HIV acquisition risk and genital inflammation associated with hormonal contraceptives is dependent on the vaginal microbiome

Laura Noël-Romas[1,2*], Michelle Perner[1,2*], Refilwe Molatlhegi[4*], Christina Farr Zuend[1,2], Amanda Mabula[5], Sarah Hoger[1,2], Alana Lamont[1,2], Kenzie Birse[1,2], Alicia Berard[1,2], Stuart McCorrister[6], Garett Westmacott[6], Al Leslie[5,7], Vanessa Poliquin[3], Renee Heffron[8], Lyle R. McKinnon[2], Adam D. Burgener[1,2,3,9]

[1]National HIV and Retrovirology Labs, JC Wilt Center for Infectious Diseases, PHAC, Canada; [2]Dept. of Medical Microbiology and Infectious Diseases and [3]Gynecology, University of Manitoba, Canada; [4]Medical Microbiology, University of Kwazulu-Natal, South Africa ; [5]Africa Health Research Institute, South Africa; [6]Mass spectrometry and proteomics core facility, National Microbiology Lab, PHAC, Canada; [7]Dept. of Infection and Immunity, University College London, UK; [8]Dept. of Global Health and Dept. of Epidemiology, University of Washington, Washington, USA; [9]Unit of Infectious Diseases, Dept. of Medicine Solna, Center for Molecular Medicine, Karolinska Institute, Karolinska University Hospital, Sweden

University of Manitoba

Public Health Agency of Canada / Agence de la santé publique du Canada

TUPDA0104 Poster Discussion: July 23, 1:00 (SR7)

Background

-Studies have found that both the progestin-only contraceptive, Depo-Medroxyprogesterone Acetate (DMPA), and vaginal microbial dysbiosis (bacterial vaginosis, BV) can lead to vaginal mucosal inflammation.

-Both DMPA use BV and are highly prevalent in Sub-Saharan Africa, where women have increased HIV acquisition risk (Fig 1).

-Recent meta-analyses suggest that DMPA may increase HIV risk by 40-50% [6,7], but these observations have been inconsistent between studies.

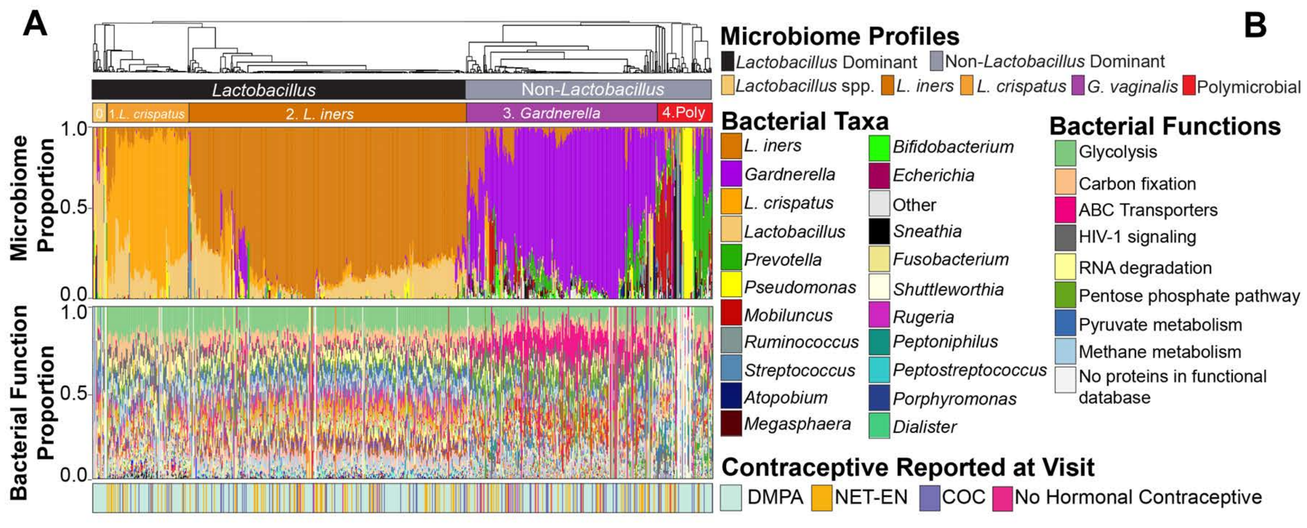
- Likewise, the presence of a non-*Lactobacillus* vaginal microbiome is commonly associated with increased mucosal inflammation and HIV acquisition risk[8-10].

