

45 Characterization of biofilm-forming microorganisms isolated from vaginal exudate in Portugal

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Bacterial vaginosis (BV) is one of the most common gynaecological conditions affecting women in the reproductive age, and can lead to increase risk gynaecological infections and pre-term labour. The aetiology of this pathology is still poorly understood, but recent reports referring to the presence of anaerobic biofilms both in the healthy and BV vagina (with different microbial compositions) have led to the theory that the microorganisms that form biofilms may be relevant for the aetiology of BV. The aim of this work was to isolate and characterise the microbial population present in the vagina of Portuguese women. For this, swabs containing samples of exudate were collected and used for the isolation of the microorganisms present. Once isolated the microorganisms were characterised for: 1) their ability to adhere to HeLa epithelial cells, 2) their biofilm forming capability and 3) their antimicrobial susceptibility. Each of the bacterial isolates was identified by sequencing the 16S gene. 13 unique isolates, from the species *Gardnerella vaginalis*, *Gemella haemolysans*,

Bifidobacterium breve, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Lactobacillus gasseri*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* and *Streptococcus salivarius*, were characterised for their initial adhesion ability to a monolayer of HeLa cells by incubating the bacteria with this monolayer and quantifying the adhesion by staining with DAPI and fluorescence microscopy. These assays revealed that *Gardnerella vaginalis* isolates had a stronger initial adhesion capability than the other isolates recovered, the weakest initial adhesion being observed with *Propionibacterium acnes*. The biofilm forming capacity was then assessed by allowing each of the strains to form biofilms under anaerobic conditions for 48 hours and using different media. It was possible to observe that isolates *G. vaginalis* also formed large amounts of biofilm while *Streptococcus agalactiae* formed little biofilm under the conditions used. Finally, the antimicrobial susceptibility was determined using MIC tests for antibiotics commonly used in the treatment of BV (metronidazole, tinidazole and clindamycin). Results showed that most microorganisms had MIC's similar to those previously reported in the literature, with a few exceptions reporting resistance to antibiotics. This work is one of the few reports on the biofilm forming capability of vaginal microorganisms other than *G. vaginalis*, *Lactobacillus* spp and *Candida* spp. thus demonstrating that other bacteria commonly found in this ecosystem also have the capability of forming biofilms and this result should be taken into account when researching the aetiology of BV.

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