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Evaluation of vaginal flora and susceptibility test of microorganisms in reproductive-age women with or without vaginitis in primary care settings

Birinci basamak sağlık kuruluşlarına başvuran vajiniti olan ve olmayan doğurganlık çağındaki kadınlarda vajinal flora ve mikroorganizma duyarlılık testinin değerlendirilmesi

Ahmet Alim, Ali Çetin, Çağlar Yıldız

Public Health Laboratory (A. Alim, PhD), Sivas Health Directorate, TR-58030 Sivas; Department of Obstetrics and Gynecology (Prof. A. Çetin, MD and Ç. Yıldız, MD), Cumhuriyet University School of Medicine, TR-58140 Sivas

Abstract

Aims. The treatment modalities of patients with vaginal discharge are generally related to their symptoms. The aim of this study was to evaluate vaginal flora and antibiogram analysis in reproductive-age women with or without vaginitis in primary care settings. **Methods.** Vaginal swabs were taken from 311 women who have vaginitis, and tested for the causative agents of vaginal discharge. The control group was 89 healthy women without vaginal discharge. Vaginal swaps were used in a commercial tests system. The tests were used for count and presumptive identification of the microorganisms and for the susceptibility testing of the urogenital mycoplasmas in the sample were interpreted by assessing the change in color of the various wells and performing a microscope examination. **Results.** Most isolated microorganisms were *Candida sp*. from 39 (12.5%)cultures, *Gardnerella vaginalis* from 32 (10.2%) cultures, *Staphylococcus aureus* from 32 (10.2%) cultures, *Ureaplasma urealyticum* from 29 (9.3%) cultures, and *Trichomonas vaginalis* from 21 (6.7%) cultures of the vaginal fluid specimens, and in 27 (8.6%) cultures, there was no isolation. In control group, *Candida* sp. was isolated from 3 (3.3%) cultures, *Gardnerella vaginalis* from 3 (3.3%) cultures, *Trichomonas vaginalis* was found in 1 (1.1%) of the vaginal fluid specimens. **Conclusion.** The results of this study confirm that culture and antibiogram are very important in the management of the vaginitis.

Keywords: Vaginal flora, antibiogram, vaginitis, reproductive age

Özet

Amaç. Vajinal akıntısı olan hastalarda tedavi modaliteleri genellikle hastaların semptomları ile ilişkilidir. Bu çalışmanın amacı, birinci basamak sağlık kuruluşlarına başvuran, vajiniti olan ve olmayan doğurganlık çağındaki kadınlarda vajinal flora ve antibiyogram analizi sonuçlarını değerlendirmektir. **Yöntem.** Vajiniti olan 311 kadından vajinal sürüntü alındı ve vajinal akıntıya neden olan ajanlar araştırıldı. Kontrol grubu vajinal akıntısı olmayan 89 sağlıklı kadından oluşturuldu. Vajinal sürüntüler ticari test sisteminde değerlendirildi. Testler mikroorganizmaların sayısı ve olası identifikasyonları için kullanıldı, örnekteki ürogenital mikoplazmaların duyarlılık analizi çeşitli kuyucuklardaki renk değişikliği ve mikroskobik değerlendirme ile yapıldı. **Bulgular.** Vajinal sıvı spesmenlerinden en çok izole edilen mikroorganizmalar sırasıyla şunlardı: 39 kültürden (%12,5) *Candida sp*., 32 (%10,2) kültürden *Gardnerella vaginalis*, 32 (%10,2) kültürden *Staphylococcus aureus*, 29 (9,3%) kültürden *Ureaplasma urealyticum* ve 21 (%6,7) *Trichomonas vaginalis*. Yirmi yedi kültürde (%8,6) mikroorganizma izole edilmedi. Kontrol grubunda vajinal sıvı spesimenlerinden 3'ünde (%3,3) *Candida sp.*, 3'ünde (%3,3) *Gardnerella vaginalis*, 1 (%1,1) *Trichomonas vaginalis* izole edildi. **Sonuç.** Bu çalışmanın sonuçları vajinit tedeavisinde kültür ve antibiyogramın çok önemli olduğunu doğrulamaktadır.

