

# Relationship of Specific Bacteria in the Cervical and Vaginal Microbiotas with Cervicitis

## Supplemental Digital Content

### **SUPPLEMENTAL METHODS**

#### ***Assessment of Co-infections***

Bacterial vaginosis was diagnosed in both studies by the presence of 3 or more Amsel clinical criteria (vaginal pH >4.5, clue cells that comprised >20% of vaginal epithelial cells on saline wet mount, amine odor on addition of potassium hydroxide (KOH), or homogeneous vaginal discharge <sup>1</sup>), or Gram staining of vaginal fluid (Nugent score  $\geq 7$ ) <sup>2</sup>. The presence of fungal elements consistent with candidiasis was determined by KOH preparation. In the Seattle study, women were universally screened for the presence of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) infection by nucleic acid amplification testing (NAAT, Aptima Combo-2 assay, Gen-Probe, San Diego, California) of a urine sample. The InPouch TV culture system (BioMed Diagnostics, White City, Oregon) was used to detect the presence of *Trichomonas vaginalis* (TV). In the Kenyan study, infection with GC, CT and TV was evaluated with NAAT testing of cervical swab specimens (Aptima Combo-2 GC/CT and TV ASR, Gen-Probe, San Diego, CA). Each Kenyan participant also underwent confidential HIV counseling and testing with enzyme-linked immunosorbent assays (primary test: Pishtaz HIV1, 2 ELISA (Pishtaz Teb Diagnostics, Tehran, Iran); confirmatory test: Vironostika HIV-1 Uni-Form II Ag/Ab (bioMérieux, Marcy l'Etoile, France)).

