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Evaluation of etiologic agents in bacterial vaginosis by molecular methods

Bakteriyel vajinoziste etiyolojik etkenlerin moleküler yöntemler ile incelenmesi

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Abstract

Aim. To investigate the distribution of etiologic agents in bacterial vaginosis (BV) by conventional and molecular methods and evaluate the pros and cons for each method. **Methods.** One hundred sexually active women with vaginal discharge and 100 sexually active healthy women were enrolled in a cross-sectional case control study. Vaginal swabs were obtained from all women, and aerobic cultures and multiplex polymerase chain reaction (PCR) was used for evaluation. The outcomes of two groups were compared for each diagnostic method. **Results.** In the study group (n=100), *G. vaginalis* (10%), *M. hominis* (5%), *U. urealyticum* (2%), *E. coli* (8%), *C. albicans* (8%), and other candida species (4%) were detected by aerobic culture; and *G. vaginalis* (72%), *A. vaginae* (39%), *M. mulieris* (6%), *B. fragilis* (2%), *M. curtisii* (2%) were detected by PCR. In the control group (n=100), *U. urealyticum* (7%), *M. hominis* (6%), *E. coli* (4%), *C. albicans* (3%), and other candida species (4%), and *Klebsiella* spp. (1%) were detected by aerobic culture; and *G. vaginalis* (68%), *A. vaginae* (25%), *M. curtisii* (12%), *B. fragilis* (6%), and *M. mulieris* (3%) were detected by PCR. *A. vaginae* was positive in 39% and 25% of women in study and control groups, respectively. (p=0.048) *M. curtisii* was positive in 2% and 12% of women in study and control groups, respectively. (p=0.012). **Conclusions.** Conventional culture is more effective for *G. vaginalis* in distinguishing between symptomatic and asymptomatic women. PCR may be a good alternative for rare BV agents, since their growth in conventional culture is difficult and anaerobic culture is time consuming.

Keywords: *Gardnerella vaginalis*, bacterial vaginosis, polymerase chain reaction, vaginal flora

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Özet

Amaç. Bakteriyel vaginoza neden olan etiyolojik ajanların dağılımını saptamak ve buna bağlı olarak ampirik tedavide kullanılabilecek antimikrobiklere yönlendirme açısından yardımcı olmak. **Yöntem.** Çalışmamızda İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi Kadın Doğum Polikliniği'ne başvuran cinsel aktif, vaginal akıntı şikayeti olan 100 kadın hasta çalışma grubu olarak alınmıştır. Herhangi bir şikayeti olmayan, kontrol amacıyla polikliniğe gelen cinsel aktif 100 kadın kontrol grubu olarak alınmıştır. Örneklere aerob kültür yapılmış ve anaerob etkenler için multiplex PZR (polimeraz zincir reasiyonu) yapılmıştır. **Bulgular.** Kültür ile hasta grubunun 10'unda (%10) *G. vaginalis*, 5'inde (%5) *M. hominis,* 2'sinde (%2) *U. urealyticum*, 5'inde (%5) *E. coli*, 8'inde (%8) *C. albicans* ve 6'sında (%6) *Candida* spp. üredi. PZR ile hasta grubunun 72'sinde (%72) *G. vaginalis*, 39'unda (%39) *A. vaginae*, 6'sında (%6) *M. mulieris*, 2'sinde (%2) *B. fragilis* ve 2'sinde (%2) *M. curtisii* pozitif olarak bulundu. Kontrol grubu kültürlerinin ise 7'sinde (%7) U. urealyticum, 6'sında (%6) *M. hominis*, 4'ünde (%4) *E. coli*, 1'inde (%1) *Klebsiella* spp., 3'ünde (%3) *C. albicans* ve 4'ünde (%4) *Candida* spp. üredi. Kontrol grubunun da PZR ile 68'inde (%68) *G. vaginalis*, 25'inde (%25) *A. vaginae*, 12'sinde (%12) *M. curtisii*, 6'sında (%6) *B. fragilis* ve 3'ünde (%3) *M. mulieris*'in pozitif olduğu saptandı. PZR yöntemiyle hasta grubunun 39'unda (%39) *A. vaginae*, 2'sinde (%2) *M. curtisii* pozitif bulunurken, kontrol grubunun 25'inde (%25) *A. vaginae*, 12'sinde (%12) *M. curtisii* pozitif olarak saptandı ve gruplar arasında istatistiksel olarak anlamlı fark bulundu. **Sonuçlar.** *G. vaginalis* enfeksiyonlarında kültür, semptomatik ile asemptomatik olguları ayırmakta PZR'ye göre daha etkindir. Nadir bacterial vaginosis etkenlerinin kültürde üretilmesinin zor olması ve özellikle anaerob kültür yönteminin zahmetli olması nedeniyle, PZR iyi bir alternatif olabilir.

