The influence of sexual debut on selected vaginal, ano-rectal and oral microbiota and vaginal inflammatory markers in Belgian adolescent girls: A cohort study

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Background

- Previously
  - characterisation of vaginal microbiota and inflammatory markers in adult sexually active women
    - in Belgium (EC grant EMPRO) *
    - in Africa (EDCTP grant Biomarkers) **

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  - adolescent girls around the time of sexual debut
    - not sexually active
    - sexually active young women
    - girls starting sexual activity

*Jespers V. *BMC Microbiol* 2012   **Jespers V. *BMC Inf Dis* 2015;   Kyongo J. *Clin Vaccine Imm* 2015

Methods (1)

- Descriptive cohort study
  - 3 study visits with 2-3 months in between
  - sample size: 100 girls between 15 and 18 years old (median first sex BE = 17,1 years old)
  - 4th and 5th grade secondary school: recruitment through schools centre of Antwerp
- Interview
- Questionnaire sexual behaviour on tablet device
- Self-sampling

- Three vaginal swabs
  - Quantitative PCR
    - Five vaginal *Lactobacillus* species, *Lactobacillus* genus
    - *G. vaginalis* and *A. vaginae*
  - Luminex: eight inflammatory markers
  - BV by Nugent score
- One oral and one ano-rectal swab
  - qPCR
    - *Lactobacillus* genus
    - *G. vaginalis* and *A. vaginae*

Results – behavioural facts

- 93 adolescents (mean age 16.2; range 14-20 years)
  - 53 (57%) were virgins
  - 35 (37.6%) vaginal penetrative sexual intercourse
  - 5 (5.4%) had engaged in non-penetrative activity*
- During follow-up, 9 (9.7%) participants had vaginal penetrative sexual intercourse for the first time

*kissing, vaginal touching: fingering, cunnilingus and use of a vaginal stimulator, giving a blow job

Results microbiota (1): BV and pH cross-sectionally

Sexual experience:	None	Non-vaginal-penetrative exposure	Vaginal-penetrative exposure	P-value Fisher exact test
	N=53	N=5	N=35	
*Bacterial vaginosis**				**0.009**
Negative (Nugent 0-3)	40 (87%)	2 (40%)	25 (73.5%)	
Intermediate (Nugent 4-6)	6 (13)	2 (40%)	4 (11.8%)	
Positive (Nugent 7-10)	0	1 (20%)	5 (14.7%)	
*pH category***				**0.099**
3-3.5	8 (16.7%)	2 (50%)	3 (8.6%)	
4	22 (45.8%)	0	13 (37.1%)	
4.5-5.5	18 (37.5%)	2 (50%)	19 (54.3%)	

*Nugent slides poor quality and unreadable for 8 participants.   **6 missing values

Results microbiota (2): qPCR species cross-sectionally

Sexual experience:	None	Non-vaginal-penetrative exposure	Vaginal-penetrative exposure	
	N=53	N=5	N=35	P-value*
Vaginal species presence: Lactobacillae				
Lactobacillus genus	50 (100%)	5 (100%)	35 (100%)	
L. crispatus	32 (64%)	4 (80%)	19 (54%)	
L. iners	28 (56%)	3 (60%)	21 (60%)	
L. jensenii	21 (42%)	3 (60%)	20 (57%)	
L. gasseri	28 (56%)	3 (60%)	17 (49%)	
L. vaginalis	25 (50%)	2 (40%)	13 (37%)	
BV related species				
Gardnerella vaginalis	13 (26%)	3 (60%)	19 (54.3%)*	0.009
Atopobium vaginae	9 (18%)	2 (40%)	13 (37.1%)*	0.051

*Logistic regression presence species with the "None sexual experience category" as reference category. Only significant results shown.

# Results microbiota (3): qPCR species cross-sectionally

Sexual experience:	None	Non-vaginal-penetrative exposure	Vaginal-penetrative exposure	
	N=53	N=5	N=35	P-value*
Ano-rectal species presence				
	N=44	N=3	N=24	
Lactobacillus genus	43 (97.7%)	3 (100%)	24 (100%)	
G. vaginalis	12 (27.3%)	1 (33.3%)	14 (58.3%)*	0.014
A. vaginae	6 (13.6%)	1 (33.3%)	11 (45.8%)*	0.005
Oral species presence				
Lactobacillus genus	53 (100%)	5 (100%)	35 (100%)	
G. vaginalis	0	0	0	
A. vaginae	0	1 (20%)	1 (2.9%)	

*Logistic regression presence species with the "None sexual experience category" as reference category. Only significant results shown.

