Pelvic Inflammatory Disease: A pilot study to identify microbial and immune biomarkers for improved diagnosis of pelvic inflammatory disease

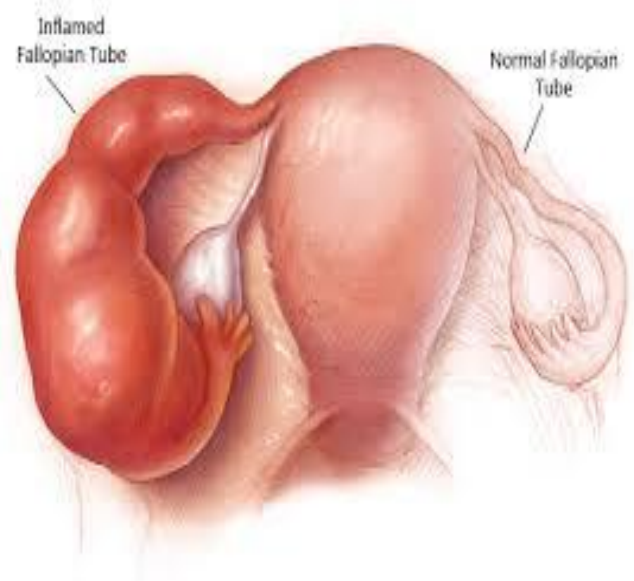
Dr Sally Sweeney – Medical Coordinator, FPNSW

e: sallys@fpnsw.org.au

Twitter @SalSweeney

PID: a syndrome of great variability….

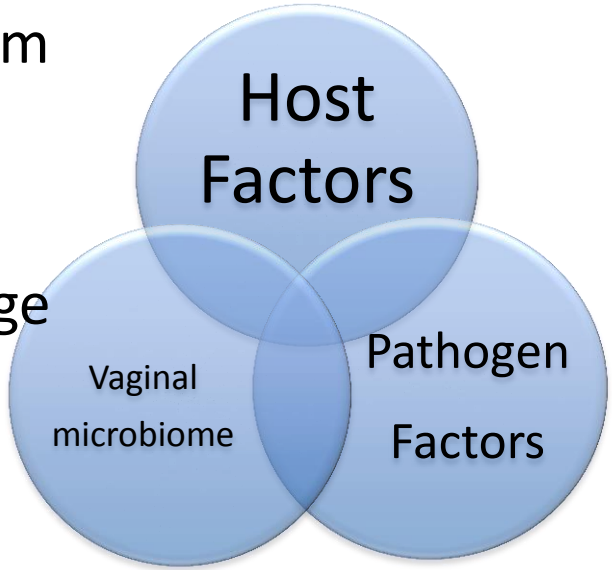
- Under-recognised cause of new onset low abdominal pain
- Spectrum of ascending inflammatory disorders affecting the upper female genital tract: endometritis, salpingitis, tubo-ovarian abscess and pelvic peritonitis
- Variation in symptoms and severity
- Prompt treatment to prevent long-term sequelae
- Symptoms may include: new onset pelvic pain, deep dyspareunia, altered vaginal discharge, abnormal bleeding, systemic features
- Examination essential to assist diagnosis



- PID in young women is mostly a sexually transmitted condition
- Always poly-microbial including vaginal bacteria
- In up to 70% no causal STI organism is identified and swabs will be negative[1,2]
- Gonorrhoea, Chlamydia, Mycoplasma genitalium detected in some cases

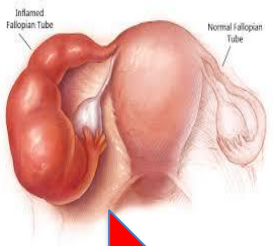
Risk Factors

- Young age 15-25 years; recent STI; recent change of partner; partner STI or symptoms
- Recent uterine instrumentation or pregnancy



1. Simms I, Stephenson JM, Mallinson H, et al. Risk factors associated with pelvic inflammatory disease. Sex Transm Infect 2006; 82: 452-457.
2. Goller JL, De Livera AM, Fairley CK, et al. Population attributable fraction of pelvic inflammatory disease associated with chlamydia and gonorrhoea: a cross-sectional analysis of Australian sexual health clinic data. Sex Transm Infect 2016 Apr 18. pii: sextrans-2015-052195. doi: 10.1136/sextrans2015-052195.

