microorganisms

*Supplementary Materials*

**Table S1.** Number of tests conducted, infections detected, and treatments by study visit.

| Time point | Baseline, N=436 n (%) | 6-Months, N=424 n (%) | 12 Months, N=397 n (%) | 18 Months, N=399 n (%) | 30 Months, N=396 n (%) |
|---|---|---|---|---|---|
| Bacterial vaginosis (BV) Tested for | 436 | 424 | 395 | 398 | 395 |
| BV Detected | 49 (11.2) | 39 (9.2) | 57 (14.4) | 56 (14.1) | 88 (22.3) |
| BV Treated | 48 (98.0) | 37 (94.9) | 55 (96.5) | 56 (100) | 79 (89.8) |
| Number of BV not detected at any prior visit | -- | 19 (48.7) | 29 (50.9) | 31 (55.4) | 39 (49.4) |
| Number of incident BV | | 20 | 28 | 25 | 49 |
| *Chlamydia trachomatis* (CT) Tests Conducted | 436 | | 395 | | 395 |
| CT Detected | 27 (6.2) | | 26 (6.6) | | 47 (11.9) |
| CT Treated | 25 (92.6) | | 26 (100) | | 44 (93.6) |
| Number of CT infections detected at a prior visit | --- | | 6 (23.1) | | 9 (6.4) |
| *Neisseria gonorrhoeae* (NG) Tests Conducted | 436 | | 395 | | 395 |
| NG Detected | 6 (1.4) | | 12 (3.0) | | 8 (2.0) |
| NG Treated | 6 (100) | | 12 (100) | | 6 (75.0) |
| Number of NG infections detected at a prior visit | --- | | 2 (16.7) | | 1 (16.7) |
| *Trichomonas vaginalis* (TV) Tests Conducted | 436 | | 395 | | 395 |
| TV Detected | 13 (3.0) | | 13 (3.3) | | 20 (5.1) |
| TV Treated | 13 (100) | | 12 (92.3) | | 15 (75.0) |
| Number of TV infections detected at a prior visit | --- | | 0 (0.0) | | 2 (10.0) |
| Composite Sexually Transmitted Infection (STI) Tests Conducted | 436 | | 395 | | 395 |
| STI Detected | 43 (9.9) | | 46 (11.7) | | 64 (16.2) |
| STI Treated | 41 (95.3) | | 46 | | 57 |
| Any[1] STI detected at any prior visit | --- | | 13 | | 22 |
| Total number incident composite STI (i.e., infections not preceded by any STI at a prior visit)[2] | | | 33 | | 42 |
| Order of incident STI and/or incident BV | Prevalent | | | | |
| Persistent negative for both (no prior infection) | 359 (82.3) | | 263 (88.9) | | 180 (81.1) |
| Incident STI (no BV prior)[2] | 28 (6.4) | | 24 (8.1) | | 17 (7.7) |
| BV before incident STI | 34 (7.8) | | 6 (2.0) | | 14 (6.3) |
| Incident STI and Incident BV | 15 (3.4) | | 3 (1.0) | | 11 (5.0) |
| CT only (no TV or NG) | | | | | |
| Incident CT (no BV prior) | | | 12 | | 9 |
| BV before incident CT | | | 3 | | 8 |
| Incident CT and Incident BV | | | 2 | | 7 |
| TV only (no CT or NG) | | | | | |
| Incident TV (no BV prior) | | | 7 | | 6 |
| BV before incident TV | | | 2 | | 3 |
| Incident TV and Incident BV | | | | | 1 |
| NG only (no CT or TV) | | | | | |
| Incident NG (no BV prior) | | | 3 | | |
| BV before incident NG | | | | | 1 |
| Incident NG and Incident BV | | | | | 1 |

[1] If a participant had TV at baseline, and then CT at 12 months or 30 months, this would not be included in analysis of incident STI. Incident STI is defined as no prior STI infection with any etiology (CT, NG, or TV). This is because the antimicrobial treatment for the first incident STI would affect VMB composition for assessment of subsequent incident STI. [2] There were n=2 participants with incident STI infections (no prior BV) and n=1 persistently negative participant that was excluded from analyses due to having <5,000 total sequence read counts.

