Bacterial vaginosis: epidemiologic, clinical and diagnostic updates

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Summary

Background and aims. Bacterial vaginosis (BV) is one the more frequently identified genital syndrome among childbearing aged women. The basic condition that generates this condition is a modification in the vaginal microbiota. The aim of this paper is to briefly review the current *status of the art* of BV and to report the results of a pilot study performed with an innovative PCR based technique.

*Materials and Methods***.** 36 samples of vaginal fluid routinely submitted for the diagnosis of BV to the Unit of Microbiology – GRHL were comparatively evaluated by standard techniques and with the HP-Vaginiti e Vaginosi NLM kit that simultaneously detects in a quantitative way specific DNA from *Candida* (*albicans*, *glabrata; krusei, tropicalis), Gardnerella vaginalis*, *Lactobacillus* spp. and *Atopobium vaginae*.

Results and conclusions. Candida spp. has been identified in 8 samples with culture and in 15 with the molecular test. 29 *G. vaginalis* were found by PCR whereas only in 7 samples a specific prescription for this microbe was present (of which 4 positive). *A. vaginae* has been identified in 20 samples by the molecular approach and *Lactobacillus* spp. was identified in 19 samples (by culture) and in 32 by PCR. The overall diagnosis of BV was made in 9 patients by standard techniques and in 7 by applying the molecular approach. (Cohen's kappa test: 0,84). The findings of this study clearly demonstrate that the joint use of the routine culture-based techniques with the multiplex PCR methods amplifies by far the sensitivity of the overall diagnostic workflow of BV.

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Key words: *Bacterial vaginosis*, *Gardenerella vaginalis*, *Lactobacillus* spp., *Atopobium vaginae*, Molecular Diagnosis.

Contributions: VS conceived the study and wrote the manuscript; GD and SZ performed the analytical step and checked the manuscript; PF supervised the study and BD supervised the molecular analytical steps. MFP and FC made the microbiologic tests.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 15 February 2018.
Accepted for publication: 15 February 2018.

Microbiologia Medica 2017; 32:7347
doi:10.4081/mm.2017.7347

Introduction

Bacterial vaginosis (BV) is among the most frequent microbiological syndromes in the population of women in their childbearing phase (1). The basic condition that is found among women suffering from BV is a substantial change in their vaginal microbiota: the commonly predominant *Lactobacillus* spp. gives way to a more complex and less characterized flora composed by several different bacterial species, including *Gardnerella* spp., *Prevotella* spp. *Atopobium* spp., *Peptostreptococcus* spp., *Mobiluncus* spp., *Sneathia* spp., *Leptotrichia* spp. and *Mycoplasma* spp. (14). This uncomfortable condition has been identified back in the 50s of the last century and since the first reporting as *bacterial vaginitis* it was considered as a member of the sexually transmissible diseases (STD) (4, 5).

Nowadays, BV is generally considered as a form of dysbiosis and it is linked, even if BV is mostly an asymptomatic condition, to increased risk of adverse pregnancy outcome, pelvic inflammatory disease (PID) and to an amplified possibility to acquire other STD, such as *Chlamydia trachomatis*, Herpes virus, *Trichomonas vaginalis* and *Neisseria gonorrhoeae* (6). BV has also been associated with an increased risk of HIV transmission and acquisition (2).

Despite this long lasting knowledge of the syndromic picture of BV, the precise reason for its onset and the large difference in its prevalence among female population in diverse geographical areas is still one the most challenging questions in the field of women health.

BV is usually clinically diagnosed based on the identification of three out of the four Amsel criteria (1): non inflammatory and homogeneous vaginal discharge; microscopic identification of clue cells; pH over 4.5 in the vaginal fluid and a positive *whiff test* (fish odor after the addition of 10% potassium hydroxide to the vaginal secretions). The application of another score system based on the microscopic semi quantitation of the presence of *Lactobacillus* in a gram stained vaginal smear, the so called Nugent criteria (11), has been proven more problematic under the diagnostic point of view, since a large proportion of women that showed a positive Nugent score are totally asymptomatic, with large differences among diverse ethnic groups. One possible

explanation of this rather imprecise score system could be found in the difficulty to correctly identify diverse bacteria based on their microscopic characteristics, as shown in a recent study by Srinivasan and co-workers (16).

