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Original Research

Agents Isolated from Vaginal Cultures in the Reproductive Period and Their Antibiotic Sensitivities (Vaginal Culture and Antibiotic Sensitivitie)

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ABSTRACT

Objective: In our study, we aimed to examine the strains isolated from vaginal swab samples sent to our laboratory from various clinics with a pre-diagnosis of vulvovaginitis and antibiotic resistance rates, retrospectively.

Methods: ethods: 90 vaginal swab samples of patientswere included into this study. Two samples were taken from each patient and one sample was used for Gram staining. The other sample was inoculated in 5% sheep blood, Eozine Methylene Blue agar and Saboraud dextrose agar. TIdentification of isolated colonies and antibiotic susceptibility studies were carried out with Vitek 2 Compact automated system. Result of the susceptibility testing are reported according to EUCAST criteria. Nugent scoring was used for bacterial vaginosis.

Results: Normal vaginal flora elements were found in 66 (73.3 %) of the specimens, and 28 organisms were found in 24(26.7 %). The distribution of the organisms is as follows: 6 (21.4%) Escherichia coli, 5(17.9%) Streptococcus agalactiae, 5(17.9%) Gardnerella vaginalis, 3 (10.7%) Candida spp., 3(10.7%) Klebsiella pneumoniae, 3(10.7%) Enterococcus faecalis. Two of the E.coli strains and one of the K.pneumoniae strains are ESBL positive. Of the agents, 21 were isolated from outpatients and 7 from inpatients. All S. agalactiae strains were susceptible to penicillin and ampicillin. E.coli and K.pneumoniae strains, which are the most frequently isolated Gram (-) organisms, are most susceptible to amikacin, carbapenems and tigecycline.

Conclusion: In patients with vaginal discharge and itching complaints, determining the organisms with microbiological culture and agent-directed treatment instead of empirical treatment will be more beneficial for cure.

Keywords: Antibiotic sensitivity, vaginitis, vaginal discharge

INTRODUCTION

Approximately 10 million patients a year present to gynecology clinics due to vaginal discharge, pruritus, and purulence (1). The three most frequently observed agents in the diagnosis of vaginitis are *Candida* spp., (CC), bacterial vaginosis (BV), and Trichomonas (TCH). However, the agent involved in vaginitis remains undiagnosed in between 7% and 72% of patients, and these soon re-present to clinics with the same symptoms (2-5). Appropriate treatment directed toward the agent should therefore be prioritized in terms of diagnosis and treatment. Various different methods are available for identifying agents of vaginitis, including vaginal discharge microscopy, examination with 10% potassium hydroxide (KOH), Gram staining, vaginal pH examination, and vaginal culture (6). Micro-organisms detected in cultures from patients of reproductive age presenting to our hospital with vulvovaginal discharge and itching and the antibiotic sensitivity and resistance rates of those micro-organisms were examined retrospectively in this research.

METHODS

Approval for the study was granted by the Clinical Research Ethical Committee on 28.01.2021 (Decision No: 03/31). Ninety vaginal culture specimens sent from patients with vaginal pruritus and discharge to the Erzincan Mengücek Gazi Training and Research Hospital, Türkiye, between January 2016 and January 2020 were included in this study. Vaginal discharge specimens collected using two sterile swab sticks from patients presenting to various clinics were placed into Stuart transport medium (Fıratmed, Türkiye) and sent to the laboratory without loss of time. One of the specimens was inoculated onto 5% sheep's blood (bioMerieux ®, France), Eosin Methylene Blue (EMB) agar, and chocolate medium (bioMerieux ®, France). The other speci-

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Table 1. Service and polyclinic distribution of agents reproduced in vaginal culture

Bacteria	Policlinic	Service	Total
	Number (%)	Number (%)	Number (%)
Escherichia coli	4	2	6 (21.4)
Streptococcus agalactiae	4	1	5 (17.9)
Gardenerella vaginalis	4	1	5 (17.9)
Candida spp.	3	0	3 (10.7)
Klebsiella pneumoniae	2	1	3 (10.7)
Enterococcus faecalis	2	1	3 (10.7)
Enterobacter aerogenes	1	0	1 (3.6)
Acinetobacter baumannii	0	1	1 (3.6)
Proteus mirabilis	1	0	1 (3.6)
Total	**21 (75)**	**7 (25)**	**28 (100)**

Results were presented as numbers (n) and percentages (%).

men was suspended with sterile saline solution and placed onto a slide. After drying, it was then Gram-stained. The blood and EMB media were left to incubate for 24-48 h at 37° C in an aerobic environment, and the chocolate medium at 37° C in an environment with 5-10% CO_2. Identification and antibiotic susceptibility studies of colonies with morphology compatible with Gram results and dominant according to flora bacteria but regarded as causative agents based on the presence of inflammatory cells were performed using a Vitek 2 Compact (Biomerieux, France) automated system. EUCAST criteria were employed in reporting the sensitivity results (7). The presence of clue cells at Gram staining, the dominance of Gram labile coccobacilli, a decrease in lactobacilli, and the absence of an increase in inflammatory cell numbers were taken into account for bacterial vaginosis.