**Table S2.** Characteristics of give participants with incident Sexually transmitted infection and incident Bacterial vaginosis detected at the same study visit and participants with incident *Chlamydia trachomatis* in the absence of other STIs and BV.

| | Incident *C. trachomatis* in the absence of other STIs and BV, N=21<br>n (%) | Incident *T. vaginalis* in the absence of other STIs and BV, N=13<br>n (%) |
|---|---|---|
| *At Baseline* | | |
| Randomization status | | |
| Control arm | 11 (52.4) | 4 (30.8) |
| Cup arm | 10 (47.6) | 9 (69.2) |
| Median Age in years | | |
| <16.9 years | 9 (42.9) | 7 (53.9) |
| >=16.9 years | 12 (57.1) | 6 (46.1) |
| Socioeconomic status score | | |
| Highest quintiles | 13 (61.9) | 9 (69.2) |
| Lowest quintiles | 8 (38.1) | 4 (30.8) |
| Water, sanitation, and hygiene score | | |
| Higher | 9 (42.9) | 7 (53.9) |
| Lower | 12 (57.1) | 6 (46.1) |
| Sexually active | | |
| No | 13 (61.9) | 8 (61.5) |
| Yes | 8 (38.1) | 5 (38.5) |
| Had transactional sex | | |
| No | 19 (90.5) | 10 (76.9) |
| Yes | 2 (9.5) | 3 (23.1) |
| Experienced coerced sex | | |
| No | 13 (61.9) | 9 (69.2) |
| Yes | 8 (38.1) | 4 (30.8) |
| Has a boyfriend | | |
| No | 19 (90.5) | 12 (92.3) |
| Yes | 2 (9.5) | 1 (7.7) |
| Vaginal Community State Type (CST) | | |
| CST-I (*L. crispatus* dominated) | 9 (45.0) | 3 (27.3) |
| CST-III (*L. iners* dominated) | 9 (45.0) | 6 (54.5) |
| CST-IV (mixed) | 2 (10.0) | 2 (18.2) |
| *At time of Incident STI* | | |
| Time of incident STI | | |
| 12 months | 12 (57.1) | 7 (53.9) |
| 30 months | 9 (42.9) | 6 (46.1) |
| Median Age in Years | | |
| <18.8 years | 13 (61.9) | 7 (53.9) |
| >=18.8 years | 8 (38.1) | 6 (46.1) |
| Socioeconomic status score | | |
| Median or higher | 13 (65.0) | 10 (76.9) |
| Below median | 7 (35.0) | 3 (23.1) |
| Ever Sexually active | | |
| No | 6 (30.0) | 6 (50.0) |
| Yes | 14 (70.0) | 6 (50.0) |
| Had transactional sex | | |
| No | 16 (80.0) | 11 (84.6) |
| Yes | 4 (20.0) | 2 (15.4) |
| Experienced coerced sex | | |
| No | 15 (75.0) | 12 (92.3) |
| Yes | 5 (25.0) | 1 (7.7) |
| Has a boyfriend | | |
| No | 11 (55.0) | 10 (76.9) |
| Yes | 9 (45.0) | 3 (23.1) |
| Vaginal Community State Type | | |
| CST-I (*L. crispatus* dominated) | 1 (4.8) | 3 (27.3) |
| CST-III (*L. iners* dominated) | 19 (90.5) | 1 (9.1) |
| CST-IV (mixed) | 1 (4.8) | 7 (63.6) |

*Not all cells sum to N due to missing data.