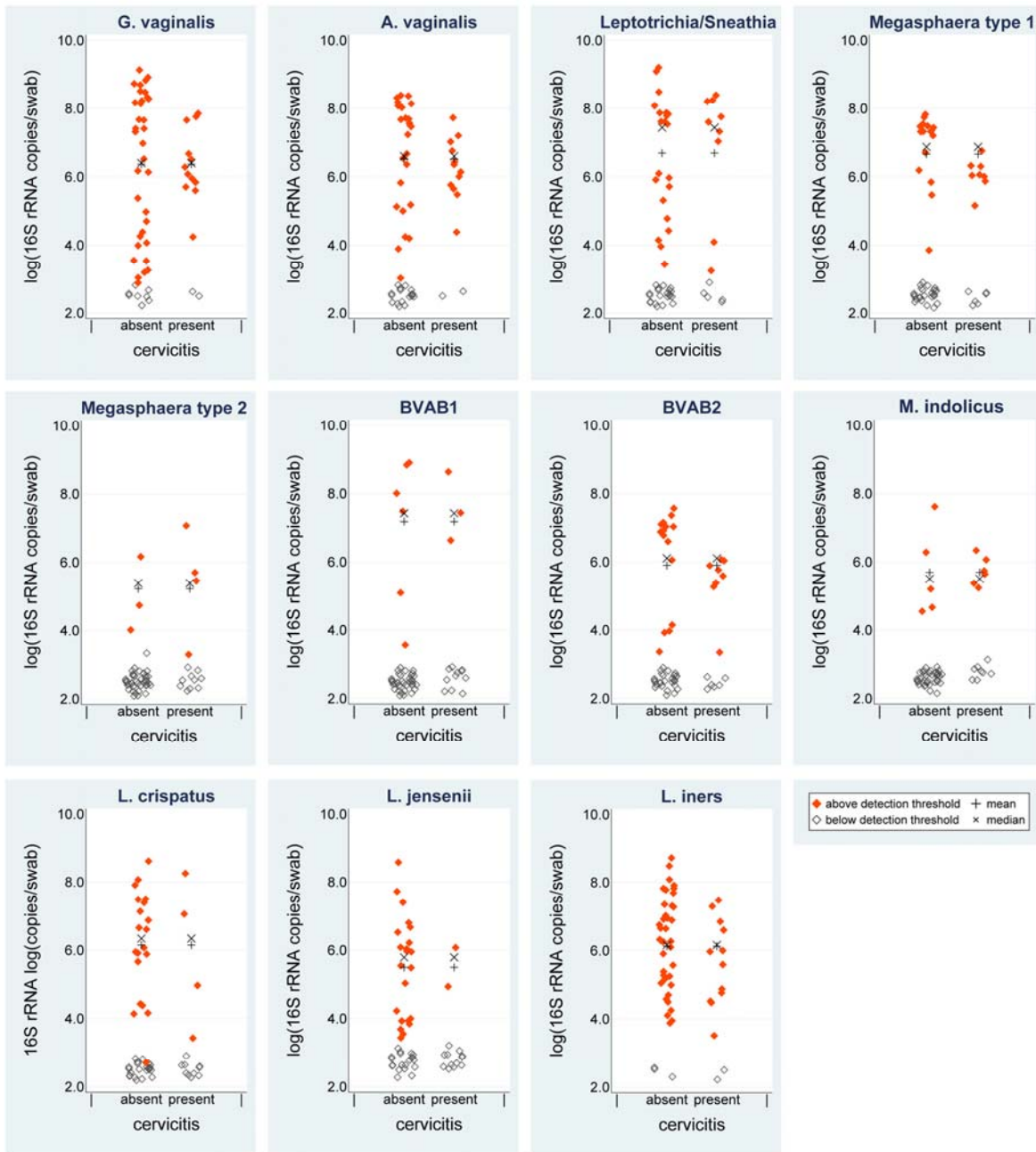
#### ***DNA Extraction***

For the Seattle study, swabs were thawed at room temperature for 5-10 min in 2 mL saline and vortexed for 2-5 min to dislodge cells. A 1 mL aliquot of the solution was centrifuged

at 18,000 x g for 10 min and the pellet extracted using either the UltraClean Soil DNA Isolation Kit or the BiOSTic Bacteremia DNA Isolation Kit (MoBio, Carlsbad, California).

