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 [1]), or Gram staining of vaginal fluid (Nugent score ≥7) [2]. 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-1Uni-Form II Ag/Ab (bioMérieux, Marcy I'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 -80ºC 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

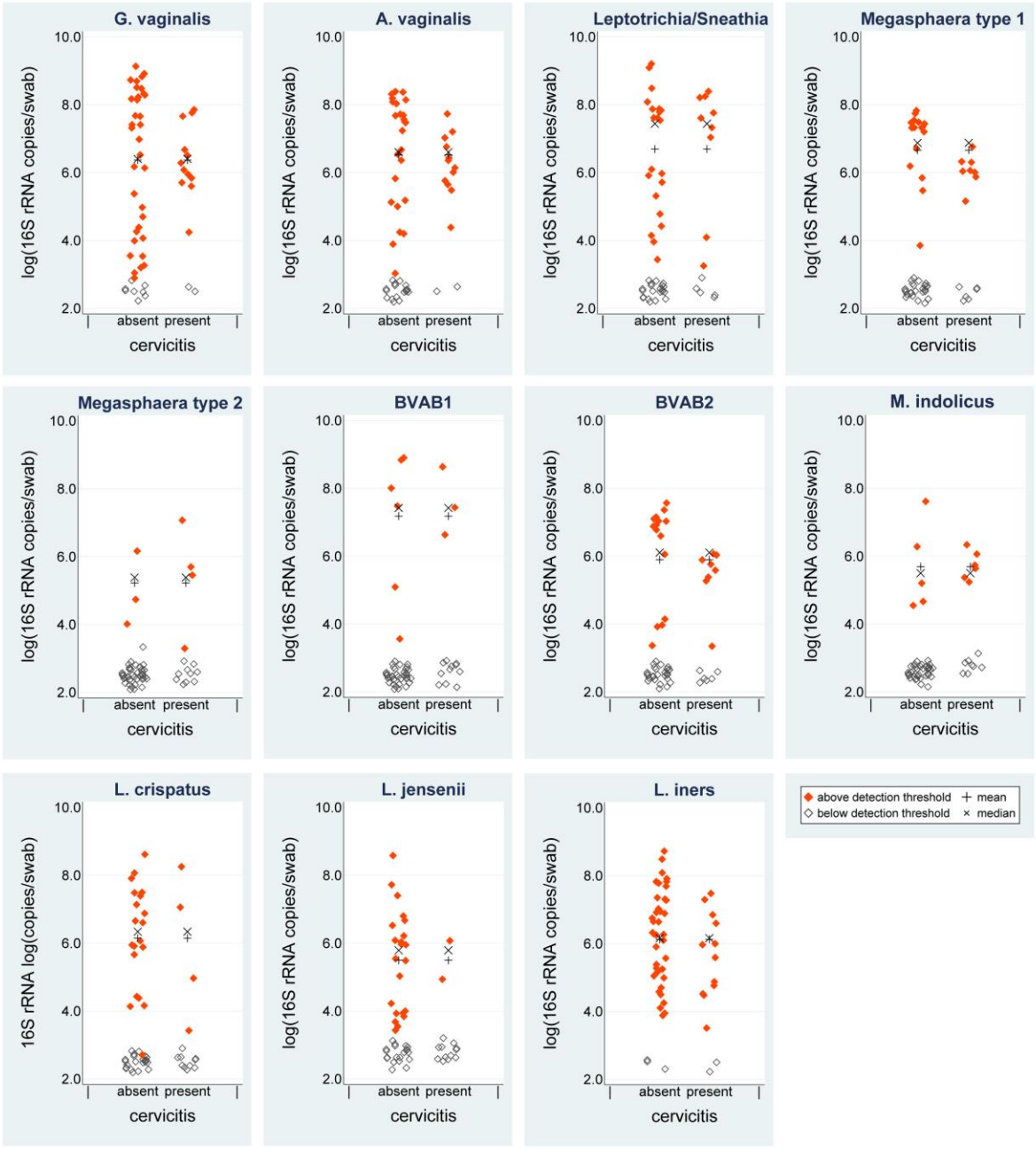

Figure S1. Cervical bacterial load (log 16S rRNA gene copies) detected using taxon-directed quantitative PCR in the Seattle cohort. Each point represents a single participant. Mean and