**Table S3.** Presence and mean relative abundance (RA) of 54 taxa by outcome status*.

| | Persistently STI and BV Negative, N=179 | | Incident STI and BV Negative, N=39 | | Incident STI and BV Before STI, N=20 | | Incident STI and BV at Same Time, N=14 | |
|---|---|---|---|---|---|---|---|---|
| | Present (%) | Mean RA (%) | Present (%) | Mean RA (%) | Present (%) | Mean RA (%) | Present (%) | Mean RA (%) |
| *Lactobacillus crispatus* | 124 (69.3) | 37.8 | 20 (51.3) | 14.7 | 5 (25.0) | 3.17 | 2 (14.3) | 0.01 |
| *Lactobacillus iners* | 117 (65.4) | 28.1 | 33 (84.6) | 38.5 | 17 (85.0) | 40.2 | 12 (85.7) | 16.1 |
| *Gardnerella vaginalis* | 94 (52.5) | 4.94 | 27 (69.2) | 6.73 | 18 (90.0) | 9.73 | 12 (85.7) | 13.9 |
| *Lactobacillus* spp. | 125 (69.8) | 4.10 | 21 (53.9) | 1.20 | 8 (40.0) | 0.23 | 4 (28.6) | 0.66 |
| *Sneathia sanguinegens* | 30 (16.8) | 2.02 | 16 (41.0) | 5.11 | 12 (60.0) | 6.81 | 10 (71.4) | 17.4 |
| *Lactobacillus jensenii* | 28 (15.6) | 2.36 | 7 (18.0) | 0.35 | 2 (10.0) | 0.62 | 0 (0) | 0 |
| Bacteria, not otherwise specified | 171 (95.5) | 1.05 | 39 (100) | 3.30 | 17 (85.0) | 3.90 | 13 (92.9) | 0.75 |
| *Prevotella timonensis* | 94 (52.5) | 1.11 | 24 (61.5) | 2.01 | 16 (80.0) | 1.52 | 13 (92.9) | 4.42 |
| *Finegoldia magna* | 154 (86.0) | 1.13 | 35 (89.7) | 1.55 | 17 (85.0) | 1.70 | 14 (100) | 4.15 |
| *Anaerococcus prevotii* | 127 (70.9) | 1.38 | 26 (66.7) | 1.36 | 15 (75.0) | 1.33 | 11 (78.6) | 1.96 |
| *Ureaplasma urealyticum* | 90 (50.3) | 1.19 | 29 (74.4) | 1.34 | 12 (60.0) | 0.92 | 7 (50.0) | 0.37 |
| *Megasphaera* spp. | 20 (11.2) | 0.50 | 5 (12.8) | 0.89 | 9 (45.0) | 4.18 | 9 (64.3) | 5.45 |
| *Prevotella bivia* | 59 (33.0) | 1.24 | 18 (46.2) | 1.08 | 9 (45.0) | 0.95 | 8 (57.1) | 1.72 |
| *Peptoniphilus gorbachii* | 119 (66.5) | 0.83 | 25 (64.1) | 0.92 | 15 (75.0) | 1.37 | 13 (92.9) | 2.58 |
| *Lactobacillus reuteri* | 97 (54.2) | 1.00 | 7 (18.0) | 0.20 | 7 (35.0) | 0.45 | 1 (7.1) | 0.01 |