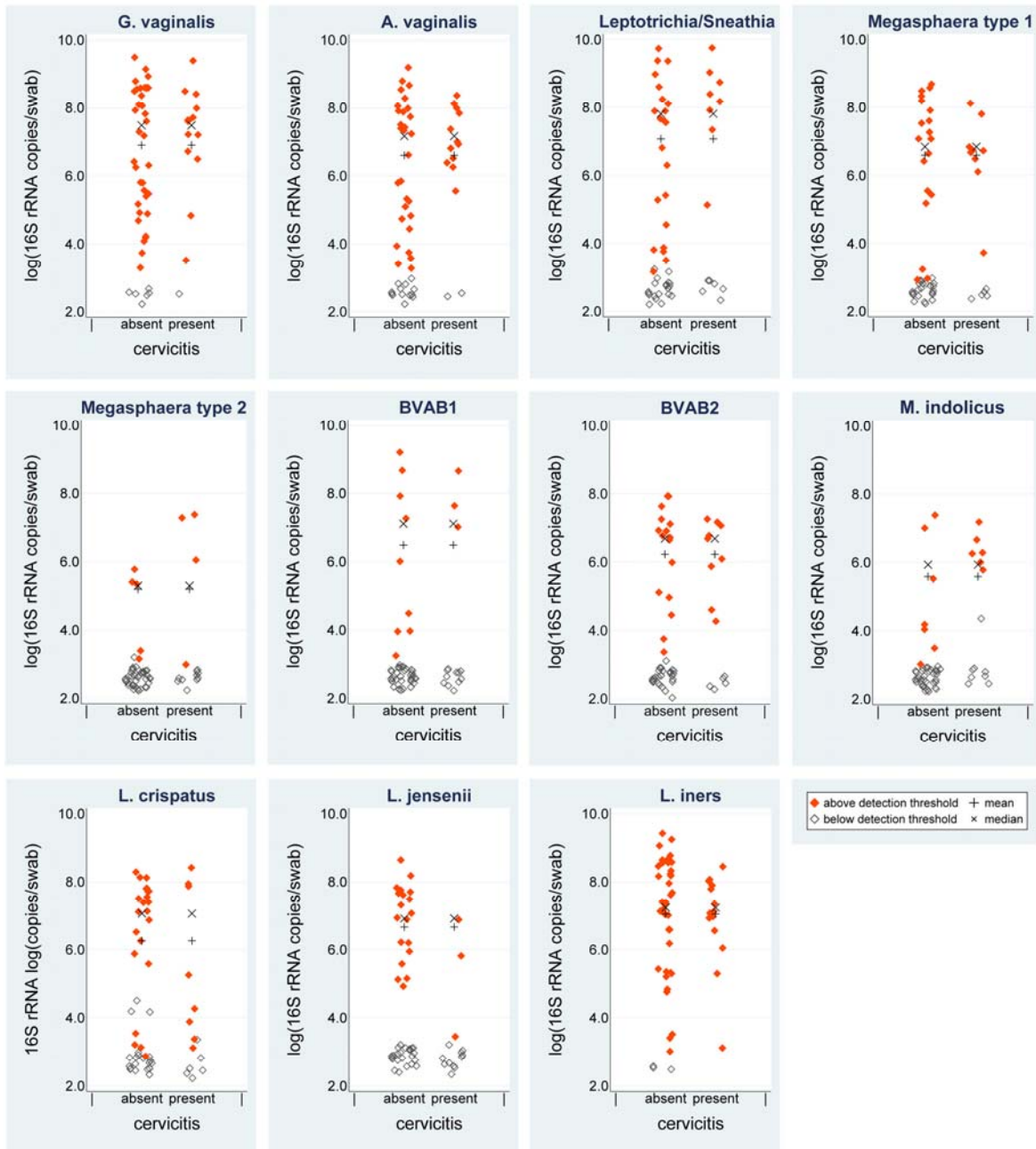
Vaginal swabs collected in Mombasa and intended for molecular detection of vaginal bacteria were frozen at -80°C and shipped to Seattle on dry ice. Swabs were prepped for DNA extraction as described above. DNA was extracted from most samples using the BiOSTic Bacteremia DNA Isolation Kit (MoBio), but 15 samples (4 cases and 11 controls) were extracted using the QIAmp DNA Mini QIAcube Kit (Qiagen, Venlo, Netherlands) as part of another project. The frequency with which *M. indolicus* was detected did not differ by extraction method and we accounted for differences in DNA yield by adjusting for extraction method in the multivariate analysis of bacterial load.

## SUPPLEMENTAL FIGURES

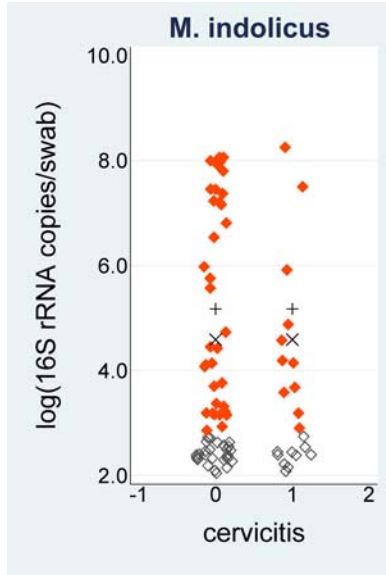


**Figure S1.** Cervical bacterial load (log 16S rRNA gene copies) detected using taxon-directed quantitative PCR in the Seattle cohort. Each point represents a single participant. Mean and

median quantities were calculated for samples in which the species/genera indicated were detected.



**Figure S2.** Vaginal bacterial load (log 16S rRNA gene copies) detected using taxon-directed quantitative PCR in the Seattle cohort. Each point represents a single participant. Mean and median quantities were calculated for samples in which the species/genera indicated were detected.