Anahtar sözcükler: Vajinal flora, antibiyogram, vaginit, üreme dönemi

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Corresponding author:

Dr. Ahmet Alim, Halk Sağlığı Laboratuvarı, Mevlana Caddesi TR-58030 Sivas. Email: alim58@gmail.com

Introduction

The normal vaginal ecosystem is a complex microenvironment with important interrelationships among endogenous microflora and their metabolic products, host metabolic products, estrogen status, and pH [1]. The microflora is largely composed of acidophilic lactobacilli, the dominant bacteria in a healthy vaginal ecosystem. Lactobacilli maintain the normal vaginal pH (3.8 to 4.2) by producing lactic acid, stabilizing the vaginal ecosystem, and hydrogen peroxide, suppressing the growth of gram-negative and gram-positive facultative and obligate anaerobes [2]. Other vaginal microorganisms include facultative anaerobes, coliforms, true anaerobes, and nonbacterial microorganisms such as *Candida albicans*, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. Many of them are commensal; they live within this ecosystem but do not harm to the host.

Lower genital tract infections are an important public health problem. These infections cause complications such as pelvic inflammatory disease, ectopic pregnancy, chronic pelvic pain, preterm birth, and infertility. In approximately 90 percent of affected women, this condition occurs secondary to bacterial vaginosis, vulvovaginal candidiasis, or trichomoniasis [3]; however, mixed infections are not uncommon. Successful treatment of vaginitis depends upon an accurate diagnosis of the causative microorganism, however in clinical practice since the symptoms and signs of vaginitis are nonspecific, the diagnosis is not reliable without aid of laboratory tests, although physicians frequently rely on empirical therapy because culture results are not rapidly available.

Vaginal complaints are a common presenting problem in primary care settings. Clinical signs and symptoms of vaginal infections vary depending on the etiology of the vaginal infection. The initial examination should include diagnostic testing and vaginal microscopic evaluation. The aim of this study was to evaluate vaginal flora and antibiogram analysis in reproductive-age women with or without vaginitis in primary care settings.

Materials and Methods

We prospectively enrolled 311 consecutive patients (mean age 34.4 ± 12.3, range 18-52 years) with complaints of vaginitis between August 2007 and June 2008. The control group consisted of 89 healthy women (mean age 33±9.5, range 21-45 years) without symptoms and clinical findings of vaginitis. Vaginal swabs from the posterior fornix were used for microbiological examination. These swabs were obtained and transported to the microbiology laboratory and vaginal fluids were extracted from the vaginal swabs. The samples were examined by staining with Gram and Giemsa. Whiff test and pH measurement were performed. The obtained specimens were tested for *Neisseriae gonorrhoeae* and *Streptococcus agalactiae (S. agalactiae)*, (Group B) using oxydase test and 7% ninhydrin, respectively. Vaginal fluids were evaluated for the presence of *Trichomonas vaginalis (T. vaginalis)* and *Candida sp.* by light microscopic analysis. Other microorganisms were determined by A. F. Genital System test (Liofilchem Diagnostics, L'Aquila, Italy).

A.F. Genital System is a 24-well system containing desiccated biochemical and antibiotic substrates for the search for, presumptive identification and susceptibility testing of microorganisms from urogenital samples. The system also provides a semi-quantitative assessment of the presence of urogenital mycoplasmas (*M. hominis* and *U. urealyticum*).

The system is inoculated with the suspension of the clinical sample and incubated at 36 ± 1°C for 18-24 hours. The tests used in the search for, count and presumptive identification of the microorganisms and for the susceptibility testing of the urogenital mycoplasmas in the sample are interpreted by assessing the change in color of the various wells and performing a microscope examination [4].

A.F. Genital System allows the search for, semi-quantitative count, presumptive identification and susceptibility testing of *M. hominis* and *U. urealyticum*, the search for and presumptive identification of the microorganisms most frequently isolated from vaginal liquid, such as: *Trichomonas vaginalis, Escherichia coli, Proteus sp./Providencia sp., Pseudomonas sp., Gardnerella vaginalis, Staphylococcus aureus, Enterococcus faecalis, Neisseria gonorrhoeae, Streptococcus agalactiae* (Group B) and *Candida sp* [4]. Some of these have not been testing antibiotic susceptibility because it was not in the commercial kit (A. F. Genital System test). Testing antibiotic susceptibility of Mycoplasmas and Ureaplasmas were carried out for tetracycline, pefloxacin, ofloxacin, doxycycline, erythromycin, clarithromycin, minocycline, josamycin, and clindamycin [4]. Data were presented n (%) of microorganism in the patients and controls.

