Value of bacterial culture of vaginal swabs in diagnosis of vaginal infections

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Abstract

Background/Aim. Vaginal and cervical swab culture is still very common procedure in our country’s everyday practice whereas simple and rapid diagnostic methods have been very rarely used. The aim of this study was to show that the employment of simple and rapid diagnostic tools [vaginal fluid wet mount microscopy (VFWM), vaginal pH and potassium hydroxide (KOH) test] offers better assessment of vaginal environment than standard microbiologic culture commonly used in Serbia. Methods. This prospective study included 505 asymptomatic pregnant women undergoing VFWM, test with 10% KOH, determination of vaginal pH and standard culture of cervicovaginal swabs. Combining findings from the procedures was used to make diagnoses of bacterial vaginosis (BV) and vaginitis. In addition, the number of polymorphonuclear leukocytes (PMN) was determined in each sample and analyzed along with other findings. Infections with Candida albicans and Trichomonas vaginalis were confirmed or excluded by microscopic examination. Results. In 36 (6%) patients cervicovaginal swab cultures retrieved several aerobes and facultative anaerobes, whereas in 52 (11%) women Candida albicans was isolated. Based on VFWM findings and clinical criteria BV (19% of women) was the most common finding, and 72 (14%) candidiasis. Of 115 women with BV and vaginitis, pH 4.5 was found in 5, and of 390 with normal findings 83 (21%) had vaginal pH 4.5. Elevated numbers of PMN were found in 154 (30%) women – in 83 (54%) of them VFWMM was normal. Specificity and sensitivity of KOH test and vaginal pH determination in defining pathologic vaginal flora were 95% and 81%, and 79% and 91%, respectively. Conclusion. Cervicovaginal swab culture is expensive but almost non-informative test in clinical practice. The use of simpler and rapid methods as vaginal fluid wet mount microscopy, KOH test and vaginal pH offers better results in diagnosis, and probably in the treatment and prevention of sequel of vaginal infections.

Key words: vaginal diseases; infection; gravity; diagnostic techniques, obstetrical and gynecological; sensitivity and specificity; serbia.

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Introduction

Some 15 years ago Wiesenfeld and Macio 1 drew attention to a surprisingly rare use of simple tests like vaginal fluid wet mount microscopy (VFWMM), 10% potassium hydroxide (KOH) test, and vaginal pH as well as a relatively high proportion of patients (17%) sampled for vaginal swab culture despite its unproven value in the diagnosis of common vaginal infections. Vaginal and cervical swab culture is still very common procedure in our country’s everyday practice whereas simple and rapid diagnostic methods have been very rarely used. Newer culture-independent techniques have shown that vagina hosts more than 300 different microorganisms of which over 95% cannot be cultured 2–5. It is not surprising if we take into account the fact that a microorganism has been displaced from its natural habitat where it has optimal amounts of nutrients and oxygen, optimal temperature and pH, and the presence of other microorganisms, into an artificial environment. Hence, the standard microbiological cultivation (with no use of special transport and growth media, anaerobic conditions, cocultivation etc.) results in the selection of rare microorganisms capable of surviving new harsh growth conditions. Consequently, aerobic and facultative anaerobic organisms are commonest growths of cervical and vaginal swab cultures although it is well-known that vagina is colonized mostly by anaerobes in the ratio 10 : 1 6–9.

The aim of the study was to show that cervical and vaginal swab culture has no value in everyday clinical practice, and that the routine use of straightforward procedures (VFWMM, vaginal pH and KOH test) may result in the better diagnosis of vaginal infections and prevention of serious complications that may follow 10–14.

