

To determine of the prevalence of *Bacterial Vaginosis*, *Candida sp*, mixed infections (*Bacterial Vaginosis* + *Candida sp*), *Trichomonas Vaginalis*, *Actinomyces sp* in Turkish women from Ankara, Turkey

Ocena częstości występowania bakteryjnej waginozy, infekcji *Candida sp.*, infekcji mieszanej (bakteryjna waginoza + *Candida sp.*), *Trichomonas vaginalis*, *Actinomyces sp.* u tureckich kobiet z Ankary, Turcja

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Abstract

Objective: To determine of the prevalence of *Bacterial Vaginosis*, *Candida sp*, mixed infections (*Bacterial Vaginosis* + *Candida sp*), *Trichomonas Vaginalis*, *Actinomyces sp* in Ankara, Turkey and analyze whether there is seasonal variation in these infectious agents.

Methods: A retrospective study on the results of 23298 cervical cytology examinations of patients which were performed in Fatih University, Faculty of Medicine, Pathology Laboratory in Ankara, Turkey from January 2007 to July 2011. Patients were included in the study if a Pap smear was performed for any reason.

Results: The prevalence of *Bacterial Vaginosis*, *Candida sp*, mixed infections (*Bacterial Vaginosis* + *Candida sp*), *Trichomonas Vaginalis*, *Actinomyces sp* was 7.76%, 2.81%, 0.32%, 0.13%, and 0.27%, respectively. A seasonal variation was not observed in the prevalence of any of the infectious agents ($p > 0.05$).

Conclusion: We conclude that cervical cytology is well suited for diagnosis of cervical infections. *Bacterial vaginosis* appears to be the predominant cause of vaginitis.

Key words: **cervical cytology** / **vaginitis** / **bacterial vaginosis** / ***Trichomonas Vaginalis*** / ***Actinomyces sp***

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Streszczenie

Cel pracy: Ocena częstości występowania bakteryjnej wagi-nozy, infekcji *Candida sp.*, infekcji mieszanej (bakteryjna wagi-noza + *Candida sp.*), *Trichomonas vaginalis*, *Actinomyces sp.* u tureckich kobiet z Ankary, Turcja i analiza możliwej sezonowości w tych czynnikach infekcyjnych.

Metoda: Badanie retrospektywne przeprowadzono w oparciu o wyniki 23298 wymazów cytologicznych pacjentek zbadanych w Fatih University, Faculty of Medicine, Pathology Laboratory w Ankarze, w Turcji od stycznia 2007 do lipca 2011. Pacjentki były włączane do badania w każdym przypadku wykonanego badania cytologicznego.

Wyniki: Częstość występowania bakteryjnej wagi-nozy, infekcji *Candida sp.*, infekcji mieszanej (bakteryjna wagi-noza + *Candida sp.*), *Trichomonas vaginalis*, *Actinomyces sp.* wynosiła 7,76%, 2,81%, 0,32%, 0,13% i 0,27%, odpowiednio. Nie obserwowano sezonowej zmienności w odniesieniu do żadnego z analizowanych czynników infekcyjnych ($p < 0,05$).

Wnioski: Cytologia jest dobrym narzędziem diagnostycznym w wykrywaniu infekcji szyjki macicy. Bakteryjna wagi-noza wydaje się być najczęstszą przyczyną infekcji pochwowej.

Słowa kluczowe: zapalenie pochwy / bakteryjna wagi-noza / *Trichomonas vaginalis* /
/ *Actinomyces sp.* /

Introduce

Vaginitis is the one of the most common problems in clinical medicine. An extensive and diverse spectrum of pathogenic agents may be observed in the vaginal microflora. Bacterial vaginosis, candidiasis and trichomoniasis are responsible for most of the cases of infectious origin [1-4]. Actinomycosis has been recognized for many years in cervicovaginal canal of women who used intrauterine device (IUD) [5].

Bacterial vaginosis (BV) is the most common vaginal infection and affects approximately 30% of women [4,6]. In this infection the normal lactobacilli-dominated vaginal flora is replaced by a combination of mixed facultative and anaerobic flora, *Gardnerella vaginalis*, or *mycoplasma hominis mobilincus sp.* BV complications can be significant. Endometritis, pelvic inflammatory disease, and preterm labor and birth in pregnant women could be seen in BV [7]. Vaginal candidiasis is the second form vaginal infection [1-4, 8]. Three quarters of all adult women experience at the last one episode of vaginal candidiasis during their life time. Another important pathogen is *Trichomonas Vaginalis* (TV). This protozoan is a considered to be sexually transmittable. It is known that trichomoniasis can lead to inflammatory pelvic disease, reproductive dysfunction and increased risk of premature rupture of fetal membranes and low birth weight [9]. *Actinomyces* are common findings in users of IUD. Because of complication of actinomycotic infection include tuboovarian abscess and pelvic inflammatory disease; it is important that *Actinomyces* microorganisms were correctly diagnosed in pathogenic specimens [10, 11].