Anahtar sözcükler: *Gardnerella vaginalis*, bakteriyel vajinozis, polimeraz zincir reaksiyonu, vajinal flora

Introduction

Vaginal flora may include lactobacilli, bacteroides, peptoccoci, peptostreptococci, staphlyococci, streptococci, corynebacteria, and *E. coli.* Rarely *C. albicans*, *G. vaginalis*, and *T. vaginalis* can be found. Bacterial vaginosis is not a real infection but rather a change in the vaginal flora along with foul smelling vaginal discharge. It is characterized by increased number of anaerobic agents, especially *G. vaginalis* and mycoplasms, and decreased number of lactobacilli in vaginal flora. Normal ratio of anaerobic/aerobic bacteria is 5, however this ratio increases up to 1000 in bacterial vaginosis [1, 2].

Bacterial vaginosis may cause pelvic inflammatory disease, infertility, and pregnancy complications; therefore it must be treated if diagnosed [3]. In clinical practice, direct microscopic examination, conventional culture, polymerase chain reaction (PCR), and immunoflorescence are used for examination of the vaginal discharge. However the anaerobic culture is not usually used for vaginal samples, therefore the etiologic agent cannot be revealed in most cases and agent-targeted therapy cannot be achieved. At this point PCR is a new method for all agents however it is mainly used for anaerobic agents.

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In our study, we investigated the distribution of etiologic agents in bacterial vaginosis by conventional and molecular methods and evaluated the pros and cons for each method.

Materials and Methods

Patient selection

Two hundred sexually active women who were admitted to outpatient service of Istanbul University Cerrahpasa Hospital between June and October 2011 were enrolled in a prospective study. The study was approved by the Human Ethics Committee of Istanbul University and supported by Istanbul University Scientific Research Projects (Project No: 8743).

The study group consisted of 100 women with vaginal discharge, and control group consisted of 100 women with no complaint of vaginal discharge. Exclusion criteria were <18 and >48 years of age, pregnant, menopausal women, menstrual disorders and any immune-suppressive systemic illnesses.

Evaluation of the samples

All the samples were taken from the vaginal posterior fornices or lateral wall of the vagina with three sterile swabs, without touching the vulva. One of the swabs was put in a protective liquid for the specimens of multiplex PCR. One of the swabs was taken for direct microscopic examination of T. vaginalis and the third swab was inoculated in an agar for aerobic culture and Gram stain was performed.

For aerobic bacteria, blood, chocolate, and Mac Conkey nutrient media were used for reproducing the different morphologic microorganisms stained by the Gram method, defined according to the shape characteristics. Fastidious Gram-negative rods that reproduce in Mac Conkey agar were inoculated in glucose, citrate Mio and TSI agar, type of the bacteria were determined according to their fermentation, motion, indole generation properties. After the recognition of rod species their antibiotic sensitivity was investigated. Definition of breeding yeasts was done according to their colony morphology and microscopic characteristics. To distinguish between the types of *Candida* species germ tube test was used, the ones who made germ tubes in two hours were identified as *C. albicans*. *Candida* species were inoculated to cornmeal agar and their producing chlamydospor property was tested. *Candida* species were seeded in chromogen agar and their subtypes have been identified according to their forming different colors. The *Candida* species were identified according to the color of the colonies that they formed after inoculation. In chromogen agar *C. albicans* produced green, *C. tropicalis* produced metallic blue and *C. kruseii* produced gray-pink colonies.