Methods

This prospective study included 505 pregnant and asymptomatic women (24–28 week of pregnancy) seen during a regularly planned appointments. A swab was taken from both the cervical canal and vaginal side wall, put into a sterile dry tube, and transported to the laboratory within 3 hours. Chocolate agar was used as growth medium. The second swab taken from the vaginal side wall was smeared onto a slide, covered by a drop of saline, and the mount viewed under a phase-contrast microscope (magnification 400×). After completion of VFWMM, a drop of 10% KOH was dripped onto one end of the mount in order to check for the presence of yeasts. All the patients underwent 10% KOH test and in all vaginal pH was determined by a test strip (Merck pH, range 4.0 to 7.0). The predominance of lactobacilli over small bacterial forms (SBF), negative KOH test, and vaginal pH < 4.5 were considered normal findings, irrespective of the polymorphonuclear (PMN) numbers (Figure 1a). The diagnosis of BV was made in patients whose VFWM had more SBF than lactobacilli (irrespective of the presence of clue cells), normal PMN numbers, and in those with pH > 4.5 and/or positive KOH test (Figures 1b and 1c). In contrast to Amsel’s criteria, the presence of clue cells and abnormal homogenous off-white vaginal discharge were not considered obligatory criteria for the diagnosis of BV 15. In patients with the predominance of SBF, elevated PMN numbers and pH > 4.5, in whom infections with Candidia albicans (10% KOH) and Trichomonas vaginalis had been excluded, the diagnosis of vaginitis was made (Figures 1d, 2a-d). The term aerobic vaginitis is not used as the diagnosis was not made on the basis of the original Donder’s criteria 16. The PMN number was assessed semi-quantitatively on at least 10 fields of view (FV) at 400× as follows (Figure 3): group 0 – PMN absent or much less numerous than epithelial cells (EC); group 1 – PMN seen on more than 50% of FV but their numbers still less than that of EC; group 3 – PMN seen on most FV and their numbers equal or higher than the numbers of EC; group 4 – PMN seen on most FV and their numbers much higher than the numbers of EC. The groups 0 and 1 were considered normal according to the PMN number, and the other two groups were considered pathological.

Fig. 1 – Vaginal fluid wet mount microscopy.

a) Normal flora: predominance of lactobacilli over small bacterial forms (SBF); b) Bacterial vaginosis (BV): “clue cells”; c) BV, “clue cells” absent, predominance of SBF, polymorphonuclear (PMN) numbers normal, pH > 4.5; d) vaginitis: predominance of SBF, elevated PMN numbers, pH > 4.5.
Fig. 3 – Vaginal fluid wet mount microscopy: large numbers of polymorphonuclear leukocytes (PMN) accompanied by the normal number of Lactobacilli.

Results

Cervicovaginal culture and vaginal fluid wet mount microscopy

In 36 (6%) patients cervicovaginal swab cultures retrieved the following bacteria: Coagulase negative staphylococcus (n = 11), Enterococcus (n = 8), Escherichia coli (n = 5), Staphylococcus aureus (n = 5), Streptococcus beta haemolyticus (n = 5), Streptococcus viridans (n = 1), and Proteus mirabilis (n = 1). In 24 (66%) of those 36 patients VFWMM was normal, vaginal pH was < 4.5 and KOH test was negative (Figure 4). BV was diagnosed in 96 (19%) of the patients [concomitant C. albicans in 28 (30%)]. Vaginitis was confirmed in 19 (4%) of the patients of whom 3 also had C. albicans (Figure 5). Solely one of 19 patients with vaginitis had normal vaginal pH, and KOH test was positive in 12/19 patients. Of 72 patients in whom we microscopically observed C. albicans, 41 had normal background flora, i.e. the majority of bacterial forms were Lactobacilli.

Vaginal pH and KOH test

Of 115 patients with BV and vaginitis, 5 had pH < 4.5 (false negative finding), whereas among 390 patients with normal findings, 83 had pH > 4.5. False negative KOH test was found in 22 (of 115) patients, whereas 34 patients within the healthy group had a positive KOH test. Original authors’ criteria were used as gold standards and specificity, sensitiv-
ity, positive and negative predictive values, and total precision for each of the two tests in relation to the two groups of patients were calculated. Similar values were obtained for both tests even when in both groups patients with C. albicans had been either retained or removed (Table 1).