PID: Remains a clinical diagnosis



Severity of infection

Individual clinician threshold for diagnosis

Antimicrobial Stewardship

http://www.sti.guidelines.org.au/syndromes/pid-pelvic-inflammatory-disease

A Pilot Study to Identify Microbial and Immune Biomarkers for Improved Diagnosis of Pelvic Inflammatory Disease

Dr Willa Huston, Clinical Associate Professor Deborah Bateson, Ms Jane Estoesta, Dr Sally Sweeney, Dr Mary Stewart, Ms Kirsteen Fleming, Ms Jodie Duggan, Mr Rami Mazraani and Dr Catherine Burke

- Pilot Case-Control Study: 30 PID cases at Newcastle Clinic; 30 controls at Penrith Clinic, 6 additional controls recruited from Newcastle
- Women aged 18-29
- Hypotheses: 1. Women who develop PID possess a unique immune and microbial profile which differs from that in women without PID.

  2. Certain chlamydial, mycoplasma genitalium or gonococcal strains detected in women with PID have pathogenic features that are more likely to lead to the development of PID and its serious sequelae than other strains.
- Study aims: To conduct a pilot case-control study comparing the pathogenic, immunological and cervical microbiome factors in women with PID compared with those in healthy controls

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- Pilot Case-Control Study: 30 PID cases at Newcastle Clinic; 30 controls at Penrith Clinic, 6 additional controls recruited from Newcastle
- Women aged 18-29
- Aims to determine pathogenic as well as host microbial and immune factors associated with a diagnosis of PID which could potentially be used to guide development of diagnostic tools for this important clinical condition
- Ultimately, the ability to generate a molecular diagnostic tool for the specific microbial agents responsible for an individual case of PID could support tailored antimicrobial treatment. This in turn would lead to the improved use of antibiotics in line with the global goal of antimicrobial stewardship.

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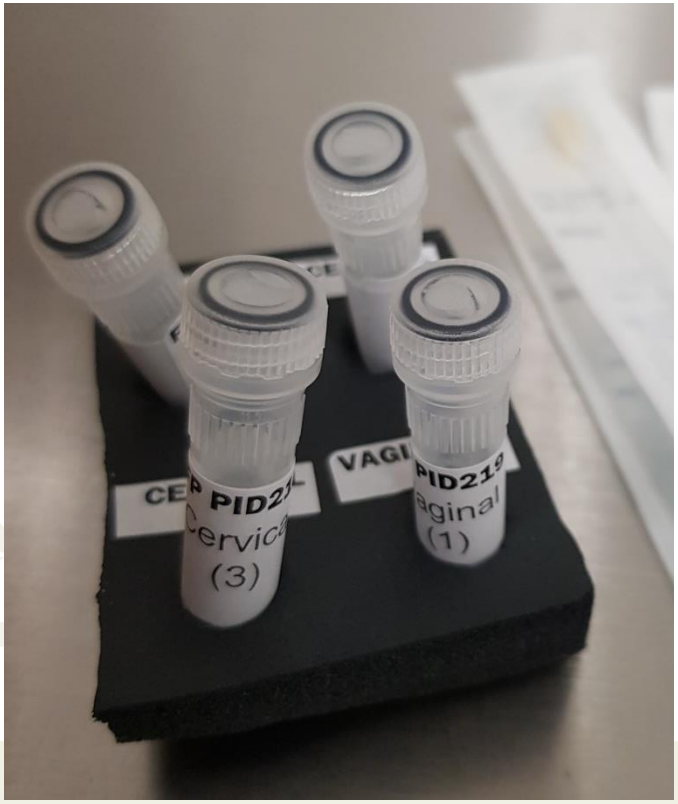