G. vaginalis was inoculated to aerobic agar. In order to identify *G. vaginalis*, presence of clue cells in Gram stain and typical morphology of the breeding colonies were used. For identification of *U. urealyticum* and *M. hominis*, the samples were inoculated in liquid *Ureaplasma* medium and were incubated at 37°C and 5-10% CO_2 medium. Twenty-four hours after, colonies were taken from the liquid agar to the solid *Ureaplasma* agar and were incubated for 2-3 days at 37°C and 5-10% CO_2 medium. Typical star-shaped colonies

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were seen when the solid ureaplasma agars were examined under light microscope. When we examine the samples that were inoculated on solid mycoplasma medium after 3 days of inoculation, the typical fried-egg colonies were seen under microscopic examination. The samples that formed typical fried-egg colonies were identified as *M. hominis*.

In order to identify anaerobic organisms South Korea Seegene Seeplex STI Master Panel 2 ACE Detection kit (SD6512X) were used. *G. vaginalis*, *B. fragilis*, *M. curtisii*, *V. atopobium*, and *M. mulieris* were identified with this kit by using multiplex PCR.

Statistical analysis

Data were presented as number (percentage). Comparison of proportions was performed using chi-square test. A p value of less than 0.05 was accepted as statistically significant.

Results

In the study group, *G vaginalis* was detected in 10 (10%), *M. hominis* was detected in 5 (5%), *U. urealyticum* was detected in 2 (2%), *E. coli* was detected in 5 (5%), *C. albicans* was detected in 8 (% 8), *Candida* spp. were detected in 6 (6%) samples. *T. vaginalis* was found neither in the study nor in the control groups. A total of 32 species were detected in the study group. In the control group, *M. hominis* was detected in 6 (6%), *U. urealyticum* was detected in 7 (7%), *E. coli* was detected in 4 (4%), *Candida* spp. were detected in 3 (3%), *C. albicans* was detected in 1 (1%), and *Klebsiella* spp were detected in 1 (1%) samples. A total of 25 species were detected in the control group. *G. vaginalis* was detected in 10 women from the study group, whereas in control group none of the women had *G. vaginalis*. In the study group, the most common agent was *G. vaginalis*, followed by *C. albicans*. In the control group *U. urealyticum* and *Candida* spp were the most common agents. Among 21 *Candida* samples, 7 were *C. albicans*, 7 were *C. krusei*, and 7 were *C. tropicalis*.

In multiplex PCR, *G. vaginalis* was detected in 72 (72%), *B. fragilis* was detected in 2 (2%), *M. curtisii* was detected in 2 (2%), *A. vagina* was detected in 39 (39%), *M. mulieris* was detected in 6 (6%) samples in the study group; whereas in the control group, *G. vaginalis* was detected in 68 (68%), *B. fragilis* was detected in 6 (6%), *M. curtisii* was detected in 12 (12%), *A. vagina* was detected in 25 (25%), *M. mulieris* was detected in 3 (3%) samples (Table 2). In the study group *G. vaginalis* was positive in 10% and 72% of the culture and PCR groups respectively (p <0.001, 95% CI: 49.9-71).

Table 1. The distribution and comparison of bacteria and yeast species.

Species	Patients (n=100)	Controls (n=100)	Significance
G. vaginalis	10	0	0.001
M. hominis	5	6	0.756
U. urealyticum	2	7	0.088
E. coli	1	4	0.733
C. albicans	8	3	0.044
Non-albicans *Candida* spp	6	4	0.774
Klebsiella spp.	0	1	0.238
Total	32	25	

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Table 2. PCR results and statistics.

PCR	Patients (n=100)	Controls (n=100)	Significance
G. vaginalis	72	68	0.537
B. fragilis	2	6	0.148
M. curtisii	2	12	0.055
A. vagina	39	25	0.033
M. mulieris	6	3	0.306

Discussion

Bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis are the most common etiologic factors of vaginal discharge in reproductive period. Bacterial vaginosis is characterized by decreased number of lactobacilli and may cause serious problems such as pelvic inflammatory disease and infertility in some women [2, 4, 5].

The prevalence of bacterial vaginosis is reported to change according to the type of the center [3, 7]. Bump et al. [7] reported the rate as 15% in sexually active women in general population. However the prevalence is as high as 33-64% in sexually transmitted disease outpatient clinics [8].