| *Fannyhessea vaginae* (*Atopobium*) | 24 (13.4) | 0.54 | 12 (30.8) | 0.46 | 13 (65.0) | 1.24 | 10 (71.4) | 3.42 |
| *Gemella haemolysans/gemella asaccharolytica* | 18 (10.1) | 0.26 | 9 (23.1) | 2.16 | 11 (55.0) | 2.28 | 9 (64.3) | 1.87 |
| BVAB1 | 8 (4.5) | 0.27 | 3 (7.7) | 0.38 | 6 (30.0) | 3.04 | 2 (14.3) | 1.57 |
| *Lactobacillus gasseri* | 42 (23.5) | 0.57 | 9 (23.1) | 1.77 | 2 (10.0) | 0.06 | 1 (7.1) | 0.10 |
| *Veillonella* spp. | 27 (15.1) | 0.51 | 8 (20.5) | 1.16 | 9 (45.0) | 0.97 | 3 (21.4) | 0.44 |
| Costridiales Family XI | 105 (58.7) | 0.73 | 24 (61.5) | 0.27 | 11 (55.0) | 0.62 | 10 (71.4) | 0.64 |
| *Prevotella spp.* | 57 (31.8) | 0.30 | 16 (41.0) | 0.43 | 10 (50.0) | 1.89 | 12 (85.7) | 2.56 |
| *Dialister succinatiphilus* | 38 (21.3) | 0.26 | 6 (15.4) | 0.11 | 12 (60.0) | 1.44 | 11 (78.6) | 3.38 |
| *Aerococcus christensenii* | 20 (11.2) | 0.16 | 11 (28.2) | 0.68 | 13 (65.0) | 1.49 | 9 (64.3) | 1.60 |
| *Prevotella corporis* | 66 (36.9) | 0.52 | 12 (30.8) | 0.19 | 6 (30.0) | 0.34 | 4 (28.6) | 0.41 |
| *Streptococcus anginosus* | 27 (15.1) | 0.32 | 7 (18.0) | 0.83 | 3 (15.0) | 0.13 | 3 (21.4) | 2.65 |
| *Prevotella disiens* | 50 (27.9) | 0.23 | 18 (46.2) | 1.59 | 11 (55.0) | 0.29 | 8 (57.1) | 0.57 |
| *Streptococcus oralis* | 11 (6.2) | 0.78 | 4 (10.3) | 0.07 | 1 (5.0) | 0.75 | 0 (0) | 0 |
| *Dialister propionicifaciens* | 73 (40.8) | 0.32 | 17 (43.6) | 0.57 | 11 (55.0) | 0.18 | 9 (64.3) | 0.54 |
| *Prevotella amnii* | 5 (2.8) | 0.15 | 3 (7.7) | 0.04 | 7 (35.0) | 1.33 | 5 (35.7) | 2.43 |
| *Sneathia amnii* | 7 (3.9) | 0.15 | 5 (12.8) | 0.8 | 2 (10.0) | 1.38 | 1 (7.1) | 0.03 |
| *Firmicutes* spp. | 37 (20.7) | 0.23 | 15 (38.5) | 0.70 | 5 (25.0) | 0.39 | 5 (35.7) | 0.21 |
| *Mycoplasma hominis* | 13 (7.3) | 0.10 | 14 (35.9) | 1.04 | 8 (40.0) | 0.27 | 7 (50.0) | 1.15 |
| *Staphylococcus hominis* | 75 (41.9) | 0.41 | 9 (23.1) | 0.17 | 4 (20.0) | 0.03 | 0 (0) | 0 |
