World Journal of Medical Images, Videos and Cases

ORIGINAL ARTICLE

Prevalence of bacterial vaginosis in patients with vaginal exudates indicating leucorrhoea in Havana, Cuba.

Abilio Ubaldo Rodríguez Pérez [1]

Introduction

Bacterial vaginosis (BV) is considered a health problem - with human and social impact - because of its high morbidity in the contemporary world. The presence of sexually transmitted diseases, multiple sexual partners and the use of intrauterine devices, are risk factors for the development of this infection, which incidence increases significantly in women of fertile age [1].

It is defined as a polymicrobial clinical syndrome, which is characterized by abnormal vaginal discharge with disorder of the vaginal ecosystem and displacement of lactobacilli by other microorganisms [2-3]. At present, it's considered a disease with well established symptoms and signs.

BV represents 60% of all vulvovaginal infections, increasing by 10 to 100 times the concentration of anaerobic pathogens (*Bacteroides* spp., *Peptostreptococcus* spp., *Gardnerella vaginalis* and *Mobiluncus*) and resulting in clinical symptoms [3]. However, it is common to obtain negative results in patients with vaginal discharge, because there are no resources which allow isolation of other microorganisms of interest than those commonly reported.

Discharge with bad odour, itching and irritation are the most common symptoms. The ammonia smell gets stronger as secretions become more alkaline after intercourse or menstruation. Redness and oedema are frequent.

To identify the presence of BV, it is important to know in advance the normal physiology of the vagina, with its microbiota a dynamic ecosystem that can be easily altered. Secretions have a complex composition, including cervical mucus and those that are associated with the vaginal wall. The amount and composition may vary greatly with age and menstrual cycle, excitation and sexual activity, contraceptives, pregnancy and emotional state.

The normal vaginal secretions are odourless, white or clear, viscous, homogeneous or flocculent with agglutinate elements, pH <4.5 and they do not slip during speculum examination. Lactobacilli predominate in the normal vaginal microbiota (up to 10 million / mL of vaginal secretions) but when there are imbalanced concentrations of facultative and anaerobic microorganisms, *Gardnerella vaginalis* can be isolated in 5-60% of cases, *Mobiluncus* can be found between 0-5% and

Mycoplasma hominis between 15-30% of sexually active healthy women [4].

However, vaginal microbiota of a patient with BV differs significantly from that of a healthy woman, presenting few lactobacilli, *Gardnerella vaginalis* in 95% of cases, Mobiluncus in 50-70% and *Mycoplasma hominis* between 60-75% with the ratio of anaerobic to aerobic microorganisms: 100 to 1000:1. The presence of leukocytes suggests the existence of a concomitant infection caused by *Neisseria gonorrhoeae* or *Chlamydia* spp. and the microbes need to be cultured. We do not recommend the routine performance of these investigations because 50-60% of women are asymptomatic patients harbouring *Gardnerella vaginalis* [1].

To make the diagnosis of BV, it's necessary to follow Amsel's clinical criteria, which are based on the presence of at least 3 of the following criteria [1, 4]:

- Homogeneous discharge: it's often described as a glass of milk poured over the vagina.
- pH over 4.5 (usually 5.0-6.0): The pH is measured with a pH strip or tape, taking care not to touch the cervical secretions, which tend to be alkaline. A vaginal pH below 4.5 excludes the diagnosis of BV.
- Positive amines liberation test (or the whiff test): fishy (amine) smell is produced by reacting vaginal discharge with 10% KOH. No odour occurs in the absence of BV. This test predicts the diagnosis of BV in 94% of patients.
- Presence of clue cells (Figure 1): these are squamous epithelial cells with bacteria on the surface that become dark. Vaginal epithelial cells generally have specific characteristics. A drop of discharge is mixed with a drop of saline solution and the cells can be highlighted by adding methylene blue to the saline solution.

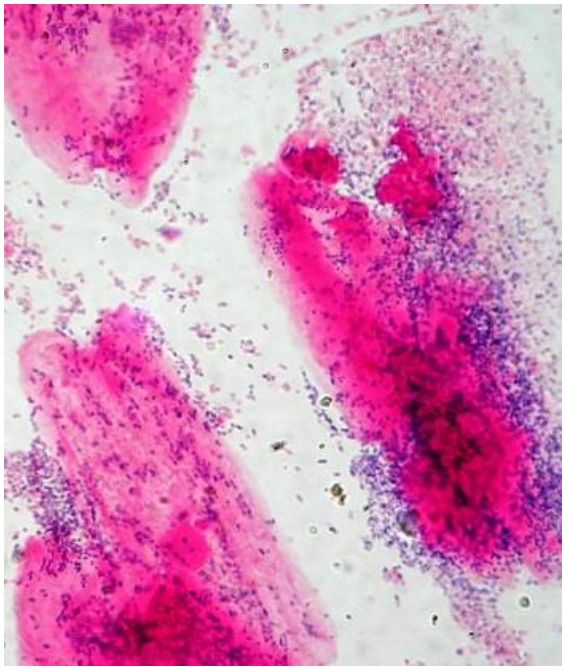

Figure 1. Clue cells, i.e. extended, vaginal epithelial cells which are covered by pathogenic bacteria. Source: Chair of Microbiology, JUMC, Cracow.

Some authors have shown that the presence of the last two criteria is enough to make the diagnosis of this condition [1, 3].

According to what was previously stated, this study was aimed at making the diagnosis of BV in three hospitals in Havana, confirming its prevalence in patients with vaginal exudates indicating leucorrhoea.