**Figure S3.** Vaginal bacterial load (log 16S rRNA gene copies) detected using taxon-directed quantitative PCR in the Kenyan cohort. Each point represents a single participant. Mean and median quantities were calculated for samples in which the species/genera indicated were detected.

## **SUPPLEMENTAL TABLES**

**Table S1.** Barcode sequences used to identify each sample in broad-range 16S rRNA gene pyrosequencing experiment.

<b>manuscript_id</b>	<b>specimen</b>	<b>primer</b>	<b>barcode</b>
S1	p7z1tr10	TR-10	TTCCAC
S2	p7z2tr10	TR-10	TTCCAC
S3	p7z2tr12	TR-12	TTGTGT
S4	p7z2tr17	TR-17	TATCAC
S5	p7z2tr18	TR-18	TATGCC
S6	p7z2tr19	TR-19	TAATCC
S7	p7z2tr22	TR-22	TACTAC
S8	p7z2tr24	TR-24	TACGTT
S9	p7z1tr24	TR-24	TACGTT
S10	p7z2tr27	TR-27	TCTTAC
S11	p7z2tr28	TR-28	TCTTGT
S12	p7z2tr29	TR-29	TCTAGC
S13	p7z2tr31	TR-31	TCAACC
S14	p7z2tr32	TR-32	TCACTC
S15	p7z2tr33	TR-33	TCGTCT
S16	p7z2tr35	TR-35	TGTTCT
S17	p7z2tr39	TR-39	TGACTT
S18	p7z2tr4	TR-4	TTACGC
S19	p7z1tr4	TR-4	TTACGC
S20	p7z2tr42	TR-42	TGGAAC
S21	p7z2tr44	TR-44	ATGCGT
S22	p7z2tr45	TR-45	ATGGAT
S23	p7z2tr5	TR-5	TTAGGT
S24	p7z1tr5	TR-5	TTAGGT
S25	p7z2tr53	TR-53	AAGTCT
S26	p7z2tr54	TR-54	AAGAAC
S27	p7z2tr55	TR-55	AAGGTT
S28	p7z2tr56	TR-56	ACTCAT
S29	p7z2tr57	TR-57	ACTGGT
S30	p7z2tr58	TR-58	ACATCT
S31	p7z2tr60	TR-60	ACAGAC
S32	p7z2tr61	TR-61	ACCTAT
S33	p7z2tr62	TR-62	ACCAAC
S34	p7z2tr63	TR-63	ACGAGT
S35	p7z2tr64	TR-64	AGTTCC
S36	p7z2tr66	TR-66	AGTGAT
S37	p7z2tr67	TR-67	AGATGC
S38	p7z2tr7	TR-7	TTCTCC
S39	p7z1tr7	TR-7	TTCTCC
S40	p7z2tr72	TR-72	CATGTT
S41	p7z2tr8	TR-8	TTCAAGT
S42	p7z1tr80	TR-80	GGTATT

**Table S2:** Relative abundance data from broad-range 16S rRNA gene pyrosequencing experiment. Samples are listed in the same order, left to right, as in Figure 1.