Results microbiota (4): Presence and amount of vaginal microbiota species by participant over the three visits
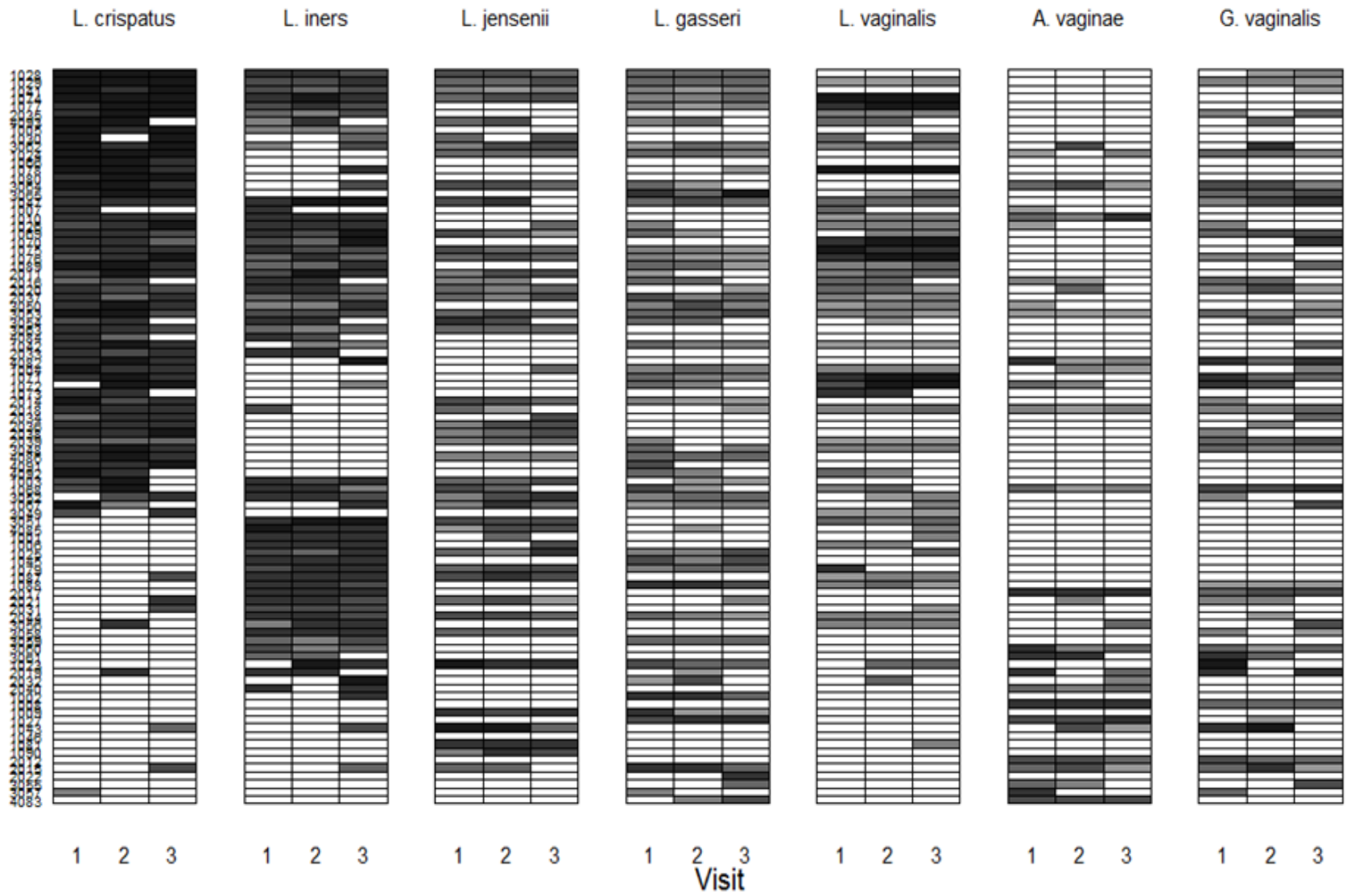
Results microbiota (5): Longitudinal analysis of microbiota - modelled