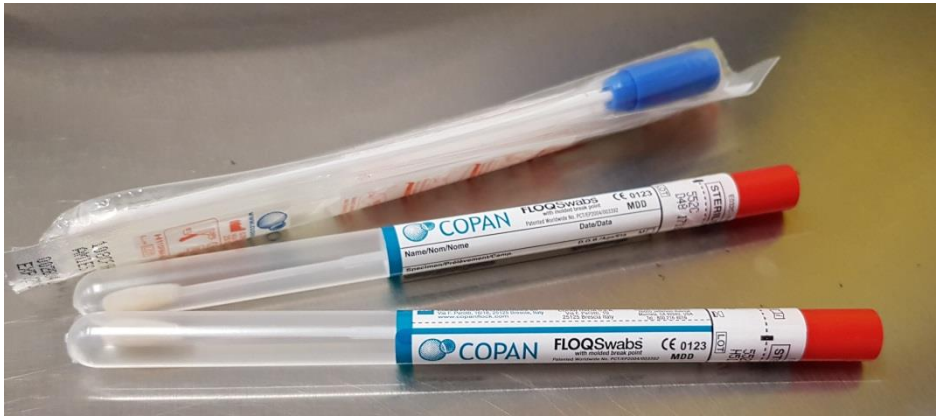

Understanding the role of the host vaginal microbiome

Vaginal microbiome of reproductive-age women

Jacques Ravel[a,1], Pawel Gajer[a], Zaid Abdo[b], G. Maria Schneider[c], Sara S. K. Koenig[a], Stacey L. McCulle[a], Shara Karlebach[d], Reshma Gorle[e], Jennifer Russell[f], Carol O. Tacket[f], Rebecca M. Brotman[a], Catherine C. Davis[g], Kevin Ault[d], Ligia Peralta[e], and Larry J. Forney[c,1]

[a]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD 21201; [b]Departments of Mathematics and Statistics and the Initiative for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, ID 83844; [c]Department of Biological Sciences and the Initiative for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, ID 83844; [d]Emory University School of Medicine, Atlanta, GA 30322; [e]Department of Pediatrics Adolescent and Young Adult Medicine, University of Maryland School of Medicine, Baltimore, MD 21201; [f]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD 21201; and [g]The Procter & Gamble Company, Cincinnati, OH 45224

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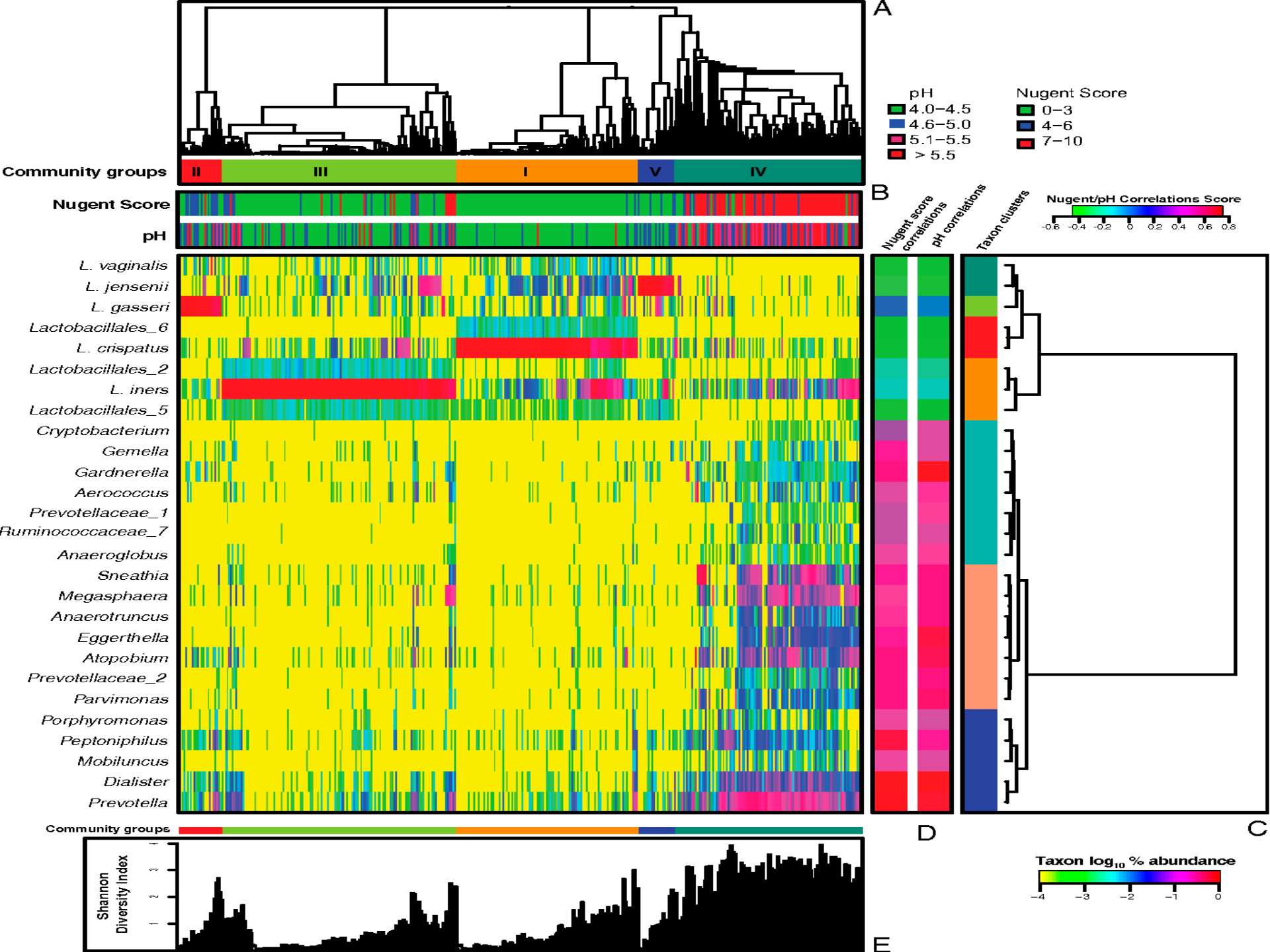
The means by which vaginal microbiomes help prevent urogenital diseases in women and maintain health are poorly understood. To gain insight into this, the vaginal bacterial communities of 396 asymptomatic North American women who represented four ethnic groups (white, black, Hispanic, and Asian) were sampled and the species composition characterized by pyrosequencing of barcoded 16S rRNA genes. The communities clustered into five groups: four were dominated by *Lactobacillus iners, L. crispatus, L. gasseri*, or *L. jensenii*, whereas the fifth had lower proportions of lactic acid bacteria and higher proportions of strictly anaerobic organisms, indicating that a potential key ecological function, the production of lactic acid, seems to be conserved in all communities. The proportions of each community group varied among the four ethnic groups, and these differences were statistically significant [$\chi^2(10) = 36.8$, $P < 0.0001$]. Moreover, the vaginal pH of women in different ethnic groups also differed and was higher in Hispanic (pH 5.0 $\pm$ 0.59) and black (pH 4.7 $\pm$ 1.04) women as compared with Asian (pH 4.4 $\pm$ 0.59) and white (pH 4.2 $\pm$ 0.3) women. Phylotypes with correlated relative abundances were found in all communities, and these patterns were associated with either high or low Nugent scores, which are used as a factor for the diagnosis of bacterial vaginosis. The inherent differences within and between women in different ethnic groups strongly argues for a more refined definition of the kinds of bacterial communities normally found in healthy women and the need to appreciate differences be-