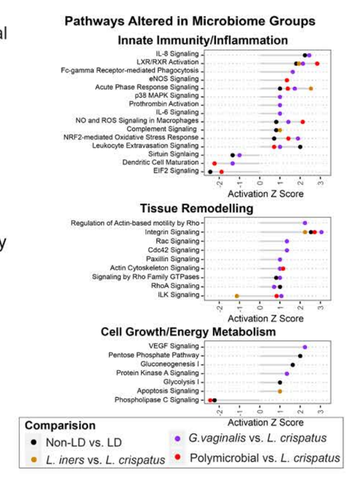
-Mass spectrometry-based proteomics, provides compositional and functional information on metabolically active bacteria. To date no one has studied the interaction between the functional microbiome and DMPA on HIV risk.

-Herein, we used a metaproteomics-based approach to estimate the interactive effect of DMPA and the vaginal microbiome on vaginal inflammation and HIV acquisition risk in 685 women from the CAPRISA-004 trial.

Results

Functionally active vaginal bacteria and host inflammation





Vaginal microbiome composition and hormonal contraceptive use

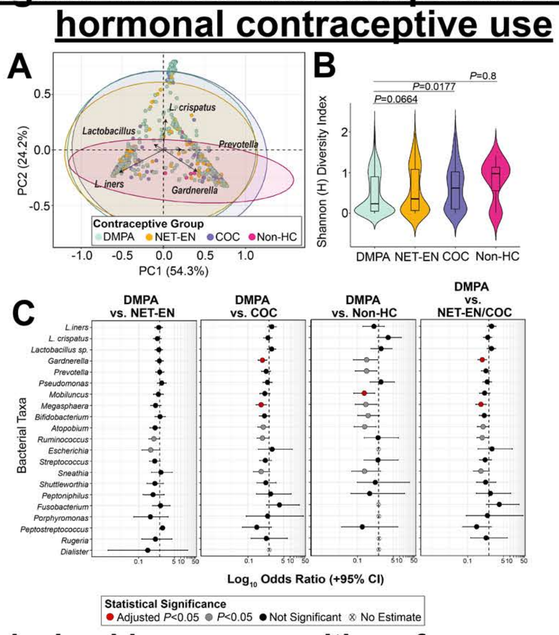


Fig 1. Inflammatory profiles in women with different vaginal microbiome composition, diversity, and function.
(A) A composition plot shows the microbiome taxa and protein functions for each sample, and the microbiome groups identified. (B) Host inflammatory pathways activated in non-*Lactobacillus* dominant profiles (Non-LD, *G. vaginalis* dominant, Polymicrobial) relative to either *Lactobacillus* or *L. crispatus*.

Fig 2. Vaginal microbiome composition of women using hormonal contraceptives as identified by mass spectrometry.
(A) Principal component (PC) analysis of bacterial proportion data showed no clustering by HC group. (B) NET-EN showed more diveristy than DMPA (P=0.017). (C) Small increases in *Gardnerella* and *Megasphaera* were seen in DMPA relative to COC.

MPA levels detected in women using hormonal contraceptives

Table 1. Proportion of subjects with detectable MPA levels by contraceptive type reported at visit.

	Proportion of Subjects (%)		
Contraceptive	**<50 pg/ml MPA**	**50-299 pg/ml MPA**	**>300 pg/ml MPA**
DMPA	2.87%	43.27%	53.87%
COC	88.89%	9.52%	1.59%
NET-EN	59.09%	36.36%	4.55%
Non-HC	100.00%	0.00%	0.00%

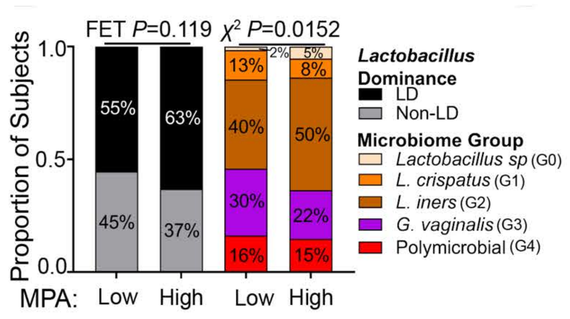


Fig 3. Microbiome group differences by MPA levels.
The proportion of women in each microbiome group for the subset of women with moderate to high levels of serum MPA (High-MPA, >300 pg/ml, n=190) compared to low or undetectable MPA (Low-MPA, ≤50 pg/ml-299 pg/ml, n=253).