The cervical cytology (Pap test) was suggested in the 1940s by George Papanicolaou, and has since been widely accepted for surveying initial cervical cancer. The cervical cytology also has been evaluated as a diagnostic test in vaginitis. The Bethesda System identifies five categories of organisms in cervical cytology [12];

- 1- Shift in flora suggestive of BV.
- 2- Fungal organisms morphologically consistent with *Candida spp.*
- 3- *Trichomonas Vaginalis* (TV).
- 4- Bacteria morphologically consistent with *Actinomyces*.
- 5- Cellular changes consistent with Herpes Simplex virus.

The purpose of this work was determine the frequency of the

causative agents of vaginitis, BV, *Candida sp*, mixed infections (BV+Candida sp), TV, and *Actinomyces sp* in cervical cytology between 2007 and 2011 in the capital city of Turkey. Comparison of seasonal variation in prevalence of vaginal infectious agents was also analyzed.

Material and methods

The study was retrospective analysis of cervical cytology results of all women attending the obstetrics and gynecology clinics of the Fatih University Medicine Faculty, Ankara over a 4.5-years period, from January 2007 to July 2011. Patients were included in the study if a Pap smear was performed for routine screening and medical necessity.

Patients with BV, *Candida sp*, mixed infections (MI), TV, or *Actinomyces sp* in the cervical cytology results were included in the study. Information related to the patients was obtained from cytology reports.

Cervical cells were collected using the PAPETTE™ (Thermo Shandon, Pittsburg, USA) cervix brush and placed immediately into a vial of PapSpin Collection Fluid (Thermo Shandon, Pittsburg, USA). Liquid based cytology (LBC) preparations were made with the Cytospin 4 cytocentrifuge (Thermo Shandon). The smears were stained using Papanicolaou's method and analyzed by three pathologists. All smears were investigated with the same diagnostic criteria based on Bethesda System/2001. The diagnosis of BV was made on mixed flora, relative absence of lactobacilli, and mature squamous cells that are covered by coccobacilli, typically extending beyond the cell margin. *Candida sp* was diagnosed when pseudo-hyphae and/or small spores were observed mixed infection was diagnosed when *Candida sp* and BV were observed at the same time. TV was diagnosed when a unicellular organism of ovoid or rounded shape which contains a single nucleus was observed. Morphological criteria for the cytological diagnosis *Actinomyces* like organisms were presence of characteristic basophile spots surrounded by intertwined filament-type formation. Eligible patients who were suspected of having *Actinomyces* infection underwent a subsequent cell block process. Cell blocks were performed if the visible sediment remaining after making the smears was sufficient to make the block. These cell blocks were stained with Gram, PAS, Grocott and Zielh Nielsen stains for confirmation of the diagnosis.

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Table I.

	Winter	Spring	Summer	Fall	P*	Total
Bacterial Vaginosis	491	453	524	334	0.083	1802
Candida Sp.	210	163	164	116	0.076	653
Mixed infections	21	23	17	14	0.666	75
Trichomonas Vaginalis	9	9	6	6	0.804	30
Actinomyces sp	14	20	19	11	0.578	64
Total	745	668	730	481		2624

* χ^2 test

Table II. Cases in which Bacterial Vaginosis, Candida sp, mixed infections (Bacterial Vaginosis +Candida sp), Trichomonas Vaginalis, Actinomyces sp were detected, stratified by age ranges.

	17-30	30-41	41-50	>=51	P*	Total
Bacterial Vaginosis	429	520	507	346	0.000	1802
Candida Sp.	223	252	115	63	0.000	653
Mixed infections	25	38	11	1	0.000	75
Trichomonas Vaginalis	5	7	12	6	0.206	30
Actinomyces sp	9	26	27	2	0.000	64
Total	691	843	672	418		2624

* χ^2 test

SPSS for Windows, version 16.0 (Statistical Package for the Social Science, Chicago, Illinois, USA) software was used for statistical calculations. The chi-squared test was used for statistical analysis. A p-value of <0.05 was considered significant for statistical evaluation.

The study was carried out according to the principles of the Declaration of Helsinki. The research was approved by the Ethics Committee for Research Involving Humans at the Fatih University Faculty of Medicine (Protocol number 04 /2012).