of samples have usually been analyzed, and the depth of sample analysis was not great.

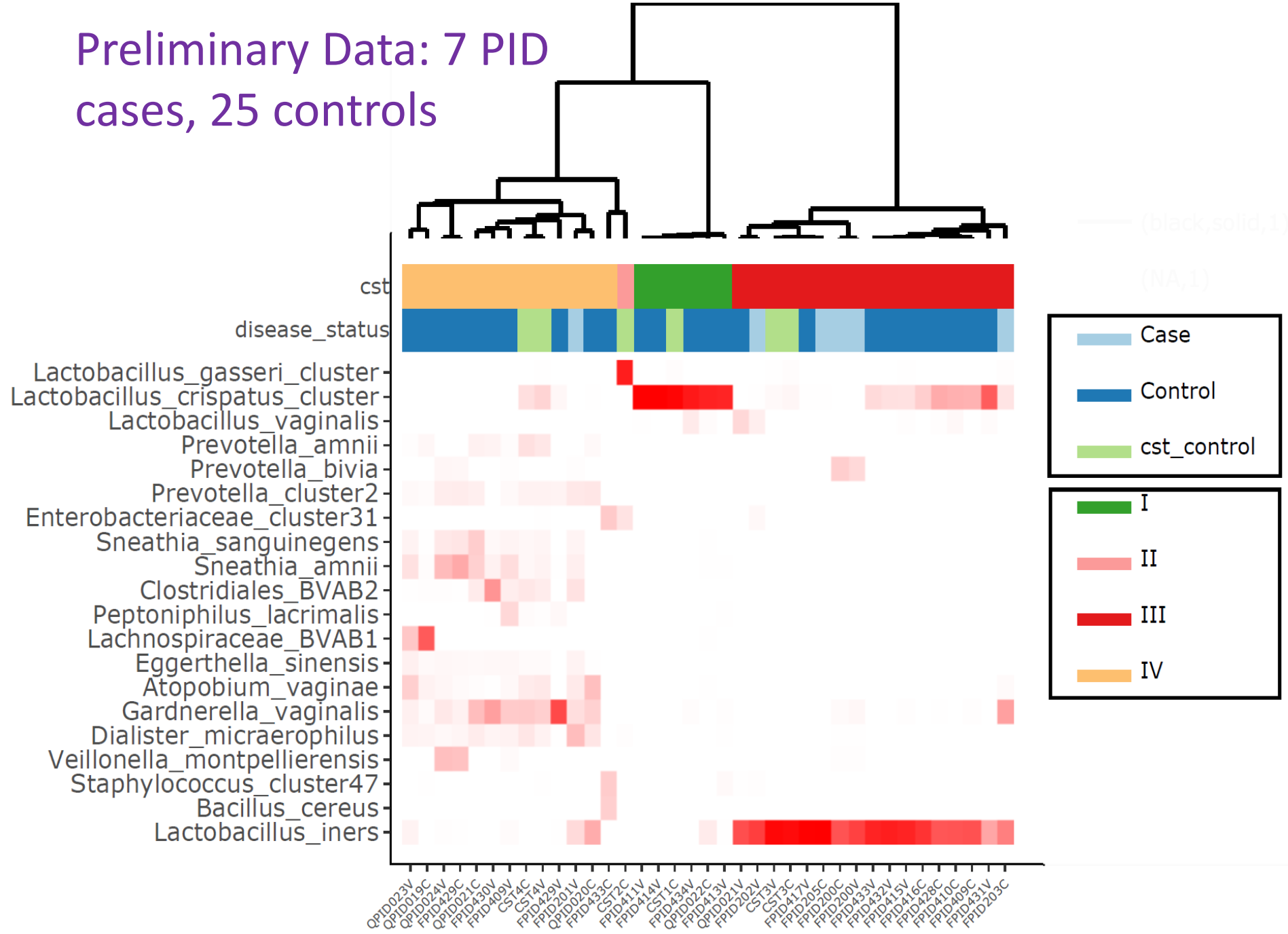
In this study we sought to develop an in-depth and accurate understanding of the composition and ecology of the vagina microbial ecosystem in asymptomatic women using a high-throughput method based on pyrosequencing of barcoded 16S rRNA genes. The data obtained are an essential prerequisite for comprehending the role and ultimately the function of vaginal microbiota in reducing the risk of acquiring diseases and identifying factors that determine disease susceptibility. Specifically we sought to characterize the vaginal microbial communities in a cohort of 396 North American women equally representing four ethnic backgrounds (Asian, white, black, and Hispanic) and further address three aims. The first was to establish whether there were correlations between community composition and vaginal pH because these would be indicative of community performance. The second was to explore how the species composition of vaginal communities was reflected in Nugent scores (25), a diagnostic factor commonly used to identify women with bacterial vaginosis (26). Finally, the third aim was to identify patterns in the relative abundances of different species because these might reflect antagonistic or cooperative interspecies interactions.

Results and Discussion

We characterized the vaginal microbiota and vaginal pH of 396


A
pH
4.0–4.5
4.6–5.0
5.1–5.5
> 5.5
Nugent Score
0–3
4–6
7–10
Community groups
II
III
I
V
IV
B
Nugent Score
pH
Nugent score correlations
pH correlations
Taxon clusters
Nugent/pH Correlations Score
-0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8
L. vaginalis
L. jensenii
L. gasseri
Lactobacillales_6
L. crispatus
Lactobacillales_2
L. iners
Lactobacillales_5
Cryptobacterium
Gemella
Gardnerella
Aerococcus
Prevotellaceae_1
Ruminococcaceae_7
Anaeroglobus
Sneathia
Megasphaera
Anaerotruncus
Eggerthella
Atopobium
Prevotellaceae_2
Parvimonas
Porphyromonas
Peptoniphilus
Mobiluncus
Dialister
Prevotella
D
C
Community groups
Shannon Diversity Index
E
Taxon log10 % abundance
−4 −3 −2 −1 0

Preliminary Data: 7 PID cases, 25 controls



Preliminary Findings:

- meta-data significantly different between case and controls in this pilot phase were those already previously correlated with PID
  - recent partner change
  - vaginal symptoms -thrush, BV

- All but 1 PID case had positive Ureaplasma on diagnostic swabs at chart review. Significance of this unknown – Ureaplasma subsequently added to pathogen culture for all samples.

- All PID cases to date had either CSTIII and CSTIV, which are microbiota states often correlated with STI risk or bacterial vaginosis

- This pilot study is sufficiently powered to detect either single immune or microbiological features that associate with PID. The results of this pilot study are being used to inform an application for a larger nationally funded study which would potentially lead to the development of immunological and microbiological diagnostic tests and other predictive tools for the development of PID.