Accuracy of the cytopathology, bacterioscopy, and vaginal flora culture

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Summary

Introduction: An over-population of vaginal microorganisms causing inflammatory processes renders it difficult to properly assess the cytopathological exam that aims to screen precedent cervical lesions. On the contrary, the occurrence of the microbial flora saprophyte does not influence correct cythodiagnosis. *Objective:* To assess the composition of vaginal tract aerobic microorganisms of asymptomatic women in menacme and post-menopause, and to analyze the accuracy of cytopathologic, bacterioscopic exams, and culturing of the flora. *Methods:* The women were first submitted to a focused anamnestic interrogatory and then submitted to gynecological exam. A sample of the vaginal fluid was collected with a culture swab and a smear was made on two glass slides for stained bacterioscopic exam (GRAM). The collection of material was then compiled in a cytopathologic smear analysis. All women signed the free and informed consent letter and the project was approved by the Ethics Research Board of Hospital São Paulo – UNIFESP. *Results:* Bacterioscopy and culture proved to be better than the cytopathologic exam in featuring the bacilli and cocci. The bacterioscopy provided a better detection of the presence of bacilli ($p < 0.001$); no statistical difference was seen between both exams with respect to the detected cocci. The β-hemolytic Streptococcus group was of significance in post-menopausal women ($p < 0.05$). *Conclusion:* In this study, the bacterioscopic and culture exams of the vaginal fluid were more effective in assessing the vaginal flora and in the detection of bacilli, compared to the cytopathological exam.

Key words: Cytology; Bacterioscopy; Culture; Vaginal content.

Introduction

The vaginal tract features a microbiota that varies according to the biological development phase of female organism and that follows the different phases of the menstrual cycle.

Peixoto [1] defined as vaginal fluid, the absolute prevalence of lactobacilli in respect to other morphotypes of microrganisms, in addition to the presence of epithelial cells in variable amount, higher than the number of leukocytes, and the absence of target cells, *Trichomonas vaginalis* and fungi.

During post-menopause, the decrease of estrogen levels leads to a lower deposit of glycogen and consequently to a decrease of lactobacilli controlling the excessive growth of potentially pathogenic bacteria.

The growth of usual flora and/or the colonization of new microorganisms introduced through sexual intercourse is liable to lead to infections characterized by vaginal discharge, frequent complaint in medical offices, in addition to the development of pelvic inflammatory disease [2]. The inflammatory process in the vaginal tract renders it difficult to properly assess the cytopathological exam intended to screen precedent lesions. The events of usual microbiota should not alter the diagnostic capacity of cytopathology.

The description of symptoms and/or observation of the aspect of vaginal contents supplies aids for the diagnosis of abnormalities in the flora, although with low sensibility and specificity, of less than 50% of cases [3].

The authors intended to assess and compare the composition of aerobic microorganisms making up the vaginal tract of women in menacme and post-menopause, by means of cytopathological exam, bacterioscopic exam with GRAM staining, and culture. The accuracy of each method was then evaluated according to their identification of microorganisms and the significant differences in the flora of both hormonal situations were then compared. The knowledge of these conditions might contribute to a better resolution of flora deviations.

Materials and Methods

A transversal and observational cohort study was performed. During the Joint-Action for Gynecological Disease Prevention offered to the female employees of Hospital São Paulo-Unifesp complex, 118 women were selected, with 59 of them in menacme and 59 in post-menopause phase. All participants signed the free and informed consent letter. The project was approved by the Research Ethics Board of Hospital São Paulo-UNIFESP.

The inclusion criteria comprised Employees of Hospital São Paulo (HSP)-UNIFESP complex who were oligo- or asymptomatic to the requirement of vaginal discharge, without a preventive exam for cervical-vaginal lesions for over one year.

Pregnant or nursing women presenting gynecological diseases formerly diagnosed and under treatment, patients clinically bearing immunosuppression, patients under therapeutic

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schedule with antibiotics for whatever infections processes, were excluded from the study.

The participants were submitted to a focused anamnestic interrogatory and a gynecological exam with vaginal specular exam. With the aid of a long swab, a sample of vaginal fluid was collected and a smear was made on two glass slides, for a stained bacterioscopic exam (GRAM). The corresponding reports were supplied by members of the staff of microbiologists at the Central Laboratory of HSP. The reports featured the number of epithelial cells, leukocytes, and microorganisms in cocci, bacilli, yeasts, and the form of groups in each of them.