Characteristics of two simple and rapid bedside tests [vaginal pH and potassium hydroxide (KOH) test] in the diagnosis of abnormal vaginal conditions as compared against vaginal fluid wet mount microscopy (VFWMM)

<table>
<thead>
<tr>
<th>Test</th>
<th>True positive</th>
<th>False positive</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4.5</td>
<td>110</td>
<td>83</td>
<td>95%</td>
<td>79%</td>
<td>56%</td>
<td>98%</td>
<td>83%</td>
</tr>
<tr>
<td>KOH</td>
<td>93</td>
<td>34</td>
<td>81%</td>
<td>91%</td>
<td>73%</td>
<td>94%</td>
<td>89%</td>
</tr>
</tbody>
</table>

PPV – positive predictive value; NPV – negative predictive value; TP – total precision.

Polymorphonuclear leukocytes

Pathological numbers of PMN were found in 154 (30%) patients, of whom 83 (54%) had normal and 71 (46%) pathological VFWMM (Figure 6). Among the latter there were: BV (n = 10), BV + C. albicans (n = 14), vaginitis (n = 19) and C. albicans (n = 28). In 72 patients with the microscopic evidence of yeast infection, 45 (63%) had a pathological number of PMN, whereas within the group of patients with C. albicans and predominantly lactobacillar flora (41 patients), 28 (68%) had the elevated number of PMN.

Fig. 6 – Vaginal fluid wet mount microscopy (VFWMM). Proportion of patients with elevated polymorphonuclear (PMN) numbers and their subdivision into those with positive and negative findings on the vaginal fluid wet mount microscopy.

Discussion

More than 30 years ago Hammerschlag et al. published results of vaginal swab microbiologic cultures in healthy, 2-month- to 15-year-old girls showing that vagina is colonized even at such an early age by a wide range of bacteria and yeasts: Diphtheroids (78%), Staphylococcus epidermidis (73%), α-hemolytic streptococci (39%), Escherichia coli (34%), Candida species (28%), Ureaplasma urealyticum (27%), Klebsiella (15%), Enterococcus species 10%, Group D streptococci (8.5%), Staphylococcus aureus (7%), Mycoplasma hominis (6%), Haemophilus influenzae (5%), Pseudomonas aeruginosa (5%), Proteus (5%). Methodologically similar studies on women in reproductive age, apart from the above bacteria, isolated, more or less frequently, other aerobes and facultative anaerobes (Peptostreptococcus, Gardnerella, Bacteroides, Veillonella, Bifidobacterium, etc.) . We cultured similar bacteria. Isolation of any of the bacte-

patients with normal vaginal flora would be probably, due to isolation of a bacterium, unnecessarily treated. Thus, in practice we would have better results if we based our diagnosis on the examination under a speculum (precision 30%) than if culture results guided our treatment decisions. Let us discuss the value of these methods in specific vaginal conditions.

Bacterial vaginosis. In contrast to most infectious diseases that we diagnose by isolation or identification of specific microorganism, commonly without its quantification, in this polymicrobial condition we cannot pinpoint a single organism so that culture findings (including isolation of Gardinerella vaginalis) are useless in the diagnosis of the syndrome. The simplest but very important step during the workup would be to perform in every woman, along with the routine gynecologic exam, the test with 10% KOH and to determine the vaginal pH . As seen from our results, this simple addition would enable us to select, in the majority of cases, women requiring further diagnostic workup. The workup generally means VFWMM or microscopy of Gram-stained samples, but not the cervicovaginal swab culture. In very few women the microscopic findings do not correlate with signs and symptoms and then we have to consider further microbiologic or molecular biologic tests in order to identify specific organisms like C. albicans, T. vaginalis, Neisseria gonorrhoeae. Considering a high prevalence of BV (12–50%), the fact that it is in half of cases completely asymptomatic but anyway may lead to serious complications, it is a high time to institute educational programs for our gynecologists to embrace VFWMM. In this way they may timely diagnose and treat BV, thus preventing its serious complications .

