0.01%), *U. canigenitalium* (1/15; <0.01%), and *U. diversum* (4/15; <0.01%) were less in abundance.

Five *Veillonella* species were identified in BV subjects, with *V. montpellierensis* found in 11 of 15 subjects accounting for 0.28% of the sequence reads. Other *Veillonella* species were less in abundance such as *V. ratti* (4/15; <0.01%), *V. magna* (6/15; <0.01%), *V. atypica* (2/15; <0.01%), and *V. criceti* (4/15; <0.01%).

Other organisms such as *Negativicoccus succinicivorans* (15/15; 0.06%), *Olsenella uli* (13/15; 1.52%), *Pediococcus cellicola* (9/15; 0.05%), *Pediococcus argentinicus* (8/15; <0.01%), *Porphyromonas uenonis* (9/15; 0.53%), *Porphyromonas asaccharolytica* (6/15; <0.01%), *Porphyromonas canis* (8/15; 0.04%), *Porphyromonas bennonis* (5/15; <0.01%), *Serinicoccus chungangensis* (13/15; 0.06%), *Arthrobacter soli* (14/15; 0.02%), and *Shewanella pneumatophori* (7/15; <0.01%), not generally associated with BV, were found in most of the BV subjects.

Discussion

We hereby report the characterized vaginal bacteriome compositions of healthy and often times annoying condition known as BV in Nigerian women. Generally, healthy non-BV women were significantly ($P = 0.05$) colonized by Firmicutes (95%) when compared with BV subjects (59%). However, the polymicrobial nature of BV was largely expressed in BV subjects as 23 different taxonomic phyla were identified in contrast to 12 phyla found in healthy non-BV women [Tables 1 and 2]. A recent review submitted that BV is polymicrobial in nature, but the etiology remains unclear as it involves the presence of a thick vaginal multispecies biofilm.[11] At the genera-level taxonomic assignments, non-BV subjects clustered together longitudinally, whereas BV genera clustered vertically as shown by the Manhattan

PCA in Figure 1. Noyes *et al.*[12] demonstrated, using Bayesian network of the associations existing between specific microbiome members, the Nugent score and vaginal pH. Taken together, the number of genera and species identified in non-BV subjects was lower than BV subjects, thus supporting previous studies that showed less abundance of microbial communities in healthy vagina. Non-BV women had higher numbers of *Lactobacillus* as shown in Figure 4, contrary to previous studies,[1,5] but in congruence with a study in a cohort of South African women showing similarity to those identified in European populations.[13] Among the non-BV subjects, there are few lactic acid–producing genera present in over two-third of the women such as *Enterococcus* and *Enterobacter*, thus signifying that *Lactobacilli* are not the only genera contributing to vaginal health. In contrast, BV subjects had avalanche of anaerobic multigenera occurring in all the tested women in conformity with other studies.[14] However, it should be noted that *Lactobacillus* was present in all BV subjects, but the caveat is that the total number of all the associated BV microbes (*Gardnerella*, *Prevotella*, *Magasphaera*, and others) outweighed or outnumbered *Lactobacillus* genera [Figure 5].

At the species taxonomic level, *Lactobacillus crispatus* was the most abundant species identified in 9 of 13 non-BV women, similar to recent studies conducted in Rwanda where they found that *Lactobacillus iners*, *L. crispatus*, and *L. gasseri* were the most abundant *Lactobacillus* species[15] in India.[16] A recent study has suggested that *Lactobacillus crispatus*, a dominant *Lactobacillus* species associated with a healthy vagina, could strongly inhibit *Candida albicans* growth, hyphal formation and regulate virulence-related gene expression,[17] and exhibit inhibitory activity against *E. coli*.[18] The maxim that *Lactobacillus*-dominated vaginal microbiota is associated with a reduced risk of acquiring and transmitting HIV and other sexually transmitted infections appears to be supporting mechanistic actions of *Lactobacillus crispatus* with protonated form of lactic acid.[19] Among the 36 *Lactobacillus* species identified in non-BV women, *L. crispatus*, *L. iners*, *L. jensenii*, *L. acidophilus*, and *L. vaginalis*, traditionally associated with healthy vagina, which is in contrast to the publication of Ravjel *et al.*[5] There are other *Lactobacillus* species found in this group of women. *L. taiwanensis*, *L. ultunensis*, *L. gallinarum*, *L. kitasatonis*, *L. thailandensis*, and *L. equicursoris* were found in over half of the healthy women [Figure 6]. The specific roles these *Lactobacillus* species play in vaginal health remain to be determined.