Results

The results of 23208 cervical cytology examinations that were performed within 4.5 years were evaluated. Among these, 2624 (11.3%) were found to have infectious agents. The prevalence of BV, Candida sp, mixed infections, TV, and Actinomyces sp were 7.76%, 2.81%, 0.32%, 0.13%, and 0.27%, respectively (Figure 1). No other microorganisms were detected in significant numbers (suspect of Chlamydia, 1 case; Herpes Virus, 2 cases). The mean patient age was 39.3 years (SD, 11.5; SE, 0.22; age range, 17-86 years).

As shown in Table I and Figure 2, seasonal variations in prevalence were found to be not statistically significant for any of the infectious agents ($p>0.05$).

Regarding the frequency of BV, Candida sp, mixed infections, TV, Actinomyces sp in different age groups, younger women had significantly higher prevalence of all infectious agents except for TV ($P<0.05$). (Table II, Figure 3).

Discussion

Cervical cytology is used for screening sexually active women to enable early detection and treatment of precancerous lesions and prevent mortality due to cervical cancer. Although not mandatory in the current Bethesda terminology, it is a common practice to give a diagnosis of infectious microorganisms seen in the cervical cytology.

Regarding BV, several studies have identified it as the leading vaginal infection [1-4,13,14]. Hart studying 5365 patients in Australia, found BV prevalence to be 13.7% [15]. Simoes-Barborasa et al, studying 142158 women in Brasilia, reported BV prevalence as 17.2% [4]. In Turkey, Karabulut et al, reported frequencies of BV to be 8.3% [3]. In our study, we found BV as the main infectious agent in 7.76% of the patients, which is similar Karabulut's result.

If left untreated, BV can cause serious complications. BV has been implicated in preterm delivery of low birth-weight infants due to chorioamnionitis, puerperal endometritis, postoperative vaginal cuff cellulitis, and pelvic inflammatory disease [7]. Since up to half of the women with BV are asymptomatic, the cervical cytology may be only means of diagnosis [16].

Some authors have suggested that the descriptive diagnosis for BV as "predominance of coccobacilli consistent with a shift in vaginal flora" is vague and probably not well-understood by most pathologists who collect routine cervical cytology [16]. Successive studies proved that the finding of "clue cell" in the cervical cytology examination had 100% sensitivity and a 96% spesitivity for the diagnosis BV [17].

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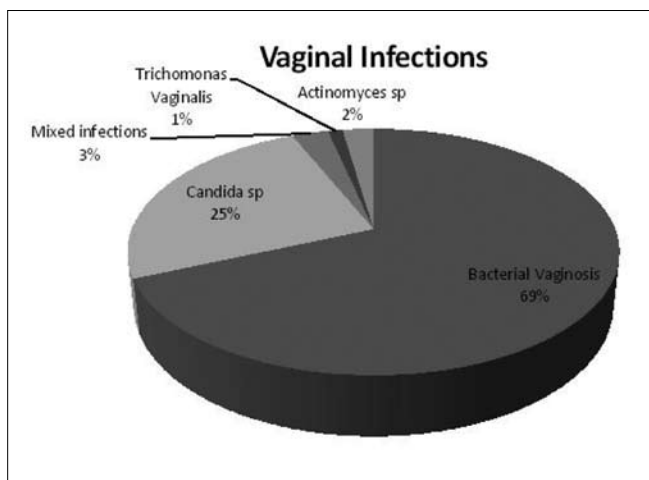


Figure 1. Frequency of bacterial Vaginosis, Candida sp, mixed infections (Bacterial Vaginosis +Candida sp), Trichomonas Vaginalis, Actinomyces sp.

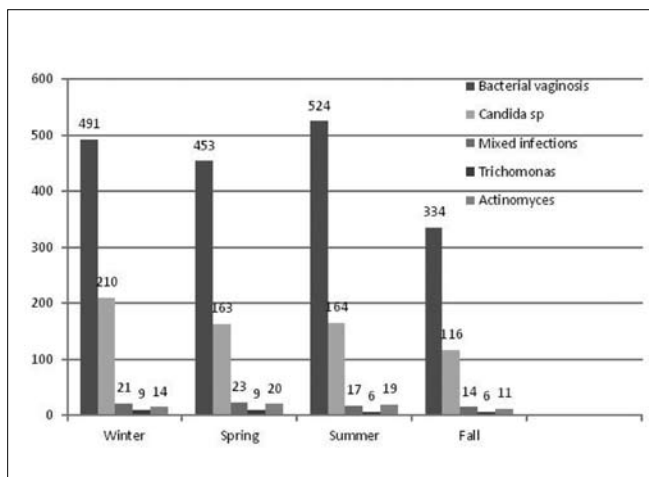


Figure 2. Prevalence of Bacterial Vaginosis, Candida sp, mixed infections (Bacterial Vaginosis +Candida sp), Trichomonas Vaginalis, Actinomyces sp.