General objective: to determine the prevalence of bacterial vaginosis in women who attended the Laboratory of Microbiology of three hospitals of Havana, Cuba between October

and December 2018, with vaginal exudates indicating leucorrhoea

Specific Objectives:
• To establish the diagnosis of bacterial vaginosis at OB/GYN Hospitals.
• To identify existing BV in patients with vaginal exudates indicating leucorrhoea.
• To apply the Amsel's clinical criteria for the identification of *Gardnerella vaginalis* in these studies.

Materials and Methods

This study was a quasi experimental cross-sectional research.

353 vaginal exudates collected towards diagnosis of bacterial vaginosis were processed in the Laboratory of Microbiology of three hospitals in Havana, Cuba, from October to December 2018, following Amsel's clinical criteria.

Technique:
• Heavy and homogeneous vaginal discharge was corroborated (whitish-gray or whitish-yellow) with bad smell at the time of sampling.
• The pH was measured with a pH tape, taking care not to touch the cervical secretions. BV pH is usually 5.0-6.0.
• Two samples of vaginal secretions were collected in each case. Each swab was placed in a separate test tube. 1 mL of 0.9% NaCl was added to one test tube and 1 mL of 10% KOH to the other one. One drop of saline solution was placed on a slide for microscopic observation. Binocular light microscope was used (Olympus, Japan) - with 10× lens for finding common microorganisms and yeasts, as well as 40× lens for organisms related to BV. Gram-stain specimens for bacteria lining the epithelial cells were observed with 100× lens (Figure 1). Presence of clue cells was verified on direct examination.
• The "fishy odour" of the whiff test was corroborated in the tube with 10% KOH (amines liberation test).

Results with discussion

We can observe in Table 1, that since the implementation of the diagnosis of BV, there is a significant increase in the positivity of these tests (63%) when compared to those made in the same period of 2017 (12%).

Table 1. Vaginal exudate results before and after laboratory diagnosis of bacterial vaginosis. Laboratories of Microbiology, three OB/GYN hospitals in Havana, Cuba. Samples collected from October - December 2018. Source: Process Record Books.

Vaginal exudates	Before diagnosis		After diagnosis	
	No.	%	No.	%
Positives	59	12	221	63
Negatives	430	88	132	37
TOTAL	489	100	353	100

Gardnerella vaginalis and *Candida albicans* were the most frequent microorganisms found (Table 2) coinciding with some authors' finds [1, 4].

Table 2. Distribution of microorganisms found in the vaginal discharge. Laboratories of Microbiology, three OB/GYN hospitals in Havana, Cuba. Samples collected from October - December 2018. Source: Process Record Books.

MICROORGANISMS	No	%
Gardnerella vaginalis **(Bacterial vaginosis)**	93	26
Candida albicans	58	17
Candida spp.	25	7
Bacillus spp. Gram-negative*	23	7
Coccus spp. Gram-positive**	17	5
Trichomonas vaginalis	5	1
No bacterial isolation	132	37
Total	353	100

Legend: * Enterobacteria and *Pseudomonas* spp. ; ** *Staphylococcus* spp. and *Streptococcus* spp.

Table 3. Diagnostic criteria for bacterial vaginosis. Laboratories of Microbiology, three OB/GYN hospitals in Havana, Cuba. Samples collected from October - December 2018. Source: Process Record Books.

CRITERIA	BV (+)	BV (-)	Sensitivity	Specificity
Clue cells	93/93	10/260	100	96
Odour (Whiff Test)	93/93	13/260	100	95
Characteristic discharge	90/93	19/260	97	93
pH	93/93	137/260	100	47
Cells + odour+ pH	88/93	0/260	95	100
Cells + odour	85/93	0/260	91	100
Odour + pH	88/93	0/260	95	100
Three of four criteria	93/93	0/260	100	100
Lactobacilli < bacteria	89/93	27/260	96	90
Total (patients)	93	260		

BV: Amsel's criteria were used with a prevalence of 23%

Table 3 shows the results obtained using the Amsel's clinical criteria for the diagnosis of BV, the presence of clue cells in the direct examination, the whiff test, and pH ≥5 were the most sensitive and specific tests; overall the prevalence of BV for the whole study was 23%.

Conclusions

• The positivity of vaginal exudates increased to 63% with the introduction of the diagnosis of bacterial vaginosis in the hospital.
• The prevalence of bacterial vaginosis was 23% in the studied population, identifying *Gadnerella vaginalis* as the most common causative agent.
• The Amsel's clinical criteria combined with clue cells, whiff test and pH value demonstrated a significant sensitivity and specificity for the diagnosis of bacterial vaginosis.

Recommendations

• To standardize the diagnosis of bacterial vaginosis in patients with vaginal discharge in these institutions.
• To broaden the study of BV risk factors, with emphasis on prevention and control.

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Conflict of interest: none declared

Acknowledgements
Thanks to staff of the Chair of Microbiology, JUMC in Cracow for the photograph.

Author's affiliation:
[1] Provincial Center of Hygiene, Epidemiology and Microbiology, Havana, Cuba

Corresponding author:
Prof. Abilio Ubaldo Rodríguez Pérez
Provincial Center of Hygiene, Epidemiology and Microbiology
102 Ave, No. 3001, among 31 and 31B Street
Marianao 14
Havana 11400
Cuba
Tel. +53 72600717
e-mail: ubaldo.rodriguez@infomed.sld.cu

To cite this article: Rodríguez Pérez AU. Prevalence of bacterial vaginosis in patients with vaginal exudates indicating leucorrhoea in Havana, Cuba. World J Med Images Videos Cases 2019; 5:e9-13.

Submitted for publication: 21 January 2019
Accepted for publication: 22 February 2019
Published on: 28 February 2019

ISSN: 2450-5773