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

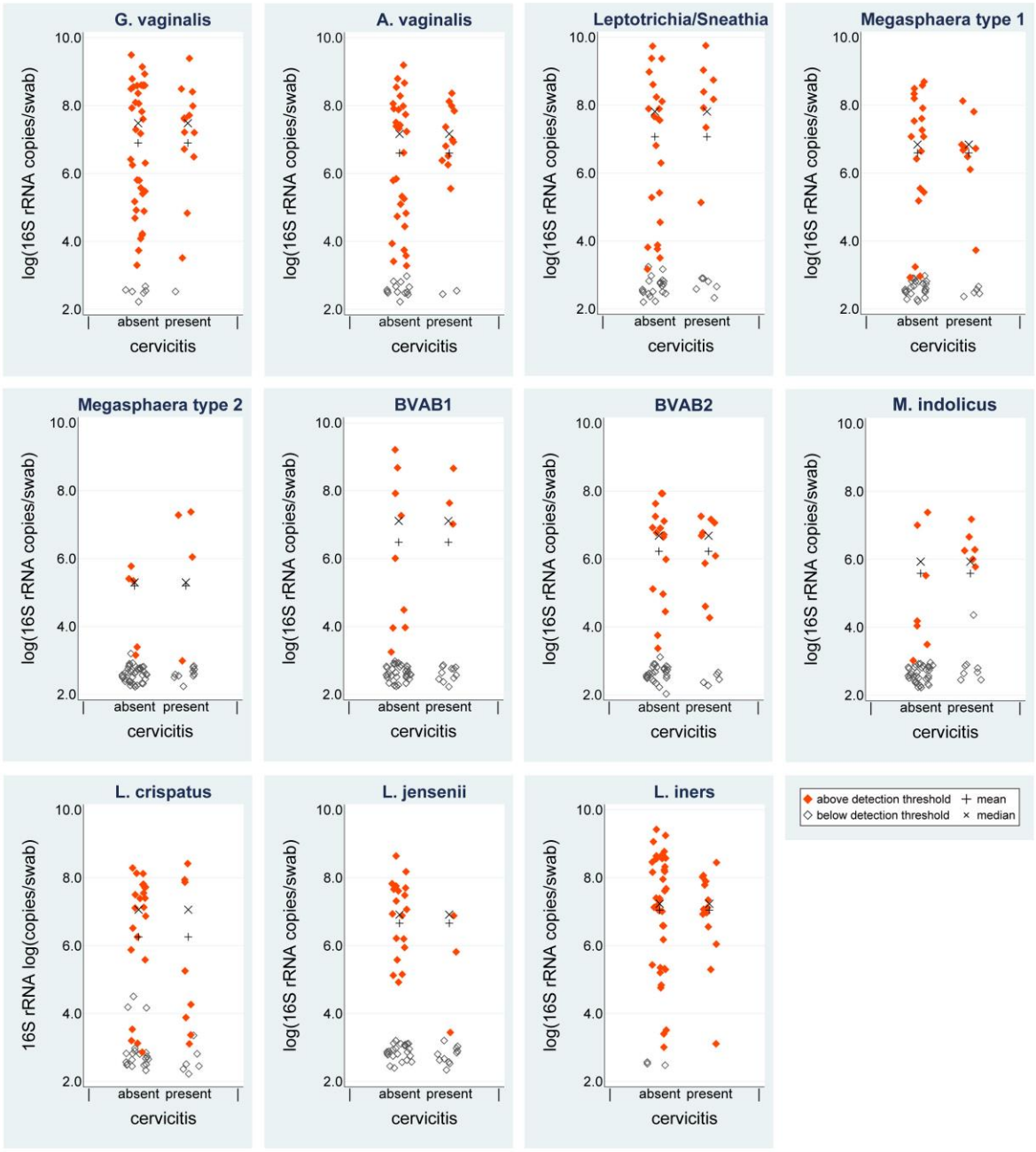

Figure S2. Vaginal bacterial load (log 16S rRNA gene copies) detected using taxon-directed quantitative PCR in the Seattle cohort. Each point represents a single participant. Mean and median quantities were calculated for samples in which the species/genera indicated were detected.

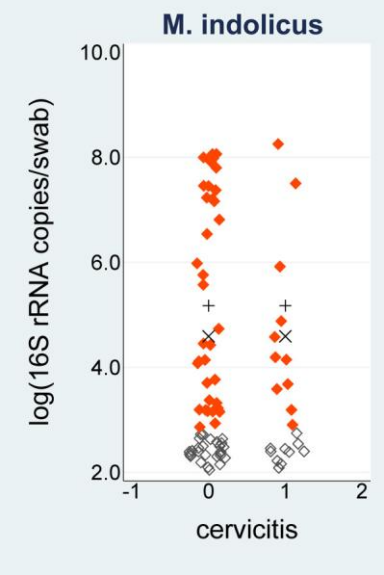

Figure S3. Vaginal bacterial load (log 16S rRNA gene copies) detected using taxon-directed quantitative PCR in the Kenyan cohort. Each point represents a single participant. Mean and median quantities were calculated for samples in which the species/genera indicated were detected.

SUPPLEMENTAL TABLES

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

Table S2: Relative abundance data from broad-range 16S rRNA gene pyrosequencing experiment.

REFERENCES

1. Amsel R, Totten PA, Spiegel CA, et al., Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med, 1983. 74(1): p. 14-22.
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.