Etiology and epidemiology

Most of the improvements achieved in the last 10 years in the field of BV have been made possible by the use of culture-independent molecular techniques. In particular, the approach based on the use of pyrosequencing and the subsequent phylogenetic analysis has shown that the vaginal microbiota (VMB) is by far more complicated and includes a large variety of different fungal and bacterial species. As a demonstration of the power held by the molecular approach to the investigation of BV, it is of note than in 2005, three novel and not previously identified bacteria were found in vaginal fluids of women with BV by using PCR and FISH. These novel microbes, identified as BVAB1 to BVAB3 showed a 16S rRNA gene sequence not related to any other: nowadays the BVABs are phylogenetically located into the phylum *Clostridium* and only BVAB3 has been recently grown in synthetic media *in vitro*. BVAB3 has been officially named as *Mageeibacillus indolicus* and it is an obligate anaerobe (14). In addition, this more powerful approach to the study of the VMB has shown that the microbial community in the reproductive female tract is largely varying depending on reproductive age and sexual activity (6).

A comprehensive evaluation of the diffusion and potential clinical differences of BV among diverse women population is still lacking in literature. One of the most complete set of data has been reviewed and published in 2013 by Kenyon and co-workers (5). At first, it must be clarified that: *BV is significantly associated with sexual contact with new and multiple male and female partners and that decreasing the number of unprotected sexual intercourse may reduce incident and recurrent infection.* Other relevant risk factors for the development of BV include: smoking, low socio-economic status, douching and recent use of antibiotic (14). Overall, the collection of epidemiologic data about BV is frequently hampered by different issues such as the diverse criteria for the selection of the population and the correlated samples and the group of women included in the surveillance. Even among different population living in a defined geographical zone BV showed assorted prevalence ratios: in the USA, BV had highest prevalence among African American women in respect to Caucasian and Asian, being the prevalence of BV among Hispanic women intermediate (5). Moving forward to an analysis of prevalence based on different Countries, the area showing the highest prevalence of BV in the Sub-Saharan African region included southern and eastern parts. Moving into the Latina America (including the Caribbean) the epidemiology of BV showed intermediate level of prevalence, with the only exception of selected small groups in Jamaica and Peru with rates above 40%. As far as Western European epidemiology is concerned, the overall prevalence of BV is quite low: the Countries showing the higher (about 20%) prevalence are Turkey, Poland and Norway. The data about BV are quite scare and scattered from the Middle East and North African Countries: the highest values (about 50%) are reported in small nomadic populations living in these areas. Overall the epidemiologic relevance of BV is low in North America (with the only exception of African Americans and aboriginal small population in Canada) as it is for women living in Asia and Australia/New Zealand.

Diagnosis

The microscopic evaluation of a vaginal smear stained according to the Gram technique is by far the most widely used method for the laboratory diagnosis of BV. The above reported score system of Nugent could be used to evaluate and interpret the microscopic observation. When Nugent criteria are applied a score of 0 to 3 corresponds to the presence of a normal vaginal flora whereas a score above 7 (up to 10) is correspondent to a defined BV. A possible variant to the Nugent score system is the Ison score that is likely more effective in identifying the intermediate BV conditions (10) based on a more comprehensive clinical and laboratory evaluation is the set of criteria proposed by Amsel, above detailed.

In the last 24 months several reports described and validated the possible use of different molecular based approaches for the laboratory diagnosis of B. (3, 7-9, 12, 13, 15). Most of the data confirm that a molecular based diagnosis could improve the overall sensitivity and specificity of the laboratory diagnosis of BV. Some points still need to be elucidated: in principle since a totally accepted definition of the microbial etiology of BV is still fluctuating, additional assays that detect with alternative molecular methodologies a panel of microbes putatively related to the development of BV are required.