specimen	p72tr55	p72tr33	p72tr18	p72tr5	p72tr4	p72tr8	p72tr61	p72tr44	p72tr62	p72tr66	p72tr54	p72tr5	p72tr58	p72tr4	p72tr17	p72tr7	p72tr12
Lactobacillus iners	3	7	11	0	4	0	97	1	88	102	768	40	42	36	460	37	0
Lactobacillus crispatus	0	0	0	0	0	0	0	0	0	0	0	0	163	0	0	0	0
Gardnerella vaginalis	448	556	229	179	504	468	1545	2275	4271	2177	2957	2112	2492	3046	1947	1005	0
Leptotrichia ammonii	181	1278	881	3998	3240	439	432	1359	299	1561	173	3	14	116	365	3218	145
Prevotella timonensis	174	1488	521	1374	158	346	662	871	1183	2847	873	26	0	297	0	0	501
BVAB1	1885	5533	52	0	0	3881	0	0	0	0	0	0	0	0	0	0	3555
Atopobium vaginae	62	117	121	46	121	121	340	202	981	1571	711	1618	2393	587	132	481	38
Prevotella amnii	0	0	0	32	1805	1451	688	486	0	0	382	1804	2838	371	0	778	646
Prevotella bivia	24	81	242	147	168	0	176	166	2698	148	3	0	0	0	0	0	0
Megasphaera sp. type 1	165	177	18	12	425	224	99	233	614	530	444	928	553	669	233	0	22
Lactobacillus jensenii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sneathia sanguinegens	10	25	399	88	158	88	274	316	1	313	11	315	12	195	18	2392	39
BVAB2	43	0	14	60	555	137	336	26	82	105	390	1	364	239	18	6	60
Eggerthella sp. type 1	101	200	59	71	96	98	109	80	304	388	163	102	166	206	124	97	10
Prevotella genogroup 4	7	0	33	0	1	0	0	11	0	0	0	0	21	358	2013	1	102
Sneathia	4	218	18	36	1489	120	12	26	9	120	17	4	4	6	119	56	3
Parvimonas micra	123	232	92	27	207	219	278	167	52	148	189	0	93	111	55	0	40
Prevotella melaninogenica	0	0	0	0	6	0	41	0	0	0	0	0	0	0	611	0	0
Dialister sp. type 2	20	26	29	1	20	21	225	90	355	151	71	14	79	244	45	0	8
Prevotella	8	20	2	6	9	13	86	7	43	7	4	3	14	6	11	2	975
Prevotella buccalis	18	26	34	12	17	70	427	175	187	0	102	0	0	30	16	0	7
Lactobacillus gasseri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aerococcus christensenii	2	6	0	2	0	3	5	21	18	32	3	88	11	23	5	0	0
Prevotella disiens	207	532	62	1	23	125	173	32	0	0	0	0	1	0	0	0	29
Peptostreptococcus anaerobius	3	0	2	19	5	191	94	25	1	0	0	0	0	0	0	0	203
Prevotella genogroup 3	259	0	234	0	0	0	0	0	0	0	0	0	0	0	0	36	578
Porphyromonas asaccharolytica	65	0	0	60	111	138	111	0	0	89	10	0	1	8	0	0	193
Dialister microaerophilus	24	58	25	21	18	24	55	71	73	64	30	14	38	26	27	23	50
Megasphaera sp. type 2	0	0	125	63	0	4	0	0	0	0	0	0	0	185	0	0	394
Gemella asaccharolytica	18	41	10	14	34	23	74	6	51	14	20	1	0	12	4	74	0
Porphyromonas	4	0	7	73	38	218	0	0	5	0	0	0	0	0	0	0	78
Magebailicus indolicus	0	0	0	53	101	0	0	0	0	0	0	0	1	147	40	0	83
Mycoplasma hominis	16	11	6	9	2	7	22	0	3	24	25	0	0	4	5	0	17
Megasphaera	3	8	10	5	21	8	2	20	30	41	9	14	33	22	15	0	14
Lactobacillus vaginalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Anaerococcus prevotii/tetradius	0	3	2	5	14	4	21	18	240	29	7	6	0	0	0	0	6
Peptostreptococcus	0	0	0	1	0	6	3	0	0	0	1	0	0	0	0	0	39
Lactobacillus	0	0	0	0	0	0	0	0	0	1	9	0	0	1	0	0	0
Veillonella montpellierensis	0	0	1	0	0	0	49	32	0	0	0	0	3	0	0	0	39
Peptoniphilus harei	17	10	3	0	16	4	49	1	9	32	78	0	0	0	15	0	1
Mobiluncus mulieris	105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	177
Porphyromonas sp. type 1	9	0	0	15	49	2	7	0	0	0	0	0	0	0	0	0	20
Atopobium	2	17	2	0	5	1	3	18	48	2	5	0	3	15	41	0	10
Streptococcus anginosus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peptoniphilus lacrimalis	62	0	0	1	0	27	41	0	13	0	1	0	0	52	0	0	10
Prevotella genogroup 7	14	6	0	4	1	5	78	4	0	31	23	0	0	0	0	0	0