| *Porphyromonas asaccharolytica* | 56 (31.3) | 0.30 | 17 (43.6) | 0.54 | 10 (50.0) | 0 | 9 (64.3) | 0.40 |
| *Brassicibacter* spp. | 39 (21.8) | 0.17 | 15 (38.5) | 1.10 | 2 (10.0) | 0.2 | 3 (21.4) | 0.08 |
| *Prevotella colorans* | 43 (24.0) | 0.24 | 12 (30.8) | 0.10 | 9 (45.0) | 0.99 | 7 (50.0) | 0.44 |
| *Fusobacterium equinum* | 9 (5.0) | 0.31 | 3 (7.7) | 0.15 | 2 (10.0) | 0.06 | 3 (21.4) | 0.37 |
| *Dialister micraerophilus* | 50 (27.9) | 0.19 | 20 (51.3) | 0.53 | 13 (65.0) | 0.42 | 13 (92.9) | 0.65 |
| *Anaerococcus* spp. | 37 (20.7) | 0.22 | 14 (35.9) | 0.23 | 2 (10.0) | 0.26 | 2 (14.3) | 0.83 |
| *Porphyromonas bennonis* | 79 (44.1) | 0.23 | 18 (46.2) | 0.33 | 7 (35.0) | 0.21 | 8 (57.1) | 0.22 |
| *Prevotella buccalis* | 60 (33.5) | 0.26 | 14 (35.9) | 0.14 | 7 (35.0) | 0.14 | 9 (64.3) | 0.85 |
| *Corynebacterium coyleae* | 62 (34.6) | 0.21 | 14 (35.9) | 0.41 | 4 (20.0) | 0.03 | 3 (21.4) | 0.38 |
| *Peptostreptococcus anaerobius* | 38 (21.2) | 0.13 | 19 (48.7) | 0.61 | 12 (60.0) | 0.44 | 5 (35.7) | 0.12 |
| *Lactobacillus coleohominis* | 49 (27.4) | 0.19 | 13 (33.3) | 0.21 | 1 (5.0) | 0.003 | 2 (14.3) | 0.07 |
| *Prevotella melaninogenica* | 7 (3.9) | 0.26 | 7 (18.0) | 0.85 | 4 (20.0) | 0.07 | 3 (21.4) | 0.34 |
| *Haemophilus influenzae* | 6 (3.4) | 0.31 | 1 (2.6) | 0.15 | 1 (5.0) | 0.003 | 0 (0) | 0 |
| *Alloprevotella rava* | 28 (15.6) | 0.34 | 4 (10.3) | 0.63 | 0 | 0 | 4 (28.6) | 0.24 |
| Clostridiales | 62 (34.6) | 0.20 | 14 (35.9) | 0.17 | 11 (55.0) | 0.17 | 7 (50.0) | 0.17 |
| *Fusobacterium nucleatum* | 19 (10.6) | 0.26 | 5 (12.8) | 0.84 | 6 (30.0) | 0.36 | 5 (35.7) | 0.26 |
| BVAB2 | 4 (2.2) | 0.02 | 3 (7.7) | 0.02 | 7 (35.0) | 0.74 | 8 (57.1) | 1.18 |
| *Porphyromonas somerae* | 40 (22.4) | 0.16 | 14 (35.9) | 0.05 | 5 (25.0) | 0.23 | 5 (35.7) | 0.23 |
| Prevotellaceae bacterium | 35 (19.6) | 0.21 | 6 (15.4) | 0.12 | 1 (5.0) | 0.01 | 0 (0) | 0 |
| *Anaerococcus hydrogenalis* | 44 (24.6) | 0.13 | 9 (23.1) | 0.16 | 5 (25.0) | 0.07 | 3 (21.4) | 0.05 |