Functionally active vaginal bacteria and host inflammation in women with detectable MPA

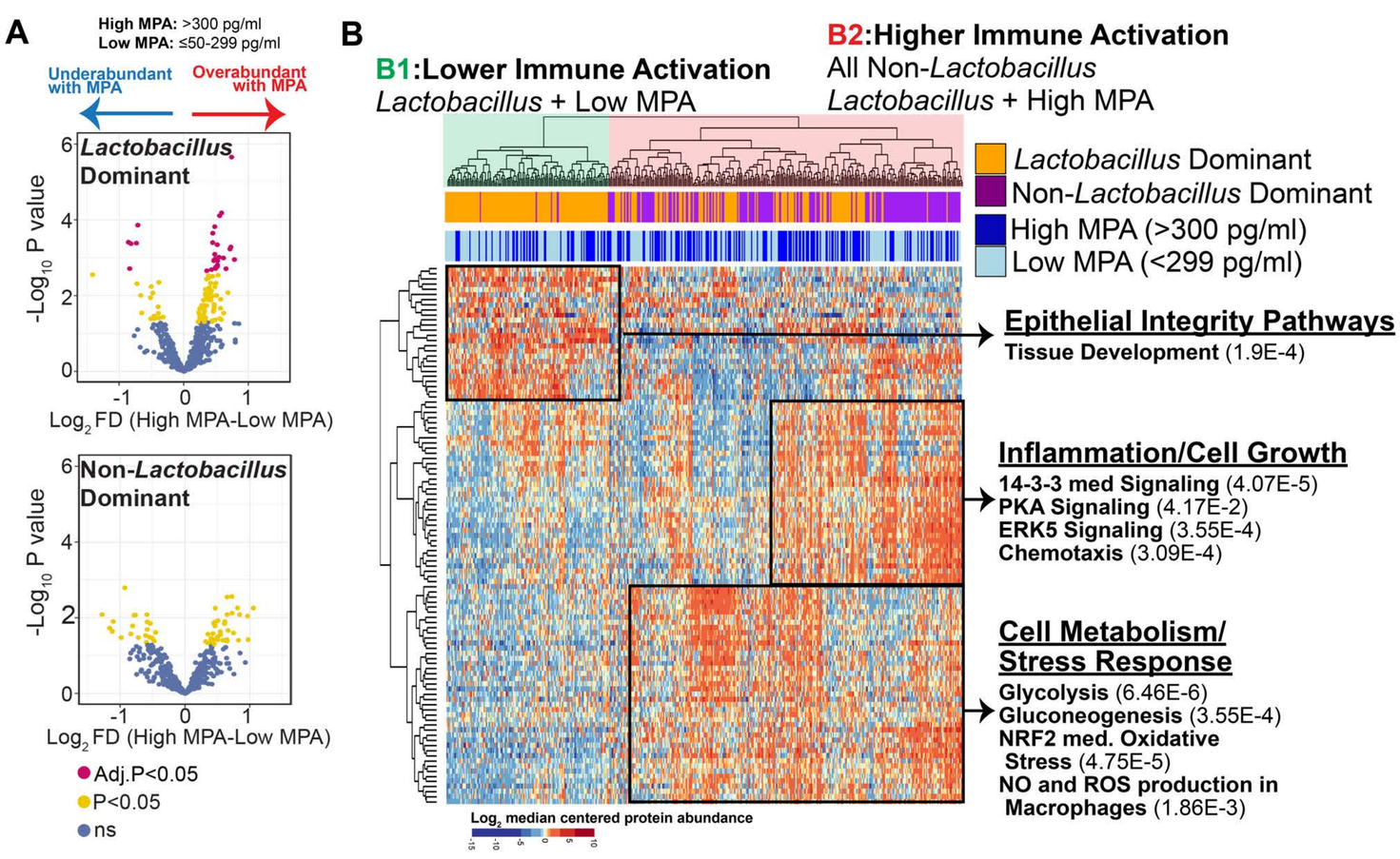


Fig 4. MPA-associated cervicovaginal proteome alterations in *Lactobacillus* dominant women.
(A) Volcano plots display cervicovaginal proteins differentially abundant in women with high vs. low MPA for each microbiome type. (B) Hierarchical clustering of proteins associated with serum MPA levels in LD women. LD/Low-MPA individuals clustered independently, and LD/High-MPA individuals clustered with non-LD women. (C) The proportion of women in each microbiome-MPA group who had lower (branch 1) or higher immune activation (branch 2). (D) Average levels of glucose metabolism biomarkers in each microbiome-MPA group. (E) Protein set enrichment analysis showed a statistical enrichment of glucose metabolism biomarkers and signatures of mucosal activated CD4+ T-cells.

Risk of HIV acquisition with DMPA by Microbiome Group

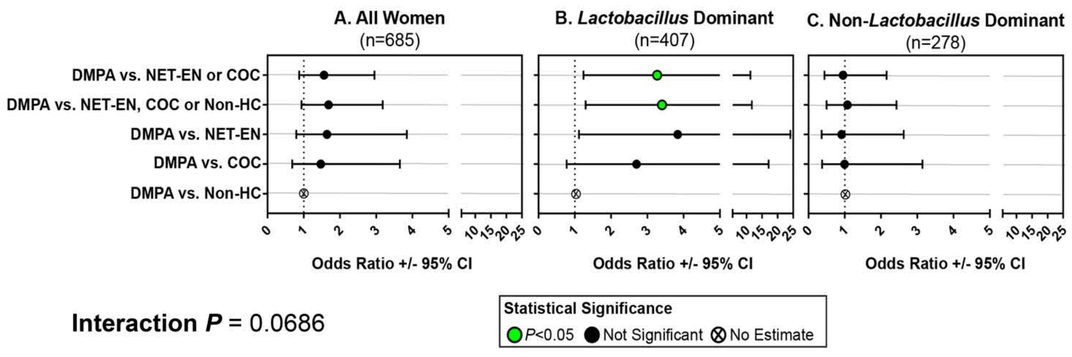


Fig 5. Probability of HIV seroconversion by microbiome type among women using different contraceptives.
Probability of HIV-seroconversion was assessed in women using DMPA relative to either hormonal (NET-EN and/or COC) and/or non-hormonal contraceptives (non-HC) across (A) all participants, (B) *Lactobacillus* dominant women or (C) non-*Lactobacillus* dominant women using logistic regression.

