

Comparative Analysis of the Vaginal Microbiome of South African Pregnant Women with and without *Chlamydia trachomatis* Infection

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ABSTRACT

Background: Chlamydia genital infections continue to be a serious health concern globally. Previous studies have reported that *Chlamydia trachomatis* infection alters the vaginal microbiota of infected women. This study aimed to investigate alterations in the vaginal microbiome of pregnant women with and without *C. trachomatis* infection.

Methods: This was a cross-sectional study among 385 pregnant women, recruited from the antenatal clinic (ANC) at the King Edward VIII Hospital in Durban, South Africa. *C. trachomatis* was detected using the Applied Biosystems™ TaqMan® Assays. A total of 40 samples, 20 *C. trachomatis* positive and 20 *C. trachomatis* negative were selected for bacterial vaginosis (BV) screening using the Allplex™ Bacterial Vaginosis plus Assay. Sequencing of the microbiomes were performed using the PacBio platform. Statistical analysis was performed on IBM SPSS version 26 (IBM Corporation, Armonk, NY).

Results: The prevalence of BV and *C. trachomatis* co-infection was 65.00%. At the genus level, *Gardnerella* was more abundant in the *C. trachomatis* infected group (32.14%) compared to the uninfected group (24.02%). Similarly, at species level, the species annotated as *Gardnerella* were more abundant in the *C. trachomatis* infected group (31.97%) compared to the uninfected group (24.03%). In addition, *L. iners* was also more abundant in the *C. trachomatis* infected women (28.30%) compared to the uninfected women. However, pairwise comparisons and the Holm-Sidak method showed no significant differences between women with *C. trachomatis* infection and the control group. The discriminant analysis showed that the class *Alpha-Proteobacteria* ($p=0.029$); order *Bacillales* ($p=0.018$); family *Enterococcaceae* ($p=0.002$); genus *Enhydrobacter* ($p=0.044$), *Enterococcus* ($p=0.004$) and *Parabacteroides* ($p=0.020$); and species *Enterococcus* spp. ($p=0.005$) and *Pseudomonasstutzeri* ($p=0.009$) were significantly associated with *C. trachomatis* infection.

Conclusion: The organisms that were significantly associated with *C. trachomatis* in this study cohort have not been previously observed in association with *C. trachomatis* infection or the vaginal microbiota. Future studies in larger cohorts that will investigate the role of these microorganisms in *C. trachomatis* infection and in the vaginal microbiota are required.

Keywords: *Chlamydia trachomatis*, Vaginal microbiota, Pregnant women, Bacterial vaginosis

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