Main Points:

- The purpose of this study was to examine micro-organisms detected in cultures from women of reproductive age with vulvovaginal discharge and pruritus and the antibiotic sensitivity and resistance rates of those micro-organisms in a retrospective manner.
- Ninety vaginal culture specimens from patients with vaginal itching and pruritus sent to the Erzincan Mengücek Gazi Training and Research Hospital microbiology laboratory between January 2016 and January 2020 were included in this retrospective study.
- The most frequently detected agents, in descending order, were E. coli (21.4%), S. agalactia (17.9%), G. vaginalis (17.9%), Candida spp. (10.7%), K. pneumoniae (10.7%), E. faecalis (10.7%), E. aerogenes (3.6%), A. baumannii (3.6%), and P. mirabilis (3.6%).
- The identification of agents using the culture method in women with vulvovaginitis and the initiation of treatment aimed at the agent rather than empiric therapy appears to be more potentially useful in terms of complete cure and preventing antibiotic resistance.

Statistical Analysis

The distribution of agents by years was analyzed using the chi-square test designed for single measurement variables. The year from which a difference derived was analyzed using the Bonferroni method with corrected p values. p values < 0.05 were regarded as significant.

RESULTS

Normal vaginal flora elements were isolated in 66 (73.3%) of the 90 vaginal smear specimens sent to our laboratory during the four-year study period, and vaginitis agents in 24 (26.7%). The patients' ages ranged between 19 and 50 years (average 37.5). Two agents grew in two patients, and 28 agents were identified in the 24 patients. Six (21.4%) of the 28 growing agents were *Escherichia coli*, five (17.9%) *Streptococcus agalactiae*, five (17.9%) *Gardnerella vaginalis,* three (10.7%) *Candida spp*., three (10.7%) *Klebsiella pneumonia, and three* (10.7%) *Enterococcus faecalis*. Lower numbers of other agents (*Enterobacter aerogenes, Acetinobacter baumannii,* and *Proteus mirabilis*) were detected. Agents detected together were *E. coli* + *S. agalactiae, E. faecalis* + *K. pneumoniae, E. coli* + *E. faecalis,* and *Candida* spp.+*A. baumannii*. Twenty-one (75%) agents were isolated from outpatients and seven (25%) from patients admitted to the ward. The distribution of agents growing in specimens from the ward and clinics is shown in Table 1. In chronological terms, the largest number of agents was detected in 2018 (n=10) and the lowest in 2016 (n=5). Examination of distribution by years using chi-square analysis revealed no statistically significant variation (p=0.249). Although more agents were detected in 2018(n=10), the difference was not significant.The distribution of agents by years is shown in Table 1.

The most frequently isolated Gram(+) bacteria were *S. agalactia* (Group B Streptococcus), followed by *E. faecalis*. The mean age

Table 2. Antibiotic resistance rates of Gram (+) bacteria (%)

Antibiotics	Bacteria (n)	
	S.agalactia (n=5)	E.faecalis **(n=3)**
Penicillin	0	–
Ampicillin	0	0
Erythromycin	2 (40)	–
Clindamycin	2 (40)	–
Vancomycin	0	0
Teicoplanin	0	0
Trimethoprm/ sulfamethoxazole	3 (60)	3(100)
Linezolid	0	0
Tetracycline	3 (60)	–
Levofloxacin	2 (40)	–
Daptomycin	0	0
Tigecycline	0	–
Ciprofloxacin	–*	1(33.3)

Results were presented as numbers (n) and percentages (%).

*-: Not tested

Table 3. Antibiotic resistance rates of gram (-) bacteria (n/%)

Antibiotics	Bacteria (n)	
	E.coli **(n=6)**	K.pneumoniae **(n=3)**
Ampicillin	5(83.3)	3 (100)
Amoxicillin/ Clavulonic acid	3(50)	2 (66.7)
Piperacillin/ Tazobactam	1(16.7)	0
Cefuroxime	2(33.3)	1(33.3)
Ceftazidime	2(33.3)	1(33.3)
Ceftriaxone	2(33.3)	1(33.3)
Amikacin	0	0
Gentamicin	1(16.7)	1(33.3)
Ciprofloxacin	1(16.7)	0
Trimethoprim/ sulfamethoxazole	2(33.3)	1(33.3)
Tigecycline	0	0
Ertapenem	0	0
İmipenem	0	0
Meropenem	0	0

Results were presented as numbers (n) and percentages (%).

of the patients in whom *S. agalactia* was isolated was 38. All the Group B streptococci were sensitive to penicillin, vancomycin, teicoplanin, linezolid, tigecycline, and trimethoprim/sulfamethoxazole. No resistance to vancomycin, ampicillin, or teicoplanin was encountered in *E. faecalis*. The antibiotic resistance results are shown in Table 2. The most frequently isolated Gram (-) bacteria were *E. coli* and *K. pneumoniae*. Two of the *E. coli* strains (33.3%) and one *K. pneumoniae* strain (33.3%) were broad spectrum β lactamase (ESBL)-positive. The antibiotics to which the Gram (-) strains were most sensitive were amikacin, carbapenems, and tigecycline (antibiotic resistance rates are shown in Table 3).

