A Fruitful Alliance: the Synergy between *Atopobium vaginae* and *Gardnerella vaginalis* in Bacterial Vaginosis-Associated Biofilm The importance of *Atopobium vaginae* in bacterial vaginosis-associated biofilm

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## Abstract

- Bacterial vaginosis (BV) is characterized by a change in the microbial composition of the vagina. The BV-associated organisms outnumber the healthassociated Lactobacillus spp. and form a polymicrobial biofilm on the vaginal epithelium, possibly explaining the difficulties with antibiotic treatment. A better understanding of vaginal biofilm with emphasis on *Gardnerella vaginalis* and *Atopobium vaginae* may contribute to a better diagnosis and treatment of BV.
- To this purpose, we evaluated the association between the presence of both bacteria by Fluorescence In Situ Hybridization (FISH) and BV by Nugent scoring in 463 vaginal slides of 120 participants, participating in a clinical trial in Rwanda (Ring Plus study Clinicaltrials.gov NCT01796613).

In half of the samples, bacterial biofilm was detected using the universal bacteria-probe. *A. vaginae* and *G. vaginalis* were part of this biofilm in resp. 54.1% and 82.0% of these samples and *A. vaginae* was almost always accompanied by *G. vaginalis*. The odds of a Nugent score above 4 was higher for samples with the presence of dispersed *G. vaginalis* and/or *A. vaginae* (odds ratio 4.5; CI: 2-10.3) and when a combination of adherent *G. vaginalis* and dispersed *A. vaginae* was present (odds ratio 75.6; CI: 13.3-429.5). When both bacteria were part of the biofilm, the odds for a Nugent score above 4 was 119 (CI: 39.9-360.8).

Table 1: *G. vaginalis, A. vaginae,* and combinations for samples analyzed with FISH by absent, dispersed only, and adherent and/or dispersed category vaginae and stratified by Nugent scoring

	Total	Nugent 0-3	Nugent 4-6	Nugent 7-10		
	Ν	N (%)	N (%)	N (%)		
FISH all bacteria						
absent	0	0 (0.0)	0 (0.0)	0 (0.0)		
dispersed only	230	197 (76.0)	19 (39.6)	14 (9.0)		
adherent and/or dispersed	233	62 (24.0)	29 (60.4)	142 (91.0)		
FISH A. vaginae (Av)						
absent	268	201 (77.6)	24 (50.0)	43 (27.6)		
dispersed only	69	41 (15.8)	10 (20.8)	18 (11.5)		
adherent and/or dispersed	126	17 (6.6)	14 (29.2)	95 (60.9)		
FISH <i>G. vaginalis</i> (Gv)						
absent	172	155 (59.8)	8 (16.7)	9 (5.8)		
dispersed only	100	71 (27.4)	15 (31.2)	14 (9.0)		
adherent and/or dispersed	191	33 (12.8)	25 (52.1)	133 (85.2)		
FISH Av and Gv combined						
Gv and Av absent	170	153 (59.1)	8 (16.7)	9 (5.7)		
Gv or Av dispersed only	101	72 (27.8)	15 (31.2)	14 (9.0)		
Gv adherent and/or Gv dispersed	51	14 (5.4)	8 (16.7)	29 (18.6)		
and Av absent						
Gv adherent and/or Gv dispersed	15	3 (1.1)	3 (6.2)	9 (5.8)		
and Av dispersed						
Gv and Av adherent and/or dispersed Gv and Av	126	17 (6.6)	14 (29.2)	95 (60.9)		

Figure 1: Superimposed CLSM images with 400 x magnification of Av + Gv biofilm, in 6 vaginal samples (A-F): vaginal epithelial cells (DAPI -blue), Av (AtoITM1 - green) and Gv (Gard162 - red)



Table 2: Association between the bacterial presence of *A. vaginae* and *G. vaginalis* by FISH and the vaginal microbiome defined by Nugent scoring

Gv and Av	absent	Gv or Av	Gv adherent	Gv adherent +/- Gv	Gv and Av adherent
combined			+/- Gv dispersed	dispersed and Av	+/- dispersed Gv and
			and Av absent	dispersed only	Av
Total=463	170	101	51	15	126
Nugent 0-3	153 (90)	72 (71.3)	14 (27.5)	3 (20)	17 (13.5)
Nugent 4-10	17 (10)	29 (28.7)	37 (72.5)	12 (80)	109 (86.5)
OR	Reference	4.5	49.2	75.6	119
(CI)		(2-10.3)	(15.9-151.8)	(13.3-429.5)	(39.9-360.8)
P-value Chi <sup>2</sup>		0.001	< 0.001	< 0.001	< 0.001
test					





Our study was not comprehensive in studying the polymicrobial biofilm in BV, but it provides strong indications regarding the importance of *A. vaginae* and the symbiosis between *A. vaginae* and *G. vaginalis* in this biofilm.

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