Results remained consistent after adjustment for potential confounding variables (age, study arm, income, education and HSV2 status), as well as partner number, sexual frequency and condom use. Sensitivity analyses within *L. iners* dominant women also showed a statistical trend toward increased MPA-associated risk. Sensitivity analysis of HC reported at baseline also showed consistent results.

Discussion

-This study shows that DMPA-associated mucosal inflammation and HIV risk may be highly dependent on vaginal microbiome communities.

-DMPA use was only associated with HIV acquisition risk in women who had *Lactobacillus* dominant microbiome communities, with a >3-fold increased rate of acquisition relative to women using other hormonal contraceptives.

-Both progestin-only hormonal contraceptives and non-*Lactobacillus* dominant vaginal communities have been shown to increase genital inflammation and alter the vaginal epithelium in women; it is possible that overlapping inflammatory mechanisms may not be additive or not influential for increased HIV susceptibility in women without vaginal *Lactobacillus*.

-This was a post hoc analysis of data collected on women from the CAPRISA-004, and contraceptive groupings were confounded by demographic characteristics such as age, condom usage and other sexual behaviours; however, adjusted analyses did not show any significant impact of these confounders on the overall results.

-Current and future studies should consider the vaginal microbiome when evaluating hormonal contraceptive safety or other products that would influence vaginal health.