	Standard Deviation Between Girls	Intercept	Girls who are sexually active		Girls who become sexually active		Visit 2		Visit 3	
			OR	P-Value	OR	P-Value	OR	P-Value	OR	P-Value
L. crispatus	3.56	2.42	1.09	0.929	26.96	0.120	1.26	0.682	1.51	0.479
L. iners	3.64	1.70	1.68	0.617	1.70	0.748	0.86	0.785	3.99	**0.022**
L. jensenii	4.02	0.76	1.98	0.551	0.08	0.214	1.19	0.783	1.06	0.923
L. gasseri	3.13	1.48	0.68	0.653	4.68	0.328	0.80	0.648	0.75	0.570
L. vaginalis	3.73	0.56	0.44	0.439	2.30	0.639	2.04	0.212	3.03	0.068
A. vaginae	3.39	0.01	34.78	**0.006**	15.83	0.122	0.93	0.898	0.81	0.730
G. vaginalis	2.11	0.13	10.25	**<0.001**	10.61	**0.022**	0.84	0.690	1.27	0.593
A. vaginae rectal	4.61	0.001	347.2	**0.002**	18.92	0.425	0.20	0.080	0.32	0.190
G. vaginalis rectal	3.17	0.04	72.39	**0.002**	24.13	0.076	0.36	0.120	0.47	0.249

The model is as follows: the intercept is the odds of having a species at visit 1 in the group who are not sexually active during the whole study. The observed odds varies among girls by the between-girls standard deviation.

Results markers (1): Distribition inflammatory markers
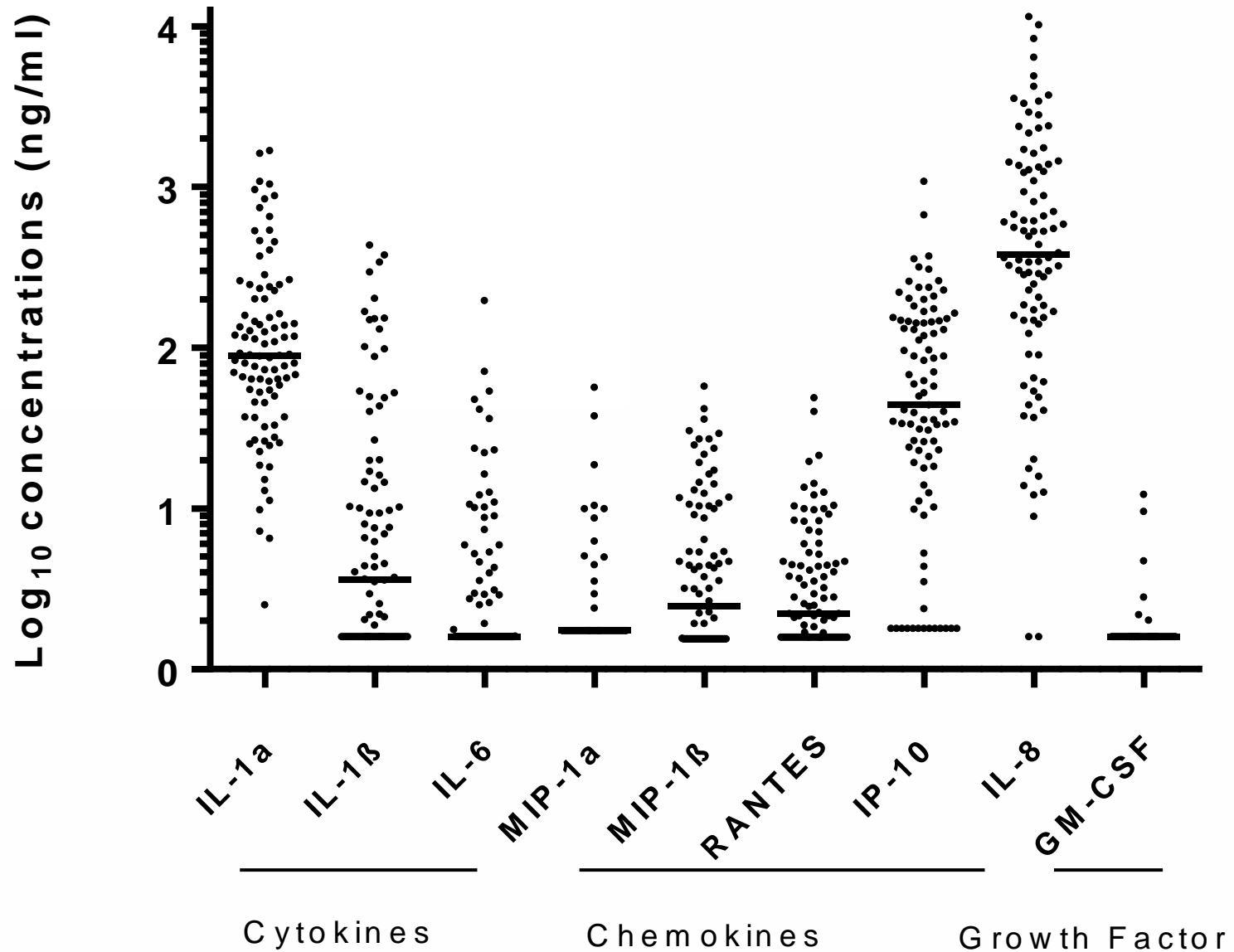
Results markers (2): cross-sectional data