Another sample of vaginal contents was properly packed for transportation for microorganism culture and sent to the Central Laboratory of HSP for seeding in an appropriate culture means for positive aerobic pathogenic microorganisms, whether pathogenic or not. The culture means in use were: chocolate agar for positive gram growth, negative gram and growth, blood agar for positive gram and negative gram growth, and teague agar (eosin methylene blue) for negative gram growth. Biochemical tests such as the use of the EPM/MILI system and immunological tests were performed. The corresponding reports were supplied by a team of microbiologists from the Central laboratory of HSP.

The collection of material for the performance of cytopathological smear analysis was made with an endocervical canal, ectocervix, and vaginal sac material, using a canal brush and wood spatulas (Ayre), respectively. The material was deposited on glass slides, identified and fixed through fixing liquid vaporization (Colpofix). It was then sent to the Cytopathology Laboratory of the Gynecology Department, to be processed, stained by the modified method of Papanicolaou, and submitted for interpretation, by an experienced cytopathologist of the service.

For the comparison of three methods, the microorganisms identified were split into three groups: cocci, bacilli, and yeasts.

The analyses were performed using the statistical package SPSS – Statistical Package for Social Sciences (v16.0). For the assessment of which of the three exams managed to identify the largest number of microorganisms, the average of organisms detected through each method was assessed, by subdividing the sample into patients according to menacme and post-menopause. For the comparison of quantitative data, the Kruskal-Wallis and the Mann-Whitney tests were used, for non-parametric data. The statistical significance value was established as 5%, or $p < 0.05$.

Results

The joint work gave priority to women aged 30 to 60 years; the average age of those in menacme was 41 years and 55 years for those in post-menopause.

The microorganisms identified in the cultures are shown in Table 1. The most frequently found bacteria comprised: Döderlein bacilli (66% in menacme and 50.8% in post-menopause), *Staphylococcus coagulase-negative* (in 40% of each hormonal situation), *beta-hemolytic Streptococcus group B* (7% of women in menacme and 27% in post-menopause), *Enterococcus spp* (in 10% of each hormonal situation), and *Escherichia coli* (8.5% in menacme and 12% in menopause). These microorganisms were then divided into two groups: cocci and bacilli. Some yeasts were also identified in the culture.

In the cytopathological report, microorganisms found were Döderlein bacilli, gram-positive bacilli, gram-negative bacilli, labile grams bacilli, gram-positive cocci, and yeasts. In addition, the number of epithelial cells, leukocytes and microorganisms were featured as rare, some, and numerous.

When the averages of agents identified in patients were analyzed through the different methods studied (through the Kruskal-Wallis method), a statistical difference was noticed among the three methods, according to the number of bacilli and cocci ($p < 0.05$). In order to identify which of the three groups would differ from each

Table 1. — *List of 118 women at menacme and post-menopause, according to microorganism.*

	Menacme		Postmenopause	
	n	%	n	%
Yeasts	3	5.1	2	3.4
Gram-positive + bacilli	8	13.6	5	8.5
Doderlein bacilli	39	66.1	30	50.8
Coagulase-negative Staphylococus	24	40.7	25	42.4
Beta-hemolytic Streptococcus Group B	4	6.8	16	27.1
Escherichia coli	5	8.5	7	11.9
Enterococcus spp	6	10.2	6	10.2
Staphylooccus aureus	3	5.1	2	3.4
Haemophilus spp	1	1.7	1	1.7
Proteus mirabilis	1	1.7	0	0
Enterobacter	1	1.7	0	0
Citrobacter spp	1	1.7	0	0
CESP	1	1.7	0	0
Pseudomonas spp	0	0	1	1.7
Streptococcus Group D non-enterococcus	0	0	1	1.7
Acinetobacter spp	0	0	1	1.7
Morganella Morganii	0	0	1	1.7

Table 2. — *Bacilli and cocci featured in the cytology, bacterioscopy, and culture in patients at menacme, analyzed "two by two" through the Mann-Whitney (U) test and the respective p values.*