At the species taxonomic level, BV subjects were not completely devoid of *Lactobacillus* species. In fact, 42 *Lactobacillus* species accounted for 12.35% of the sequence reads with *Lactobacillus iners* prevailing, while non-BV group had 36 *Lactobacillus* species accounting for more than 96% of the sequence reads. The complex nature and diversity of microbiota found in BV subjects was similar to previous studies in West Africa[20] and United States.[21] This study revealed the presence of other bacterial organisms. Notable species found in over half of the BV cohort were *Aerococcus christenenii*, *Arthrobacter soli*, *Bifidobacterium cuniculi*, *Clostridium cadaveris*, *Coriobacterium glomerans*, *Gemella asaccharolytica*, *Jeotgalicoccus coquina*, *Negativococcus succinivorans*, *Olsenella uli*, *Serinicoccus chungangensis*, and *Veillonella montpellierensis*. Most of these bacteria are found in oral and gut niches. For example, *Olsenella uli* is frequently isolated from dental plaque in periodontitis patients and can cause primary endodontic infection.[22] The presence of these microbes in the vagina of BV women may suggest translocation during oral–genital sexual practices since they are infrequently found in sexually inactive women.

There are limitations associated with the interpretation of the findings, in terms of what constitute a healthy vagina as the definition of BV is still shrouded in obscurity.[23] Absence of Lactobacilli especially *Lactobacillus crispatus* may not necessarily constitute BV as some subjects had other lactic acid–producing bacteria present in the vagina. Some subjects identified as non-BV had bacterial organisms associated with BV as previously reported,[24] while some subjects identified as BV had *Lactobacillus crispatus* in varying proportions.

Conclusion

We have demonstrated that the vagina of healthy, non-BV Black African, Nigerian women is colonized by *Lactobacillus*-dominated bacterial communities, in addition to the presence of other lactic acid–producing bacteria as revealed by 16S rRNA metagenomics. Women with BV are colonized by polymicrobial communities, similar to studies done in other continents of the world. However, a future large-scale vaginal metagenomics study may be warranted.

Financial support and sponsorship

The 16S rRNA metagenomics sequencing was done by uBiome Inc. San Francisco, USA, under a grant-in-kind award to Dr. Kingsley C Anukam.

Conflicts of interest

There are no conflicts of interest.

References

1. Antonio MA, Hawes SE, Hillier SL. The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. J Infect Dis 1999;180:1950-6.
2. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial

vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991;29:297-301.
3. Fiscella K, Klebanoff MA. Are racial differences in vaginal pH explained by vaginal flora? Am J Obstet Gynecol 2004;191:747-750.
4. Fettweis JM, Brooks JP, Serrano MG, Sheth NU, Girerd PH, Edwards DJ, *et al.* Differences in vaginal microbiome in African American women versus women of European ancestry. Microbiology 2014;160:2272-82.
5. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, *et al.* Vaginal microbiome of reproductive-age women. Proc Nat Acad Sci 2011;108(Suppl 1):4680-7.
6. Borgdorff H, van der Veer C, van Houdt R, Alberts CJ, de Vries HJ, Bruisten SM, *et al*. The association between ethnicity and vaginal microbiota composition in Amsterdam, the Netherlands. PLoS One 2017;12:e0181135.
7. Anukam KC, Osazuwa EO, Ahonkhai I, Reid G. *Lactobacillus* vaginal microbiota of women attending a reproductive health care service in Benin City, Nigeria. Sex Trans Dis 2006;33:59-62.
8. Anukam KC, Osazuwa EO, Ahonkhai I, Reid G**.** Association between absence of vaginal lactobacilli PCR products and Nugent scores interpreted as bacterial vaginosis. Trop J Obstet Gynaecol 2005:22:103-7.
9. Vasquez A, Jakobsson T, Ahrne S, Forsum U, Molin G. Vaginal *Lactobacillus* flora of healthy Swedish women. J Clin Microbiol 2002;40:2746-9.
10. Jespers V, Kyongo J, Joseph S, Hardy L, Cools P, Crucitti T, *et al.* A longitudinal analysis of the vaginal microbiota and vaginal immune mediators in women from sub-Saharan Africa. Sci Rep 2017;7:11974.
11. Machado D, Castro J, Palmeira-de-Oliveira A, Martinez-de-Oliveira J, Cerca N. Bacterial vaginosis biofilms: Challenges to current therapies and emerging solutions. Front Microbiol 2016;6:1528.
12. Noyes N, Cho KC, Ravel J, Forney LJ, Abdo Z. Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis. PLoS One 2018;13:e0191625.
13. Pendharkar S, Magopane T, Larsson PG, de Bruyn G, Gray GE, Hammarström L, *et al.* Identification and characterisation of vaginal *Lactobacilli* from South African women. BMC Infect Dis 2013;13:43.
14. McMillan A, Rulisa S, Sumarah M, Macklaim JM, Renaud J, Bisanz JE, *et al*. A multi-platform metabolomics approach identifies highly specific biomarkers of bacterial diversity in the vagina of pregnant and non-pregnant women. Sci Rep 2015;5:14174.
15. McMillan A, Rulisa S, Gloor GB, Macklaim JM, Sumarah M, Reid G. Pilot assessment of probiotics for pregnant women in Rwanda. PLoS One 2018;13:e0195081.
16. Madhivanan P, Alleyn HN, Raphael E, Krupp K, Ravi K, Nebhrajani R, *et al*. Identification of culturable vaginal *Lactobacillus* species among reproductive age women in Mysore, India. J Med Microbiol 2015;64:636-41.
17. Wang S, Wang Q, Yang E, Yan L, Li T, Zhuang H. Antimicrobial compounds produced by vaginal *Lactobacillus crispatus* are able to strongly inhibit *Candida albicans*growth, hyphal formation and regulate virulence-related gene expressions. Front Microbiol 2017;8:564.
18. Ghartey JP, Smith BC, Chen Z, Buckley N, Lo Y, Ratner AJ, *et al. Lactobacillus crispatus* dominant vaginal microbiome is associated with inhibitory activity of female genital tract secretions against *Escherichia coli*. PLoS One 2014;9:e96659.
19. Tyssen D, Wang YY, Hayward JA, Agius PA, DeLong K, Aldunate M, *et al.* Anti-HIV-1 activity of lactic acid in human cervicovaginal fluid. mSphere 2018;3:pii:e00055-18.
20. Pépin J, Deslandes S, Giroux G, Sobéla F, Khonde N, Diakité S, *et al.* The complex vaginal flora of West African women with bacterial vaginosis. PLoS One 2011;6:e25082.
21. Dols JA, Smit PW, Kort R, Reid GM, Schuren FH, *et al.* Microarray-based identification of clinically relevant vaginal bacteria in relation to bacterial vaginosis. Am J Obstet Gynecol 2011;204:305e1-7.
22. Göker M, Held B, Lucas S, Nolan M, Yasawong M, Del Rio TG, *et al*. Complete genome sequence of *Olsenellauli* type strain (VPI D76D-27C^{T}). Stand Genomic Sci 2010;3:76-84.
23. Reid G. Is bacterial vaginosis a disease? Appl Microbiol Biotechnol 2018;102:553-8.
24. Shipitsyna E, Roos A, Datcu R, Hallén A, Fredlund H, *et al.* Composition of the vaginal microbiota in women of reproductive age – Sensitive and specific molecular diagnosis of bacterial vaginosis is possible? PLoS One 2013;8:e60670.