Sexual experience:	None	Non-vaginal-penetrative exposure	Vaginal-penetrative exposure	
	N=53	N=5	N=35	P-value*
Cytokines				
IL-1α	1.87(1.51-2.09)	2.06(1.83-2.57)	2.30(1.85-2.69)*	<0.001
IL-1β	0.32(0.20-0.97)	0.41(0.20-1.72)	0.80(0.20-2.00)*	0.007
IL-6	0.20(0.20-0.45)	0.20(0.20-0.77)	0.29(0.20-1.01)*	0.043
Chemokines				
MIP-1α	0.24(0.24-0.38)	0.24(0.24-0.24)	0.24(0.24-1.00)	
MIP-1β	0.19(0.19-0.70)	0.62(0.19-1.02)	0.65(0.19-1.16)*	0.010
RANTES	0.32(0.20-0.64)	0.20(NA)	0.47(0.33-0.84)*	0.018
IP-10	1.85(1.10-2.17)	1.56(0.81-2.30)	1.79(1.54-2.37)	
IL-8	2.38(1.75-2.81)	2.82(2.48-3.09)	2.85(2.48-3.37)*	<0.001
Growth factor				
IL-6	0.20(0.20-0.45)	0.20(0.20-0.77)	0.29(0.20-1.01)*	0.043

*Regression analysis with the "None sexual experience category" as reference category. Only significant results shown.

Results markers (3): Longitudinal analysis of markers - modelled

	Standard Deviation			Girls who are sexually active		Girls who become sexually active		Visit 2		Visit 3	
	Between girls	Within girls	Average value	Estimate	P-Value	Estimate	P-Value	Estimate	P-Value	Estimate	P-Value
IL-1α	0.27	0.42	1.81	0.40	**<0.0001**	0.29	**0.030**	0.02	0.776	0.03	0.589
IL-1β	0.53	0.49	0.65	0.40	**0.004**	0.25	0.256	0.004	0.954	0.08	0.272
IL-6	0.32	0.34	0.39	0.24	**0.006**	0.10	0.464	0.02	0.654	-0.00	0.923
MIP-1α	0.20	0.32	0.40	0.14	0.127	0.01	0.951	0.09	0.193	0.04	0.580
MIP-1β	0.32	0.30	0.50	0.24	**0.004**	0.01	0.934	-0.03	0.510	-0.04	0.355
RANTES	0.24	0.27	0.40	0.25	**0.0002**	0.10	0.321	-0.003	0.934	-0.01	0.835
IP-10	0.57	0.49	1.50	0.19	0.190	0.23	0.325	0.10	0.167	-0.05	0.540
IL-8	0.59	0.53	2.33	0.51	**0.001**	0.25	0.297	0.02	0.796	0.03	0.688
GM-CSF	0.08	0.14	0.23	0.08	**0.029**	0.001	0.984	0.004	0.888	-0.04	0.229

Average value in $\log_{10}$ ng/ml concentrations.
The model is as follows: the average value is the expected value at visit 1 in the group who are not sexually active during the whole study. The observed average value varies among girls by the between-girls standard deviation and between observations for the same girl by the within standard deviation.

Conclusions

- BV
  - by Nugent score was not present before sexual debut
  - was present for the sexually experienced non-vaginal penetration category
- Species
  - The presence of *Lactobacillus* species was not affected by sexual debut and were stable within participants over time
  - *G. vaginalis* and *A. vaginae* presence vaginally and ano-rectally was positively associated with sexual debut
  - Girls who became sexually active over time had an inreased odds for the presence of vaginal *G. vaginalis*
- Markers
  - IL-1α, IP-10 and IL-8 were nicely distributed
  - In the sexually active girls, several markers were increased compared to the non-sexually active
  - Adolescents becoming sexually active were likely to have increased IL-1α

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Thank you for listening

Acknowledgement

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