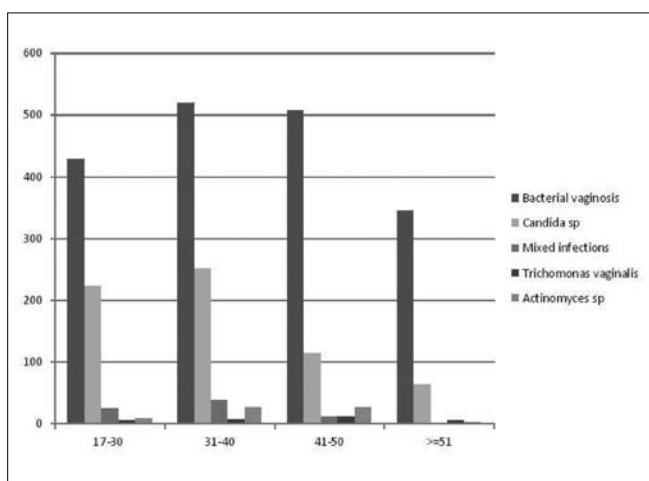


Figure 3. Prevalence of Bacterial Vaginosis, Candida sp, mixed infections (Bacterial Vaginosis +Candida sp), Trichomonas Vaginalis, Actinomyces sp, according to age ranges.

Clue cells have a decreased frequency in older women because in this age range, the pH is less variable and BV is more frequent in young women that are related pH alteration [18]. Also in our study, younger women had a significantly higher prevalence of BV.

Cervicovaginal candidiasis is the most common fungal disease in the world. Approximately 75% of women are affected from vulvovaginal candidiasis at some point in their life time. Hart, found candidiasis as a main infectious agent with 17.6% prevalence rate [15]. Toloj et al reported Candida in 26% [19]. Adad et al reported the infection by Candida sp has presented a large increase over the last decade (8.1% in 1988 to 22.5% in 1998) while Kent reported a fall in the frequency of Candida albicans in USA and a stabilization of infection by this agent in Skandinavia [1,20]. In our study Candida sp was detected in 2.78% of all of the cervical cytology specimens.

TV infection is an unpleasant and irritating disease of the female urogenital system and is one of the most common sexually transmitted diseases worldwide. The frequency of TV has diminished in Brasilia (17.3% in 1978 down to 3.4% in 1998) [1]. In addition, in the USA and Scandinavia, incidence of TV has gone down in the past years [20]. In the east of Turkey, Karaman et al found TV prevalence to be 0.75% [21]. In the west of Turkey, Karabulut et al found a low frequency of TV (0.32%). Our observations were even lower than these reported values (0.12%)[3].

Mixed infections (BV+Candida sp) were not determined previously in the literature but were encountered in practice of cervical cytology from time to time.

The frequencies of all infectious agents in our study were much lower than those published in the literature. It is probable that lower indices are related to socioeconomic power of the patients, since Fatih University Hospital is a private funded hospital. The conservative lifestyle in our country may contribute to lower rates in sexually transmitted disease.

Actinomyces species are obligate anaerobic bacteria that demonstrate branching and filamentous growth. First report of Actinomyces in cervical cytology was detected by Gupta et al in 1976. They found this agent in 3 of 13 patients who were IUD users. They claimed that Actinomyces sp could be easily identified on cervical cytology [22]. In literature many of published studies confirmed authors' findings via use of culture or immunofluorescent antibodies [23].

For microbiologists, cervicovaginal cultures are the most difficult cultures to be evaluated in a clinical microbiology practice Because of the necessity of some expensive, difficult, and very complicated processes for the diagnosis, especially of Actinomyces sp. In this study, we prepared cell blocks for detection of Actinomyces sp and stained with histochemical dyes for confirmation of diagnosis. This method is easier for diagnosis of Actinomyces compared to culture.

There have been a few studies published regarding seasonal variation in the detection of microorganisms in the cervical canal. The results varied from study for study. Although some authors reported no seasonal variation [24], others reported seasonal variation of infectious agents. Some of them showed that Candida sp exhibited higher incidence during fall, or during winter season [25,26]. Rietveld et al and Sodhani et al reported that TV exhibited a higher incidence during winter and lower

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incidence during summer [26, 27]. In our study, seasonal variation wasn't detected.

The cervical cytology was suggested in the 1940's, and has since been widely accepted as one of the screening tests. We conclude that cervical cytology examinations were helpful in the diagnosis of cervical infections. Cell block may be a useful method for evaluation in cervical cytology.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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