In our study, in culture *G. vaginalis* was detected in 10 women from the study group, whereas in control group none of the women had *G. vaginalis*. In multiplex PCR *G. vaginalis* was positive in 72 and 68 women in study and control groups, respectively. Therefore we see that culture showed better results to differentiate women with and without discharge. Considering our findings, as suggested by Ling et al. [9], *G. vaginalis* should not be used as indicator organism in the diagnosis of bacterial vaginosis.

M. hominis was positive in 5 and 6 women in the study and control groups, respectively. *M. hominis* can be found in genital flora of women in reproductive period [10,11]. Pingmin et al. [12] reported that 10-35% of the normal women had *M. hominis* in their flora. Yavuzdemir et al. [13] reported that 40-70% of the women with vaginitis had *M. hominis*. Holst et al. [14], accepted *M. hominis* as one of the four major agents of bacterial vaginosis and reported the detection rate as 76% and 9% of the study and control groups. Findik et al. [11] isolated *M. hominis* in 13.3% of 60 women with vaginal discharge.

In our study, *U. urealyticum* was detected in 2 and 7 women in study and control groups, respectively. The association between *U. urealyticum* and bacterial vaginosis is controversial since it was isolated from both healthy women and women with bacterial vaginosis [15, 16]. Haznedaroglu et al. [17] isolated *U. urealyticum* from 73 and 53 of the study and control groups, respectively. Yavuzdemir et al. [13] reported that *U. urealyticum* was detected in 33% of women with vaginitis, however they failed to show the association with bacterial vaginosis. Ermis et al. [18] reported that *U. urealyticum* was detected in 27 (22%) of 119 women without vaginal discharge.

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In our study *E. coli* was found in 5 and 4 women in the study and control groups, respectively. *E. coli* is known to be a part of the normal flora. However colonization of the vagina may cause pelvic inflammatory disease, urinary system infections and neonatal septicemia [8]. For this reason, it should be treated whenever necessary.

We detected that *M. curtisii* was found in 2 and 12 women in study and control groups, respectively. *A. vaginae* was detected in 39 and 25 women in study and control groups, respectively. In recent studies on use of PCR method in bacterial vaginosis, *A. vaginae* and *M. curtisii* were detected in high rates in bacterial vaginosis. Ling et al. [9] suggested that especially *A. vaginae* was more specific for bacterial vaginosis compared to *G. vaginalis*. Our study is the first to show *A. vaginae* in bacterial vaginosis by PCR in Turkey. The detection rates of *M. mulieris* and *B. fragilis* were not significantly different between study and control groups.

Non-albicans *Candida* spp. were detected in 6 and 4 women in the study and control groups, respectively. Twenty percent of women are known to have asymptomatic colonization. Erdem et al. [8] reported that *C. albicans* and non-albicans *Candida* spp. are detected in 27-60% and 6-12%, respectively, of women with vaginal discharge. In another study [4], *C. albicans* was found in 50% to 76% of women with vaginitis.

Comparing the results of aerobic culture and PCR, the latter seems to be more specific for the agents that cause bacterial vaginosis. Since anaerobic culture is not used in daily gynecologic practice, anaerobic bacteria which cause bacterial vaginosis cannot be identified and therefore remain untreated. However PCR method is not used due to its high cost.

In conclusion, PCR method is more sensitive compared to aerobic and anaerobic culture methods in diagnosis of bacterial vaginosis, moreover it identifies all the agents such as. *G. vaginalis, B. fragilis, M. curtisii, M. mulieris,* and *A. vaginae* at once. Failure of aerobic culture to show rare agents leads to treatment failure. PCR method could be used in women with recurrent treatment failure.

Conflict of Interest

Authors declare that there is no conflict of interest.