Mobiluncus curtisi	1	0	0	0	0	0	12	0	0	13	39	0	0	77	0	0	2
Aerococcus	1	7	0	0	0	1	1	2	0	26	20	2	1	7	3	0	0
Clostridiales Family XI, Incertae Sedis	2	4	4	1	0	10	26	5	1	3	4	0	3	0	1	0	56
Moryella	0	0	0	0	10	9	0	0	0	0	0	0	0	8	0	0	78
Fusobacterium	0	7	0	34	0	0	74	0	0	0	0	0	0	1	0	0	2
Porphyromonas asaccharolytica/uenonis	4	0	0	11	8	13	31	0	0	3	0	0	0	0	0	0	20
Actinomycetes	4	8	0	0	22	5	19	7	18	5	0	0	0	9	0	2	1
Fusobacterium nucleatum	0	11	0	46	0	0	89	0	0	0	0	0	0	1	0	0	0
Clostridiales	0	0	0	2	1	5	3	0	0	0	0	0	1	1	0	0	25
Streptococcus agalactiae	0	0	0	0	0	0	1	0	4	0	0	0	0	0	0	0	0
Dialister	3	1	0	2	0	1	43	3	7	3	0	0	0	2	3	2	2
Bacteria	0	0	3	0	0	0	0	0	0	0	0	0	77	0	0	0	0
Peptoniphilus	37	0	1	0	0	0	7	0	0	3	0	0	0	0	0	0	27
Neisseria gonorrhoeae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80	0	0
Campylobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	77
Prevotella bergeiensis	0	6	0	1	0	0	4	3	0	0	0	0	0	0	1	0	0
Eubacterium	1	8	0	3	0	1	0	16	0	0	0	1	0	0	0	0	2
Lactobacillus reuteri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactobacillus gallinarum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mobiluncus	15	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	36
Jonquetella	0	0	0	0	0	48	1	0	0	0	0	0	1	0	0	0	0
Lactobacillus acidophilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Finegoldia magna	5	0	0	0	2	0	0	0	2	1	0	0	0	0	0	0	14
Peptostreptococcus stomatis	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	6
Dialister propionicifaciens	0	0	0	0	0	0	3	26	0	0	0	0	0	0	0	0	0
Haemophilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anaerococcus	4	0	0	0	0	0	7	0	0	1	0	0	1	7	0	0	0
Atopobium minimum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anaerococcus vaginalis	0	0	0	0	0	0	4	0	1	0	0	0	0	0	0	0	4
Gemella	1	4	0	2	2	0	2	0	0	1	0	0	0	4	0	2	0
Staphylococcus epidermidis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus spp.	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eggerthella	0	1	0	0	1	0	0	2	2	2	0	0	3	1	0	0	0
Lactobacillus helveticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Firmicutes	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Bacteroides	0	1	3	0	1	0	0	0	0	0	0	0	1	0	0	0	5
Campylobacter ureolyticus	0	1	0	1	0	0	3	2	0	0	0	0	0	0	0	0	0
Bifidobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0
Actinomycetes turicensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacilli	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Porphyromonas uenonis	3	0	0	0	3	0	1	0	0	1	0	0	0	0	0	0	1
Prevotella corporis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Bacteroides	0	0	0	0	1	0	5	0	0	0	0	0	0	0	0	0	1
Clostridium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veillonella	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0
Lachnospiraceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0
Porphyromonas benoniis	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
Micrococciaceae	0	0	0	0	2	1	1	1	0	1	0	0	0	0	0	0	0
Betaproteobacteria	11	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0
Lactobacillales	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Kocuria	0	0	0														

## **REFERENCES**

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2. Nugent RP, Krohn M, and Hillier S, Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *Journal of Clinical Microbiology*, 1991. 29: p. 297-301.