	U	*p*
Cytology x Bacterioscopy		
Total bacilli	965.50	< 0.001
Total cocci	1,268.50	0.003
Cytology x Culture		
Total bacilli	1,526.50	0.12
Total cocci	1,075.00	< 0.001
Bacterioscopy x Culture		
Total bacilli	1,153.50	< 0.001
Total cocci	1,523.00	0.18

Table 3. — *Bacilli and cocci featured in the cytology, bacterioscopy, and culture in patients at post-menopause, analyzed "two by two" through the Mann-Whitney (U) test and the respective p values.*

	U	*p*
Cytology x Bacterioscopy		
Total bacilli	886.50	< 0.001
Total cocci	1,209.50	< 0.001
Cytology x Culture		
Total bacilli	1,495.50	0.13
Total cocci	935.50	< 0.001
Bacterioscopy x Culture		
Total bacilli	1,117.50	< 0.001
Total cocci T	1,385.50	0.03

other, a "two by two" analysis was performed, through the Mann-Whitney method and the respective values of p appear in Tables 2 (menacme) and 3 (post-menopause). The results of multiple comparisons for each variable were submitted to Bonferroni correction in order to eliminate possible bias concerning false positives, and the *p* value was then significant, when below 0.02.

The authors could affirm that there was a significant difference in the averages of bacilli in the "cytological vs bacterioscopy" and "bacterioscopy vs culture" methods ($p > 0.001$). When the averages of cocci were considered, a significant difference was noticed among the "cytological vs bacterioscopy" and "cytological vs culture" methods ($p < 0.01$). The averages of cocci can be considered equal between the "bacterioscopy vs culture" methods, since the value of p was $p > 0.02$.

For the detection of yeasts, no statistical difference was noticed between the number of microorganisms and the utilized method ($p > 0.05$).

Discussion

Bacterioscopy proved to be a worthy examination tool, since it informs the physician about the number of *Döderlein* bacilli of epithelial cells and leukocytes in respect to the other microorganisms, which facilitates to identify the flora as normal or abnormal, as shown by Espiegel [4]. Gram-staining was also the method that better identified the bacilli, in addition to being comparable to the culture for identification of cocci. This type of identification and the option of fresh exam are the best forms for the diagnosis of vaginitis caused by aerobics [5]. Nugent *et al*. [6] demonstrated that the diagnosis of vaginosis through bacterioscopy is liable to be reproduced among different diagnostic centers and microbiologists, with no great discrepancies.

As shown in the reports, when the clinical diagnosis is doubtful, the culture can differentiate vaginosis from vaginitis caused by aerobic flora and can aid in the differentiation of infection by multiple microorganisms [5]. Considering that the analysis of aerobics is focused, the results in the culture have shown the presence of bacteria such as *beta-hemolytic Streptococcus Group B* in a larger group of women and isolated cases such as *Citrobacter, Proteus sp* which described as present in floras and deemed to be normal [7], can however be the cause of inflammatory processes. The same authors also described the *Döderlein bacilli* and *coagulase-negative Staphylococcus*, as it also occurred in the present study.

The significant difference between the floras in both hormonal situations was the identification of *beta-hemolytic Streptococcus Group B* in women at post-menopause ($p < 0.05$), which occurred in the Hillier and Lau [8] paper that also demonstrates that gram-negative anaerobics and gram positive cocci are the microorganisms most found in this hormonal phase.

Dicacciati *et al*. [9] demonstrated that the cytopathological exam can be very useful in the identification of bacterial vaginosis – a vaginal ecosystem unbalance. However, the fact that cytopathologists focus their attention on alterations of the epithelium decreases the sensibility of the method to assess the vaginal flora. In this study, the cytopathological exam was less effective than bacterioscopy and culture in identifying the flora.

The reports demonstrates and discusses the complexity of the vaginal flora and its changes according to the hormonal stage of each woman, similarly to this study, with the three exam being discussed. In view of these facts, the different microorganisms causing inflammatory process and discharges cannot always be treated the same way. As already seen, the abnormal flora can lead to a difficult cytological assessment. The present authors can then conclude that the aids in bacterioscopy proved to better identify the bacilli. Culturing might help the physician to understand what is occurring in this complex flora, treating the patients in a more specific and individual form, and thus allowing a better screening of precedent lesions to be made.

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