Here we report about the use of a multiplex PCR based test (HP-Vaginitis & Vaginosis kit - AU27117, Nuclear Laser Medicine - NLM, Settala, Italy) that identifies and quantifies the presence of specific DNA fragments from *Candida* (*albicans*, *glabrata; krusei, tropicalis)*, *G. vaginalis*, *A. vaginae* e *Lactobacillus* spp. in vaginal fluid specimens.

Materials and Methods

A total of 36 vaginal fluid samples, obtained from women with age ranging from 19 to 47 years, routinely evaluated at the Unit of Microbiology of the Great Romagna Hub laboratory were included in the study. For all of the patients the clinical picture suggested a possible diagnosis of BV according to the Amsel criteria. The standard diagnostic workflow was performed and included the microscopic evaluation of the vaginal smear after Gram staining associated with culture based techniques for the identification of *G.vaginalis* and *Candida* spp.. The HP-Vaginiti e Vaginosi (AU27117 - NLM) kit is based on two different steps: the first is a multiplex target amplification that basically could enrich the targets content of the sample while the second step is based on a nested reaction that further amplifies and identifies the germs. As controls a human derived DNA and an artificial sequence, that is used as reference for the quantitation of the different targets thus allowing the automated calculation of the final score, (SPIKE) are included so that the complete cycle of the reaction could be checked and monitored. The final and overall evaluation of the results is achieved by the software MT-PCR Analysis that provides the user with an automated score correlated with the different expression of the vaginal flora from intermediate alteration to BV.

Results

The results obtained are summarized in Table 1. In detail, *Candida* spp. has been identified in 8 samples with culture and in 15 with the molecular test (Cohen's kappa test: 0,57). 29 *G. vaginalis* were found by PCR whereas only in 7 samples a specific prescription for this microbe was present (of which 4 positive). *A. vaginae* is not routinely identified but this bacterium has been identified in 20 samples by the molecular approach. Finally, *Lactobacillus* spp. was identified in 19 samples (by culture) and in 32 by PCR (Cohen's kappa test: 0,25). The overall diagnosis of BV

Table 1. Comparative summary of the results obtained by standard culture based methods and the HP-Vaginiti e Vaginosi NLM kit.

Microbe	Culture/microscopy, n. positive samples	HP-Vaginiti e Vaginosi NLM kit, n. positive samples
Candida (albicans, glabrata; krusei, tropicalis)	8	15
Gardnerella vaginalis	4	29
Atopobium vaginae	ND	20
Lactobacillus spp.	19	32

was made in 9 patients by standard techniques and in 7 by applying the molecular approach. (Cohen's kappa test: 0,84).

Discussion and Conclusions

Despite the still wide use of culture and microscopic methods for the laboratory diagnosis of BV, the implementation of a molecular approach has become more and more common in the last years. Among the most important factors that prompted the enlarging use of PCR based techniques for the diagnosis of BV, the first place is due to the technical improvement that nowadays allows the multiplex detection of several different genetic targets within one reaction, thus perfectly fitting the syndromic character of BV. An additional relevant issue that contributed to the wider use of PCR based techniques is the reduction of cost of these methods strictly linked with the availability of automated instrumentation that consequently allows the simple and rapid processing of large number of samples.

One should also not forget that in recent years a more precise clinical and epidemiological definition of BV has been made available thus enlarging the request for a more focused diagnostic workflow. The findings of this study, even if obtained with quite a limited set of patients clearly demonstrate that the joint use of the routine culture based techniques with the multiplex PCR methods amplifies by far the sensitivity of the overall diagnostic workflow of BV: in particular by using the HP-Vaginiti e Vaginosi NLM kit an extended number of samples positive for all the detectable germs. In addition this technique has also allowed the identification of *A.vaginae* that is likely playing a relevant role in the pathogenesis of the condition of unbalanced microbial flora behind BV. In particular this molecular assay allows an accurate and specific measurement of marker bacterial specie with a comparative quantification of these microbes. This feature allows the calculation of a ratio, similar to the one used for the Nugent score. Additional calculation of the human cell number and estimation of the ratio between human cells and anaerobic bacteria is used as a molecular surrogate for the microscopic clue cells detection.

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