Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier

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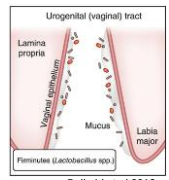
Cervicovaginal microbiome (VMB)

- Healthy composition: dominated by lactobacilli
- Dysbiosis associated with:
  - Bacterial vaginosis (BV)
  - Increased risk of HIV acquisition and other adverse reproductive health outcomes
- Mechanisms largely unclear
  - Cervicovaginal inflammation and other changes to mucosal barrier thought to have important roles

Cervicovaginal mucosal barrier

- Mechanical barrier
  -> mucus, epithelium
- Innate immune response
  -> cytokines, antimicrobial peptides and enzymes
- Adaptive immune response



Belkaid et al 2013

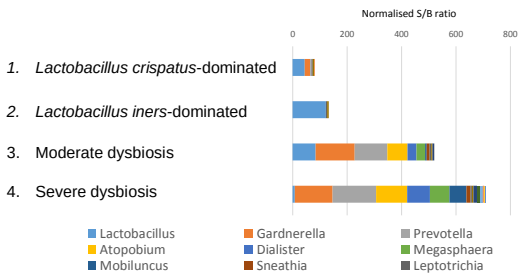
- Strengthened by a *Lactobacillus*-dominated VMB (lactic acid, other antimicrobial products).

Methods

- Comparison of cervicovaginal proteome among four VMB groups
- CVLs of 50 women from a cohort of Rwandan female sex workers.
- Mass spectrometry
- Targeted approach (pre-defined mucosal barrier proteins)
- Untargeted approach (differentially abundant proteins, adjusted for multiple comparisons)

Methods

Four groups, in order of increasing bacterial diversity:



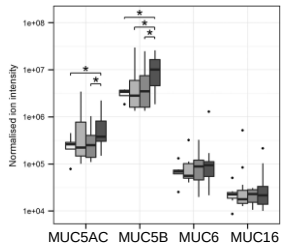
Results: study groups

	L. crispatus-dominated (n=7)	*L. iners*-dominated (n=11)	Moderate dysbiosis (n=14)	Severe dysbiosis (n=18)
Median age [IQR]	30 [26-36]	32 [26-40]	30 [23-34]	27 [24-30]
Consistent condom use	4 (57%)	2 (22%)	1 (8%)	6 (43%)
Hormonal contraceptive use	3 (43%)	10 (91%)	13 (83%)	16 (89%)
Median day of menstrual cycle [IQR]	31 [27-112]	15 [12-33]	19 [16-35]	16 [11-29]
BV by Nugent	**0 (0%)**	**0 (0%)**	**9 (64%)**	**19 (100%)**
Any viral STI	**1 (14%)**	**9 (82%)**	**12 (86%)**	**16 (89%)**
HIV	1 (14%)	4 (36%)	8 (57%)	10 (56%)
Leukocytes (+++)	1 (14%)	1 (9%)	2 (14%)	8 (44%)

Targeted analysis: Mechanical barrier

With increasing bacterial diversity:

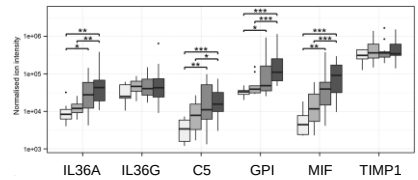
- MUC5AC↑, MUC5B ↑
- Keratins↓
- LDHA and LDHB ↑



Targeted analysis: Innate immunity

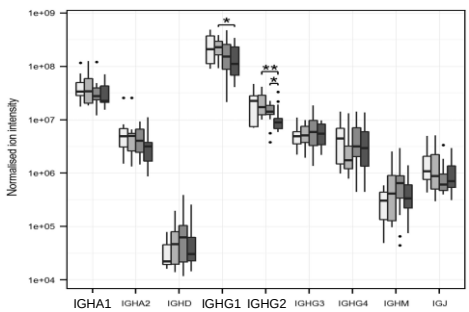
With increasing bacterial diversity:

- Antimicrobial peptides: CSTA↓, LYZ↓, RPS27A↓, S100A7↑, S100A9↑, histones↑
- Increase pro-inflammatory cytokines:



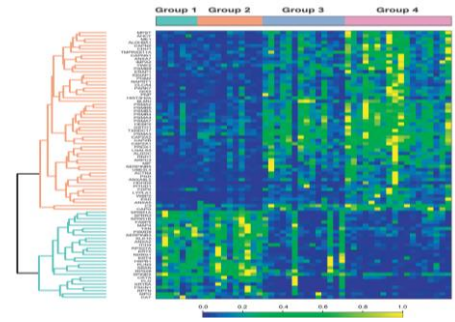
Targeted analysis: Adaptive immunity

With increasing bacterial diversity:



Untargeted analysis

82/549 proteins differentially abundant



Untargeted analysis

82/549 proteins differentially abundant

Proteasome subunits

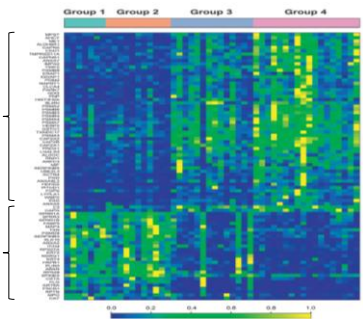
Proteases

Catabolic enzymes

Actin-organizing proteins

Protease inhibitors

Epithelial proteins



Limitations

- Only Rwandan women at high risk
- Cross-sectional data
- Limited sample size

5. Conclusions

Conclusions

- Strong relationship between the VMB and cervicovaginal human proteome
- With increasing bacterial diversity: mucus alterations, cytoskeleton alterations, increasing cell death, increasing proteolytic activity, altered AMP balance, increasing pro-inflammatory cytokines, and decreasing IgG1/2.
- Supports hypothesis that dysbiosis causes cervicovaginal inflammation and other detrimental changes to the mucosal barrier
- Systems biology approaches should be incorporated into larger epidemiological and intervention studies

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Rinda Ubuzima team Rwanda

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