C. albicans. In this study, with blood agar cultivation, C. albicans was isolated in 56 patients (not shown), whereas fungal elements were seen on VFWMM (10% KOH) in 72 (14%) women. Obviously, special media are better suited for C. albicans isolation. It is known that some 15–30% patients have mixed infections. In line with this, 38% of our patients with BV had concomitant C. albicans. Moreover, as 63% women with C. albicans had elevated PMN numbers despite the fact that the majority (68%) had a predominant lactobacillar flora, it is obvious that elevated PMN numbers necessitate a search for C. albicans.

Vaginitis. Many forms of vaginitis have been described in the literature so far, from aerobic, cytolytic, atrophic, desquamative, and inflammatory to ulcerative but here we de-

cided to use simply the term vaginitis. 18. The crucial single feature by which we may microscopically differentiate vaginitis from BV is, in our opinion, elevated and normal PMN number, respectively. Of course, only after a prior exclusion of C. albicans and T. vaginalis infections. In both BV and vaginitis the bacterial background is very similar: more small bacterial forms than Lactobacilli. Microscopically during VFWMM it is impossible to make a distinction between the flora of BV (anaerobes, e.g. Atopobium vaginae) and aerobic vaginitis (aerobes, e.g. Coccus). Moreover, Lactobacillus iners and Gardnerella vaginalis, the two most common bacterial species detected by molecular biological studies, are so similar phenotypically that cannot be differentiated on VFWMM under the 400× magnification. We also think that the presence of clue cells should not be a prerequisite for the diagnosis of BV during VFWMM as, by our experience, many women fulfilling other three Amsel’s criteria have no clue cells. This is probably the reason why the diagnosis of BV is made more frequently according to the Nugent’s criteria – most of the women are actually classified into the intermediary group 19. Moreover, in the study, 18 of 19 women diagnosed with vaginitis had a patho-

logical vaginal pH strengthening the view that the microscopic examination is the only way to differentiate between BV and vaginitis.

Polymorphonuclear leukocytes. Although it is a widespread belief that PMNs seen under the microscope represent a form of inflammation, a disease, it is common to encounter many women with elevated PMN and normal numbers of Lactobacilli. In our cohort of patients, 54% with elevated PMN had predominant lactobacillar flora. Verhelst et al. 20 have delineated a separate group of women in their new classification of Gram-stained sample analysis (so-called Claeys’ criteria). Their microscopic findings are characterized by a large number of PMN accompanied by a normal number of Lactobacilli (Figure 3). In our study 16% of the patients had such findings without detectable C. albicans confirming that this group of women should be paid a special attention in future studies in order to find a cause of such unusual constellation of microscopic findings. Finally, analysis of PMN should become a part of routine VFWMM.

Until recently most of our knowledge about vaginal microbiology has come from studies based upon the culture. However, as about 95% of microorganisms within the vaginal flora cannot be cultivated, other, mostly molecular biology-based techniques are increasingly used to define vaginal microflora. 21–23. These techniques will undoubtedly help us to understand dynamics and intricate interactions among the microorganisms in maintenance of healthy vaginal environment or development of pathological conditions. This will make us to redefine terms of normal and pathologic within the vaginal ecosystem. 4–8. Bacterial communities should be analyzed not only qualitatively and quantitatively but also functionally so that we are able to define healthy vaginal microbiome of an individual woman (personalized medicine). Till that time, for everyday practice, VFWMM and/or Gram-stained vaginal samples along with vaginal pH and KOH test should be routine methods to assess the vaginal flora and discriminate between healthy and diseased.

Conclusion

Despite abundant evidence dispelling a reasonable value of culturing vaginal and/or cervical swabs in clinical practice, it is still by large the most frequent diagnostic procedure in this country, and, not uncommonly, a major tool to distinguish between normal and pathological vaginal flora. Yet, the deployment of only two simple tests (vaginal pH and KOH test) when there is no resources for microscopic examination offer much better results than standard swab cultures. Moreover, the techniques are simple, rapid and inexpensive, and may be easily performed even in the setting of primary healthcare level. The wider use of the techniques would result in better management of vaginal infections and their consequences.

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