Results

The study group consisted of 311 symptomatic and 89 asymptomatic women. Identificated microorganisms were listed in Tables 1 and 2. Most isolated microorganisms were; *Candida sp*. from 39 (12.5%) cultures, *G. vaginalis* from 32 (10.2%) cultures, *Staphylococcus aureus* from 32 (10.2%) cultures, *U. urealyticum* from 29 (9.3%) cultures, *T. vaginalis* was found in 21 (6.7%) of the vaginal fluid specimens, there was 27 (8.6%) cultures which has no isolation. In control group *Candida sp*. was isolated from 3 (3.3%) cultures, *G. vaginalis* from 3 (3.3%) cultures, *T. vaginalis* was found in 1 (1.1%) of the vaginal fluid specimens.

After analysis of antibiograms, only in 4 patients with vaginitis, we found josamycin resistance of *Mycoplasma sp*. and *U. urealiticum*. In other patients and controls, there was no antibiotic resistance of studied microorganisms.

Discussion

Vaginitis caused by bacterial, fungal, or protozoal agents can be related to altered vaginal discharge, odor, pruritus, vulvovaginal irritation, dysuria, or dyspareunia, depending on the type of infection. Vaginal discharge with non-malodorous, mucous, white or yellowish characteristics called physiological leucorrhoea [5]. It is not eligible to use only the characteristics of the vaginal discharge for the diagnose [6]. Changes in cervicovaginal secretions by estrogen are the causes of physiological leucorrhoea. In reproductive aged women, normal vaginal discharge consists of 1 to 4 mL fluid (per 24 hours), which is white or transparent, thick, and mostly odorless, which is a combination of mucoid endocervical secretions with sloughing epithelial cells, normal bacteria, and vaginal transudate [7]. The dominant bacteria of the vagina are lactobacillus that maintains the normal vaginal acidic pH with their product lactic acid.

Bacterial vaginosis (BV) is the most common cause of vaginal discharge in women of childbearing age, accounting for 40 to 50 percent of cases [8]. Approximately 50 to 75 percent of women with BV are asymptomatic [9-11]. Homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls, vaginal pH greater than 4.5, positive Whiff-amine test (defined as the presence of a fishy odor when 10 percent potassium hydroxide (KOH) is added to a sample of vaginal discharge), clue cells on saline wet mount are criteria (Amsel criteria) and three of these criteria are necessary for the diagnosis.

Group A streptococcus (GAS) is an uncommon cause of vulvovaginitis, in one series of almost 7000 pregnant women, only 0.03 percent were colonized with GAS, Group B streptococcus (GBS) commonly colonizes the vagina and causes neonatal sepsis and

maternal upper tract infection (endometritis), 20 percent of pregnant women were colonized with GBS [12].

Table 1. Identification of microorganisms in patients.

No	Microorganisms	Identification number	%
1	*Neisseriae gonorrhoeae*	1	0.32
2	*Gardnerella vaginalis*	32	10.28
3	*Candida sp.*	39	12.54
4	*Streptococcus faecalis*	19	6.10
5	*Escherichia coli*	22	7.07
6	*Proteus sp.*	7	2.25
7	*Staphylococcus aureus*	32	10.28
8	*Pseudomonas sp.*	5	1.60
9	*Ureaplasma urealyticum*	29	9.32
10	*Mycoplasma sp.*	6	1.92
11	*Trichomonas vaginalis*	21	6.75
12	*Streptococcus faecalis + Streptococcus agalactiae*	6	1.92
13	*Streptococcus faecalis + Escherichia coli*	6	1.92
14	*Streptococcus faecalis + Candida sp.*	5	1.60
15	*Streptococcus faecalis + Staphylococcus aureus*	3	0.96
16	*Streptococcus faecalis + Ureaplasma urealyticum*	6	1.92
17	*Staphylococcus aureus + Candida sp.*	5	1.60
18	*Ureaplasma urealyticum + Mycoplasma sp.*	27	8.60
19	*Ureaplasma urealyticum + Escherichia coli*	3	0.96
20	*Ureaplasma urealyticum + Streptococcus faecalis +Mycoplasma sp.*	3	0.96
21	*Gardnerella vaginalis + Streptococcus faecalis + Klebsiella sp.*	4	1.28
22	*Ureaplasma ürealyticum + Streptococcus faecalis + Escherichia coli + Mycoplasma sp.*	3	0.96
23	No growth	27	8.68
Total		311	100.0