Methods

DMPA: n=447 (65.1%)
NET-EN: n=123 (18%)
COC: n=97 (14.1%)
Non-HC: n=16 (2.3%)

} **HIV seroconverters**: n=63
HIV- controls: n=622

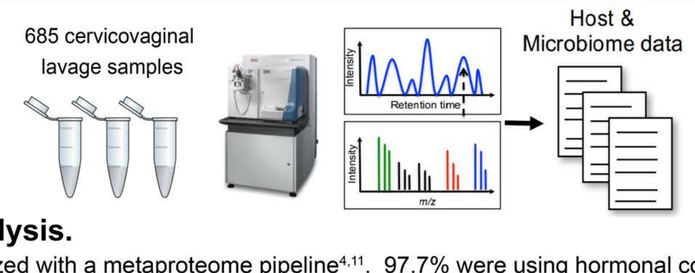


Fig 6. Study design and metaproteomic analysis.
Samples from women in the CAPRISA 004 study were analyzed with a metaproteome pipeline[4,11]. 97.7% were using hormonal contraceptives; 91.7% did not change method from baseline. Samples were analyzed by label-free mass spectrometry for bacterial and host proteins. Bacterial proteins were binned to the genus level, and functionally annotated with the KEGG database. Logistic regression models stratified by contraceptive and microbiome type were used to estimate risk of HIV infection.

Acknowledgements

We thank the CAPRISA 004 study team for providing access for samples in this study, especially Salim S. Abdool Karim, Quarraisha Abdool Karim, Leila Mansoor, Natasha Samsunder, and Jo-Ann Passmore. We are grateful to all the women who participated in the CAPRISA 004 for their willingness to contribute samples for this study. Funding for this research was provided by the Canadian Institutes of Health Research (CIHR) (A.D.B., L.R.M., S.S.A.K.) (grant TMI 138658), and the Public Health Agency of Canada (A.D.B, G.W.). M.P. is a recipient of a studentship of the CIHR and the Manitoba Health Research Council. We thank S. McCorrister, M. Abou, and C. Miller for technical assistance. We also thank all investigators of the CIHR Team Grant, including K. Broliden, K. Arnold, D. Lauffenburger, K. Hasselrot, and A. Tjernlund. The funding agencies did not have any role in the study design; the collection, analysis, and interpretation of data; or the writing of the paper or its submission for publication. The CAPRISA 004 tenofovir gel trial was funded principally by the U.S. Agency for International Development, grants through FHI360, and CONRAD for product manufacturing, with support from the South African Department of Science and Technology (DST). We thank the DST and the South African National Research Foundation for supporting the specimen repository; A. Kashuba for tenofovir concentration data; the CAPRISA 004 study team, including J. Frohlich, A. Kharsany, K. Mlisana, C. Baxter, T. Gengiah, N. Samsunder, and S. Sibeko, as well as the study clinicians, counselors, pharmacists, statisticians, and fieldwork, data quality, laboratory and administrative staff for their contributions; and the women who participated in the CAPRISA 004 trial.

CIHR IRSC
Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

References

1 Butler, A. R. et al. Modelling the global competing risks of a potential interaction between injectable hormonal contraception and HIV risk. AIDS. (2013).
2 Borgdorff, H. et al. Lactobacillus-dominated cervicovaginal microbiota associated with reduced HIV/STI prevalence and genital HIV viral load in African women. ISME. (2014).
3 Hummelen, R. et al. Deep sequencing of the vaginal microbiota of women with HIV. PLoS One.(2010).
4 Klatt, N. R. et al. Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women. Science. (2017).
5 Anahtar, M. N. et al. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. Immunity.(2015).
6 Morrison, C. S. et al. Hormonal contraception and the risk of HIV acquisition: an individual participant data meta-analysis. PLoS Med. (2015).
7 Polis, C. B. et al. An updated systematic review of epidemiological evidence on hormonal contraceptive methods and HIV acquisition in women. AIDS. (2016).
8 Shannon, B. et al. Distinct Effects of the Cervicovaginal Microbiota and Herpes Simplex Type 2 Infection on Female Genital Tract Immunology. J Infect Dis . (2017).
9 Zevin, A. S., McKinnon, L., Burgener, A. & Klatt, N. R. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. Curr Opin HIV AIDS. (2016).
10 Gosmann, C. et al. Lactobacillus-Deficient Cervicovaginal Bacterial Communities Are Associated with Increased HIV Acquisition in Young South African Women. Immunity. (2017).
11 Abdool Karim, Q. et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science. (2010).