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 Bacteroidetes (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
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, Megaspheera, 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 Lactobacillus-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, and 16S 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

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**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    | 252 | 803 | 107 | 558 | 451 | 119 | 803 | 216 | 989 | 331 | 2168 | 3177 | 2619 | 7349 | 8710 | 4222 | 12350 | 4363 | 21164 | 3849 | 91559 | 95.00% |
| Proteobacteria| 130 | 103 | 103 | 613 | 452 | 67  | 49  | 48  | 79  | 13  | 15   | 15   | 10   | 12   | 38   | 127  | 112  | 1355 | 2795 | 40959 | 3.78% |
| Actinobacteria| 283 | 201 | 257 | 24  | 167 | 11  | 2   | 146 | 16  | 31  | 15   | 78   | 21   | 1355 | 10   | 112  | 127  | 112  | 3849 | 7957  | 0.74% |
| Bacteroidetes | 56  | 4   | 28  | 1   | 77  | 3   | 13  | 2   | 12  | 6   | 16   | 23   | 242  | 1355 | 10   | 112  | 127  | 112  | 3849 | 7957  | 0.06% |
| Tenericutes   | 26  | 1   | 1   | 0   | 1   | 8   | 0   | 10  | 0   | 0   | 6    | 2    | 55   | 1355 | 10   | 112  | 127  | 112  | 3849 | 7957  | 0.01% |
| Spirochaetes  | 5   | 0   | 0   | 0   | 0   | 2   | 0   | 0   | 0   | 0   | 0    | 7    | 0.01% |
| Cyanobacteria | 4   | 4   | 0   | 0   | 0   | 2   | 2   | 0   | 0   | 0   | 2    | 1    | 1    | 4    | 21   |     |      |      |      |     | 87.73% |
| Chlorobacter | 0   | 0   | 0   | 0   | 0   | 2   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 2    |     |      |      |      |     | 87.73% |
| Chloroflexi  | 3   | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |     |      |      |      |     | 87.73% |
| Thermi       | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 1   | 0   | 0    | 0    | 0    | 0    | 2    |     |      |      |      |     | 87.73% |
| Fusobacteria | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    |     |      |      |      |     | 87.73% |
| Verrucomicrobiota | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1    | 0    | 3    | 0    | 1    | 0    | 0    | 0    |     | 87.73% |

**Non-BV subjects**

| NV1 | NV2 | NV3 | NV4 | NV5 | NV6 | NV7 | NV8 | NV9 | NV10 | NV11 | NV12 | NV13 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|
| 9   | 6   | 5   | 3   | 6   | 7   | 5   | 6   | 4    | 5    | 7    | 6    |      |
| 15  | 12  | 10  | 11  | 13  | 12  | 10  | 9   | 10   | 13   | 14   | 12   |      |
| 26  | 17  | 18  | 19  | 17  | 21  | 16  | 15  | 12   | 16   | 16   | 26   | 12   |
| 50  | 32  | 35  | 27  | 29  | 40  | 25  | 29  | 21   | 28   | 24   | 43   | 12   |
| 81  | 44  | 55  | 35  | 36  | 56  | 30  | 34  | 22   | 38   | 29   | 61   | 42   |
| 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      | 10        | 479       | 2248      | 2250      | 492745    | 19%         |
| Actinobacteria          | 85270     | 88515     | 13512     | 8519      | 8742      | 51085     | 4957      | 60878     | 7387      | 10161     | 23939     | 38194     | 7188      | 5426      | 240       | 419917      | 15.81%      |
| Fusobacteria            | 45106     | 1580      | 1321      | 42502     | 0         | 408       | 978       | 8159      | 3962      | 1375      | 3         | 909       | 2280      | 0         | 108566    | 4.08%       |
| Proteobacteria          | 1330      | 1318      | 264       | 739       | 765       | 2299      | 75        | 1295      | 584       | 1979      | 28751     | 1382      | 25        | 415       | 116       | 39317       | 1.48%       |
| Acidobacteria           | 187       | 27        | 0         | 9         | 0         | 0         | 2         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 225         |             |
| Thermi                  | 24        | 17        | 3         | 14        | 0         | 1         | 0         | 7         | 5         | 0         | 1         | 0         | 1         | 0         | 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         | 5936      | 220       | 1009      | 19        | 14314     | 65        | 7718      | 633       | 1585      | 1669      | 289       | 1         | 271       | 0         | 33198       | 1.25%       |
| Cyanobacteria           | 3         | 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         | 0           | 5           |
| Thermodesulfobacteria   | 6         | 3         | 0         | 2         | 6         | 0         | 0         | 1         | 0         | 0         | 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         | 4         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 12          |
| Nitrospirae             | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 0         | 1         | 0         | 0         | 0         | 0         | 0         | 1           |             |
| Chlorobi                | 0         | 0         | 0         | 0         | 0         | 2         | 0         | 0         | 0         | 0         | 1         | 0         | 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         | 1         | 0         | 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           |
| Chloramidia             | 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: 14 16 9 14 6 16 6 14 10 6 11 10 8 7 5
Total phylum-level identified: 423 239 140 141 116 270 89 195 145 128 352 192 48 201 76
Total class-level identified: 11 13 6 11 10 10 8 16 18 18 12 22 13 15 16
Total order-level identified: 24 24 13 16 11 15 12 13 10 11 11 11 5 5 4
Total family-level identified: 36 39 19 23 14 17 15 15 11 12 13 12 6 5 3
Total genus-level identified: 56 56 30 33 21 27 20 21 16 17 19 17 8 7 5
Total species-level identified: 72 72 43 46 30 37 29 30 22 24 27 24 12 10 6
BV, Bacterial vaginosis
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), *Ureaplasm* (0.67%; 12/15), *Mycoplasma* (0.62%; 12/15), *Veillonella* (0.56%; 12/15), *Peptostreptococcus* (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), *Serinococcus* (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), *Bacteroides* (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; 19.25%), *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%), and *L. kitasatonis* (5/13; 0.15%), and others. Eleven *Enterococcus* and four *Enterobacter* species were present in over two-thirds 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*
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. eldenii* (0.01%; 3/15), *M. geminatus* (<0.01%; 2/15), *M. sp. UPII 335-2 (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. tetraliois* (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. microaerophilus* (15/15; 0.6%) identified in all the BV subjects. This was followed by *D. succinatophilus* (9/15; 2.54%), *D. invius* (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...
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. curtisi (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. lagogenous (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. gpaA181A (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 (11/15; <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. crici (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

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

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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.[19] 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.

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**Conflicts of interest**
There are no conflicts of interest.

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