Table 2. Identification of microorganisms in controls.

No	Microorganisms	Identification number	%
1	*Gardnerella vaginalis*	3	3.37
2	*Candida sp.*	3	3.37
3	*Streptococcus faecalis*	4	4.49
4	*Escherichia coli*	4	4.49
5	*Staphylococcus aureus*	2	2.24
6	*Ureaplasma urealyticum*	2	2.24
7	*Trichomonas vaginalis*	1	1.12
8	*Streptococcus faecalis + Streptococcus agalactiae*	1	1.12
9	*Streptococcus faecalis + Escherichia coli*	1	1.12
10	*Streptococcus faecalis + Candida sp.*	1	1.12
11	*Staphylococcus aureus + Candida sp.*	1	1.12
12	No growth	66	77.5
Total		89	100.0

Vaginal discharge, pruritus, burning, soreness, odor, dyspareunia, bleeding, and dysuria are the symptoms of vaginitis. When the adnexal tenderness accompanies to vaginitis it is suggestive of pelvic inflammatory disease.

Physical examination and some diagnostic studies are necessary because the history is not enough to detect the cause. Appearance of vulva, characteristics of the vaginal discharge,

the presence of cervical inflammation and adnexal or cervical tenderness are the important points of the physical examination. Erythema and edema at vulva are seen in candidiasis and trichomoniasis but in bacterial vaginosis, it has a normal appearance. Trichomonas is classically associated with a greenish-yellow purulent discharge; candidiasis with a thick, white, adherent, "cottage cheese-like" discharge; and bacterial vaginosis with a thin, homogeneous, “fishy smelling" gray discharge. However, the appearance of the discharge is extremely unreliable and should never form the basis for diagnosis [5]. At reproductive ages, vaginal pH measurement is an important finding for the etiology. If the vaginal pH is 4-4.5 it suggests candidiasis, when the pH >4.5 it suggests trichomoniasis or bacterial vaginosis. Candidal buds or hyphae, motile trichomonads, epithelial cells studded with adherent coccobacilli (clue cells), and polymorphonuclear cells (PMNs) are the findings about microscopic examination with saline. When microscopy is negative in symptomatic patients, vaginal culture is an alternative for microscopy.

Karaduman et al. [13] studied on 229 pregnant women for detecting the vaginal infectious agents and found 43.3% of the group was infected. They detected 34% *Candida sp*. and 3.4% *T. vaginalis* at the vaginal swabs.

Ertabaklar et al. [14] studied 220 cases and found in 12 (5.45%) of these 220 samples *T. vaginalis* was detected by both saline wet mount preparations and the trypticase-yeast extract-maltose (TYM) culture method. Sixteen (7.27%) out of all the patients were found to be positive by TYM cultivation. They showed that culture is the most sensitive method for diagnosis of trichomoniasis.

Randomized clinical trials of 5-nitroimidazole drugs (metronidazole or tinidazole) have generally reported cure rates of 90 to 95 percent [15-18].

The Centers for Disease Control and Prevention [15] recommends annual screening for all sexually active women aged 25 and younger for *C. trachomatis* and screening of high-risk women for *N. gonorrhoeae*. Several studies have reported younger age as a primary risk factor for sexually transmitted infections and *C. trachomatis* rates for incarcerated adolescent females have been reported as high as 24.7% [19-22]. Raising sensitivity among primary health care workers/employees on disease control, Intersectoral information sharing, increasing laboratory use and facilities, setting up/establishing a surveillance system including specialties of behavior are the major preventive measures for vaginal infection agents.