References

1. Atasu T, Sahmay S. [Article in Turkish] Jinekoloji (Kadin Hastaliklari), Nobel Tip Kitabevleri,2001; 231-241.
2. Balci O, Capar M. [Article in Turkish] Vaginal enfeksiyonlar. Turk Jinekoloji ve Obstetrik Dernegi derg. 2005; 2(5): 14-20.
3. Duran N, Culha G, Cetin M, Zeteroglu S, Gungoren A, Hakverdi AU. [Article in Turkish] Genital enfeksiyon suphesi olan adolesan ve yetiskin kadinlarda enfeksiyon etkenleri. Tip arastirmalari dergisi 2005; 3(2): 13-19
4. Aydin F, Tosun I, Kaklikkaya N, Yazici Y. Frequency of bacterial vaginosis among women attending for intrauterine device insertion at an inner-city family planning clinic. Eur J Contracept Reprod Health Care. 2003 Sep; 8(3): 135-138.

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5. Barbone F, Austin H, Low WC, Alexander WJ. A follow-up study of methods of contraception, sexual activity and rates of trichomoniasis, candidiasis and bacterial vaginosis. Am J Obstet Gynecol 1990; 163: 510-514.
6. Borekci B, Ingec M, Aktas O. [Article in Turkish] Jinekolojik sikayetleri olan hastalarda vaginal mikroflora uzerine bir arastirma. MJAU 2003; 35: 57-60.
7. Bump RC, Buesching WD. Bacterial vaginosis in virginal and sexually active adolescent females: evidence against exlusive sexual transmission. Am. J Obstet Gynecol. 1988; 158: 935-939.
8. Erdem H, Cetin M, Timuroglu T, Cetin A, Yanar O, Pasha A. Identification of yeasts in public hospital primary care patients with or without clinical vaginitis. Aust NZJ Obset Gynaecol, 2003; 43(4): 312-6.
9. Ling Z, Kong J, Liu F, Zhu H, Chen X, Wang Y, Li L, Nelson KE, Xia Y, Xiang C. Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. BMC Genomics. 2010;11 :488.
10. Bahar H, Torun MM, Ocer F, Kocazeybek B. Mobiluncus species in gynaecological and obstetric infections: antimicrobial resistance and prevalence in a Turkish population. Int J Antimicrob Agents. 2005; 25(3): 268-271.
11. Findik D, Guler L, Soysal S. [Article in Turkish] Vagina akintisi orneklerinin mycoplasma ve nonspesifik bakteriler yonunden degerlendirilmesi. Infeksiyon Dergisi 1996; 410(1): 51-54.
12. Pingmin W, Yuepu P, Jiwen Z. Prevalence survey on condom use and infection of urogenital mycoplasmas in female sex workers in China. Contraception. 2005 Sep;72(3):217-20.
13. Yavuzdemir S, Bengisun S, Gungor C, Ciftcioglu N, Ozenci H, Vardar G. [Article in Turkish] Vaginal akintisi olan kadinlarda G. vaginalis, Mikroplazma ureaplazma T. vaginalis, maya N. gonorrhoeae ve diger bakterilerin sikligi. Mikrob Bult. 1992; 26: 139 -143.
14. Holst E., Goffeng AR, Andersch B. Bacterial vaginosis and vaginal microorganisms in idiopathic premature labor and association with pregnancy outcome. J Clin Microbiol. 1994; 32(1): 176-86.
15. Hillier SL., Krohn MA., Rabe LK, Klebanoff SJ, Eschenbach DA. The normal vaginal flora H2O2 producing lactobacili and bacterial vaginosis in pregnant women. Clin. Infec. Dis. 1993; 16: (Suppl) 273-278.
16. Hillier SL, Cassen E, Easterling TR, Rabe LK, Eschenbach DA. The role of bacterial vaginosis and vaginal bacteria in amniotic fluid infection in women in preterm labor with intact fetal membranes. Clin. Infect. Dis. 1995; 20(Suppl. 2): 276-278.
17. Haznedaroglu T, Hakbilen S, Baysallar M, Kisa O, Basustaoglu A, Gun H. [Article in Turkish] Vaginit etkeni olabilecek mikroorganizmalarin sikligi ve tani yontemlerinin karsilastirilmasi. Infek. Derg. 1995; 9: 121-124
18. Ermis H, Baysal B, Isildaksoy N, Ibrahimoglu L. [Article in Turkish] Normal vaginal floranin servikal erozyon ve bazi kontraseptif yontemlerle degisimi. Haseki Tip Bulteni, 1995; 33(1): 13-16.

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