DISCUSSION

Genital infections in women can lead to local discomfort during sexual relations and pain or pelvic inflammatory disease by causing vaginal discharge and mucosal ulceration. Persistent infection of the upper genital system can even result in infertility, ectopic pregnancy, and chronic pelvic pain. Physical examination must be performed after history has been taken from patients presenting with symptoms of vaginitis. Inspection of the vulva and speculum examination can be indicative in terms of several vaginal agents. Mild, transparent discharge may be observed in asymptomatic patients with a normal vaginal flora. Changes occur at physical examination as a result of vaginitis agents. An off-white vaginal discharge with a fishy odor may be expected in bacterial vaginosis. Vaginal discharge resembling cottage cheese, erythema, and vulvar edema may be present in Candida infections. A foam-like, yellow discharge, erythema, and petechiae may be observed in Trichomonas infections (5). Investigation in terms of the vaginal agent may be required in addition to vaginal examination in complicated and refractory infections (8). One of the methods applied to identify the vaginal micro-organism is vaginal culture. Although many micro-organisms in human vaginal flora do not grow in culture, molecular studies have been used as an addition to molecular methods in some studies (9-10). FISH and PCR methods have also been employed to identify vaginal infections in recent studies (11-13). The most frequently detected agents in this study, in descending order, in this study were *E. coli* (21.4%), *S. agalactia* (17.9%), *Gardnerella vaginalis* (17.9%), Candida spp. (10.7%), *K. pneumoniae* (10.7%), *E. faecalis* (10.7%), *E. aerogenes* (3.6%), *A. baumannii* (3.6%), and *P. mirabilis* (3.6%). The agents identified by Mohhamed Kadir et al. using a similar method were *S. aureus* (12.4%), *E. coli* (11.6%), *C. albicans* (8.0%), β hemolytic streptococci (2.8%), *Klebsiella* spp. (1.2%), *N. gonorrhoeae* (0.8%), and *Pseudomonas* spp. (0.4%) (14). In another study, coagulase negative staphylococci (n=11), enterococci (n=8), *S. aureus* (n=5), β hemolytic streptococci (n=5), *S. viridans* (n=1), and *P. mirabilis* (n=1) were isolated in vaginal cultures from 505 pregnant patients with no symptoms (6). *G. vaginalis, E. coli*, group B streptococci, *Mycoplasma* spp. and *C. albicans* are frequently seen in normal vaginal flora (5). In the light of the results of these studies, not all growth in culture may require treatment. However, positive culture results in addition to a patient history

and physical examination findings can be a useful guide in terms of treatment options. The detection of growth in culture alone in patients with no complaints and with no significant examination findings does not require treatment directed toward the agent in question. The most common Gram (+) bacterium in vaginal culture specimens in this study was *S. agalactia*. In terms of antibiotic resistance rates, this was 100% sensitive to penicillin, vancomycin, teicoplanin, and linezolid. Bolukaoto et al. reported similar results (15). Another study involving different patient groups also detected similar antibiotic sensitivity rates (16). The Gram (-) bacteria most frequently isolated in this study were *E. coli* and *K. pneumoniae*. Two (33.3%) of the *E. coli* isolates and one *K. pneumoniae* isolate (33.3%) were broad spectrum β lactamase (ESBL)-positive. ESBL positivity reduces the therapeutic options since it bestows the ability to hydrolyze broad spectrum cephalosporins and monobactams. Several studies have shown that ESBL positivity has an adverse effect on therapeutic options (17-19). The antibiotics to which Gram (-) bacteria were most sensitive in this study were carbapenems, amikacin, and tigecyclines. Similar studies have also reported comparable antibiotic resistance rates (17). Another study described carbapenems as the antibiotic to which *K. pneumoniae* isolates were most sensitive (20). Recurring urinary tract infections are also frequently seen in women with refractory vaginitis infection (21).

CONCLUSION

In conclusion, the number of patients presenting to gynecology clinics with vaginal discharge is high. Identifying the agents concerned using the culture method and the initiation of treatment aimed at the agent rather than empiric therapy, in addition to history and examination findings in these patients, appears to be more useful in terms of complete cure.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erzincan Binali Yıldırım Üniveristesi (January 28.2021, Decision No: 03/31).

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Conflict of Interest: No conflict of interest was declared by the authors.

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