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THE ROLE OF *GARDNERELLA VAGINALIS* IN MIXED SPECIES BIOFILMS OCCURRENCE IN BACTERIAL VAGINOSIS AND ITS PREVALENCE IN PORTUGAL

Nuno Cerca¹

¹CEB-IBB, Universidade do Minho

Bacterial vaginosis (BV) is the most common gynaecological clinical condition in reproductive -age women in reproductive age, and it has been associated with an increased risk of development of preterm labour, spontaneous abortion, and several sexually transmitted diseases such as HIV. Despite being of worldwide importance, no studies, to date, were performed in Portugal. Furthermore, 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. The first advances on BV pointed *Gardnerella vaginalis* as the infectious causative agent of BV but soon after it was found that *G. vaginalis* was also present in healthy women. 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. A couple of years ago the first descriptions of multispecies biofilm communities were described in BV. 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.

With this state of the art, I've hypothesized that strains of *G. vaginalis* that were able to form biofilms could be the causative agent of BV and proposed to conduct the first epidemiological survey of this condition in Portugal. To test my hypothesis, my research group collected near 300 vaginal samples and then 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 known virulent genes. We also developed a novel PNA based multiplex methodology that was able to improve molecular diagnostic of BV and was then used to quantify the initial adhesion interactions between different bacterial species.

From the results gathered in the past 3 years, my research group was able to demonstrate that near 20% of Portuguese women had past or present BV episodes, and near 40% were colonized with *G. vaginalis*. Furthermore, BV associated *G. vaginalis* outcompeted all the other BV associated bacterial species in the initial adhesion to the epithelial cells and cytotoxicity assays. Furthermore, when comparing BV-associated *G. vaginalis* strains to strains isolated from healthy women, we found that all 7 strains from BV were more virulent than the 7 strains colonizing healthy women. 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. Also, no significant differences were found in antimicrobial resistance profiles between the two groups. 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 revealed that BV is a prevalent condition in Portugal, and 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.

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