Functional analysis of virulence potential from *Gardnerella vaginalis* and other anaerobes commonly associated with Bacterial vaginosis

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In the past half century, bacterial vaginosis (BV) has been a controversial topic in medical microbiology, and despite the wealth of information on this topic, the etiological agent has not yet been definitively identified [1]. The first advances on BV pointed *Gardnerella vaginalis* as the infectious causative agent of BV [2] but soon after it was found that *G. vaginalis* was also present in healthy women [3]. Additionally, *G. vaginalis* was not able to cause BV consistently. Furthermore, other microorganisms started to be associated with BV, and this resulted in a shift in the paradigm to that of a multispecies infection. However, epidemiological data revealed inconsistencies with this latter theory [4]. A couple of years ago the first descriptions of multispecies biofilm communities were described in BV [5]. Interestingly, *G. vaginalis* was present in most cases and accounted for the majority of the biofilm biomass. Further studies demonstrated that biofilm-forming *G. vaginalis* presented higher tolerance to external stresses [6].

Taking these data into consideration, we hypothesized that strains of *G. vaginalis* that were able to form biofilms could be the causative agent of BV. To test our hypothesis, we isolated more than 30 bacterial species from BV patients and also several strains of *G. vaginalis* from healthy women, and tested biofilm forming ability, initial adhesion to human vaginal cells, cytotoxicity activity, antimicrobial resistance and gene expression of know virulent genes.

Our results revealed that *G. vaginalis* outcompeted all the other bacterial species in the initial adhesion to the epithelial cells. Furthermore, when comparing BV-associated *G. vaginalis* strains to strains isolated from healthy women, we found that all 7strains from BV were more virulent than the 7 strains colonizing healthy women, as measured by the higher cytotoxicity and the higher initial adhesion to epithelial cells. No significant differences were found in antimicrobial resistance profiles. Interestingly, no significant differences in expression of known virulence genes were detected, suggesting that the higher virulence of the BV-associated *G. vaginalis* was due to a yet unknown virulence determinant. We then tested virulent *G. vaginalis* against other known BV-associated anaerobe pathogens, namely *Mobiluncus mulieris*, *Atopobium vaginae*, *Prevotella bivia* and *Fusobacteria nucleatum* in mixed biofilm formation quantification. Interestingly, while the other tested anaerobes did not reveal a higher initial adhesion, they did enhance biofilm formation by *G. vaginalis*.

Overall, our data suggests that virulent variants of *G. vaginalis* have the potential to be the etiological agent of BV, while acknowledging that other anaerobes do enhance *G. vaginalis* virulence.

Keywords: Bacterial vaginosis; multi-species biofilms; virulent variants; gene expression; citotoxicity assays

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