In summary, our study demonstrates that management of women with vaginitis needs to use of vaginal culture at least in women with recurrent vaginitis. Low probability of antibiotic resistance will be helpful to select antibiotics for patients with vaginitis.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Verstraelen H. Cutting edge: the vaginal microflora and bacterial vaginosis. Verh K Acad Geneeskd Belg 2008; 70: 147-74.
2. Plourd DM. Practical Guide to Diagnosing and Treating Vaginitis. Medscape Womens Health. Available at: http://www.medscape.com/viewarticle/408848 (accessed on February 17, 2009).
3. Egan ME, Lipsky MS. Diagnosis of vaginitis. Am Fam Physician 2000; 62: 1095-1104.
4. A.F. GENITAL SYSTEM. System for the search, count and susceptibility testing of pathogenic urogenital germs. REF.74156. Available at: http://www.frilabo.pt/fcms/images/stories/afgenitalsystem.pdf (accessed on February 21, 2009).
5. Anderson MR, Klink K, Cohrssen A. Evaluation of vaginal complaints. JAMA 2004; 291: 1368-79.

6. Gunay T, Aksakoglu G. Bakteriyel Vaginozis. Sted 2002; 11: 369-370.
7. Anderson M, Karasz A, Friedland S. Are vaginal symptoms ever normal? A review of the literature. Med Gen Med 2004; 6: 49.
8. Morris M, Nicoll A, Simms I, Wilson J, Catchpole M. Bacterial vaginosis: a public health review. BJOG. 2001; 108: 439-50.
9. Yen S, Shafer MA, Moncada J, Campbell CJ, Flinn SD, Boyer CB. Bacterial vaginosis in sexually experienced and non-sexually experienced young women entering the military. Obstet Gynecol 2003; 102: 927-33.
10. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis: Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983; 74: 14-22.
11. Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu KF, Andrews WW. Vulvovaginal Symptoms in Women With Bacterial Vaginosis. Obstet Gynecol 2004; 104: 267-72.
12. Mead PB, Winn WC. Vaginal-rectal colonization with group A streptococci in late pregnancy. Infect Dis Obstet Gynecol 2000; 8: 217-19.
13. Karaduman A, Al FD, Aksu G, Haberal A. Distribution of Agents of Vaginal Infection in Pregnants. Turkish Journal of Infection 2006; 20: 171-175.
14. Ertabaklar H, Ertug S, Kafkas S, Odabasi AR, Karatas E. Vaginal Akıntılı Olgularda *Trichomonas vaginalis* Arastirmasi. T Parazitol Derg 2004; 28: 181-184.
15. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines 2006. MMWR Recomm Rep 2006; 55(RR11): 1–94.
16. Hager WD, Brown ST, Kraus SJ, Kleris GS, Perkins GJ, Henderson M. Metronidazole for vaginal trichomoniasis. Seven-day vs single-dose regimens. JAMA 1980; 244: 1219-20.
17. Manorama HT, Shenoy DR. Single-dose oral treatment of vaginal trichomoniasis with tinidazole and metronidazole. J Int Med Res 1978; 6: 46-9.
18. Forna F, Gulmezoglu AM. Interventions for treating trichomoniasis in women. Cochrane Database Syst Rev 2003; 2: CD000218.
19. Newman SB, Nelson MB, Friedman HB, Gaydos CA. Should female federal inmates be screened for Chlamydial and gonococcal infections? J Correctional Health Care 2005; 11: 137-55.
20. Centers for Disease Control and Prevention. High prevalence of Chlamydial and gonococcal infection in women entering jails and juvenile detention centers-Chicago, Birmingham, and San Francisco, 1998. JAMA 1999; 282: 1417-8.
21. Mertz KJ, Voigt RA, Hutchins K, Levine WC. The Jail STD Prevalence Monitoring Group. Findings from STD screening of adolescents and adults entering corrections facilities, implications for STD control strategies. Sex Transm Dis 2002; 29: 834-839.
22. Robertson AA, Thomas CB, St Lawrence JS, Pack R. Predictors of infection with *Chlamydia* or *gonorrhea* in incarcerated adolescents. Sex Transm Dis 2005; 32: 115-22.