* Taxa presence and relative abundances are reported at time of infection, or at time of sampling if persistently negative.

**Table S4.** Distribution of Network properties by Outcome: Persistently negative for STI and BV and BV prior to incident STI.

| Network properties | Persistently Negative, No STI and No BV N=179 | BV Prior to Incident STI N=20 | Absolute Difference |
|---|---|---|---|
| **Largest Connected Component (LCC)** | | | |
| Relative LCC size | 0.438 | 0.438 | 0 |
| Clustering coefficient | 0.320 | 0.0 | 0.320 |
| Modularity | 0.309 | 0.441 | 0.132 |
| Positive edge percentage | 78.9 | 78.6 | 0.376 |
| Edge density | 0.209 | 0.154 | 0.055 |
| Natural connectivity | 0.101 | 0.096 | 0.004 |
| Vertex connectivity | 1.00 | 1.00 | 0 |
| Edge connectivity | 1.00 | 1.00 | 0 |
| Average dissimilarity | 0.687 | 0.691 | 0.005 |
| Average path length | 2.33 | 3.63 | 1.30 |
| **Whole network** | | | |
| Number of components | 9 | 8 | 1 |
| Clustering coefficient | 0.309 | 0 | 0.309 |
| Modularity | 0.561 | 0.727 | 0.166 |
| Positive edge percentage | 88.2 | 76.0 | 12.2 |
| Edge density | 0.069 | 0.050 | 0.018 |
| Natural connectivity | 0.039 | 0.038 | 0.002 |
| **Degree Centrality** [1] | | | |
| *Fannyhesea vaginae (Atopobium)* | 0.194 | 0 | 0.167 |
| *Sneathia amnii* | 0.161 | 0 | 0.161 |
| *Gemella haemolysans/ Gemella asaccharolytica* | 0.129 | 0 | 0.129 |
| *Firmicutes* spp. | 0 | 0.097 | 0.097 |
| *Prevotella bivia* | 0 | 0.065 | 0.065 |
| *Prevotella* spp. | 0 | 0.065 | 0.065 |
| *Prevotella colorans* | 0 | 0.065 | 0.065 |
| *Porphyromonas somerae* | 0 | 0.065 | 0.065 |
| *Porphyromonas asaccharolytica* | 0 | 0.065 | 0.065 |
| *Lactobacillus* spp. | 0.065 | 0 | 0.065 |
| **Betweenness Centrality** [2] | | | |
| *Megasphaera* | 0 | 0.628 | 0.628 |
| *Porphyromonas somerae* | 0 | 0.513 | 0.513 |
| *Lactobacillus jensenii* | 0 | 0.462 | 0.462 |
| *Firmicutes* spp. | 0 | 0.410 | 0.410 |
| *Fannyhesea vaginae (Atopobium)* | 0.385 | 0 | 0.385 |
| *Prevotella amnii* | 0.192 | 0.474 | 0.282 |
| *Gemella haemolysans/ Gemella asaccharolytica* | 0.218 | 0 | 0.218 |
| *Sneathia amnii* | 0.205 | 0 | 0.205 |
| *Streptococcus oralis* | 0.179 | 0 | 0.179 |
| *Sneathia sanguinegens* | 0 | 0.154 | 0.154 |
| **Closeness Centrality** [3] | | | |
| *Fannyhesea vaginae (Atopobium)* | 0.704 | 0 | 0.704 |
| *Sneathia amnii* | 0.671 | 0 | 0.671 |
| *Gemella haemolysans/ Gemella asaccharolytica* | 0.593 | 0 | 0.593 |
| *Veillonella* spp. | 0.498 | 0 | 0.498 |
| *Lactobacillus* spp. | 0.478 | 0 | 0.478 |
| *Porphyromonas somerae* | 0 | 0.450 | 0.450 |
| *Firmicutes* spp. | 0 | 0.444 | 0.444 |
| *Gardnerella vaginalis* | 0.443 | 0 | 0.443 |
| *Dialister succinatiphilus* | 0.426 | 0 | 0.426 |
| *Prevotella bivia* | 0 | 0.423 | 0.423 |
| **Eigenvector Centrality** [4] | | | |
| *Fannyhesea vaginae (Atopobium)* | 1.00 | 0 | 1.00 |
| *Sneathia amnii* | 0.850 | 0 | 0.850 |
| *Prevotella bivia* | 0 | 0.546 | 0.546 |

| | | | |
|---|---|---|---|
| *Gemella haemolysans/ Gemella asaccharolytica* | 0.517 | 0 | 0.517 |
| *Porphyromonas somerae.* | 0 | 0.493 | 0.493 |
| *Megasphaera* | 0.493 | 0.958 | 0.464 |
| *Prevotella* spp. | 0 | 0.439 | 0.439 |
| *Prevotella colorans* | 0 | 0.405 | 0.405 |
| *Gardnerella vaginalis* | 0.311 | 0 | 0.311 |
| *Lactobacillus* spp. | 0.304 | 0 | 0.304 |

[1] Jaccard P-value =0.263. [2]Jaccard P-value =0.088. [3]Jaccard P-value =0.263. [4]Jaccard P-value =0.461.