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Evaluation of Trichomonas vaginalis and Candida albicans alongside with pathogenic bacteria in Kirkuk females

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A B S T R A C T

One of the most prevalent infections of the female reproductive system is (vulvovaginitis). Vaginitis has three forms of infectious, namely (trichomoniasis, valvovaginal candidiasis, and bacterial vaginosis). During the period from December (2021) to April (2022) in the Women conduct Children Hospital in Al-Nasr neighborhood and in private outpatient clinics in Kirkuk city in Iraq. The number of specimens examined was 160 samples (106 from healthy and 54from infected women) and the ages ranged between (14-50) years. After the pairs swabs were taken, the direct examination and staining and culturing on the different culture media to diagnose many pathogenic microorganism (bacteria spp, candida spp, trichomonas vaginalis parasite). The bacteria and candida diagnosis conforming by vitex-2 system. From the 54 sample positive, the commonest organisms was bacteria (33.75%gram negative and gram positive 31.25%), trailed by Candida species 14.37%, Trichomniasis as (5%), and mixed infection with those 3 agents in (17.5%). Escherichia coli 15(27.77%) and Staphylococcus aureus 16(32%) was the major bacteria isolate and Candida albicans (13.12%) was the major of candida spp. isolate. However, the findings of the vulvovaginitis and age relationship revealed that women aged (36-45years) 20(12.5%) are more common infected. This study revealed that among women suffering from vaginal discharge, bacterial vaginosis occurs often. In rare instances, trichomoniasis and bacterial vaginosis have been documented as co-infections. It can be present alone or in conjunction with candidiasis.

Introduction

Estrogen, vaginal pH, metabolic byproducts of flora and pathogens, and Lactobacilli interact dynamically with one another to form the typical vaginal environment, female genital infections are a common health problem in developing and developed countries, as pathogens replace the natural flora in the vagina, which is mainly represented by Lactobacilli, which plays an important role in protecting the vagina from the invasion of pathogens [1&2]. Vaginitis is an inflammation of the vagina; When a pathogen is introduced or the vaginal environment evolves, the vaginal flora is affected, which leads to vaginitis [3].Vaginal infection is one of the most common gynecological infections , that consist of the three vaginal bacterial, trichomoniasis, and candidiasis illnesses. Candidasis is the second most prevalent infectious cause of vaginitis after bacterial vaginosis, which is characterized by an instability in the vaginal environment [4]. Vaginal and occasionally vulvar symptoms including burning, irritation, itching, odor, and vaginal discharge are all part of the category of disorders known as vaginitis [5]. Gram-positive and Gram-negative bacteria form the majority of the flora in bacterial vaginosis (BV), which is marked by a significantly reduced number of obligate anaerobes and extremely low levels of lactobacillus [6&7]. There are main factors that lead to vaginitis, which are the hormonal imbalance that occurs in women, or because of the use of detergents that lead to irritation and sensitivity, or through the use of contraceptives [8&9]. Although many instances of BV are asymptomatic or only manifest as foul-smelling vaginal discharge without other inflammatory symptoms, BV is sometimes referred to as "vaginosis" rather than "vaginitis"[10&11]. Just around 20 of the more than 200 Candida species were thought to be significant human pathogens[12]. The primary frequent isolated species was Candida albicans, but other species including Candida tropicalis, C. glabrata, C. krusei, and C. parapsilosis were also emerging as significant sources of Candidal infections. It is thought to be the second most prevalent cause of vaginitis after BV [13]. The majority of candida infections are opportunistic and originate from changes in the host, including altered immune systems, chronic medication use, pregnancy, diabetes, and malnutrition. These factors all help to speed up the growth of pathogenic candida and produce UTIs in the host[14,15&16]. Trichomonas vaginalis is a common sexually transmitted parasite that causes vaginal infections in women as well as urethritis in men. It is a flagellated protozoan parasite [17]. Most individuals show no symptoms of illness, particularly in cases of minor or severe infections. [18]. In chronic infections, women feel abdominal pain, burning during urination, itching, and yellowish-greenish smelly vaginal discharge[19]. Whereas in women, symptoms might range from offensive vaginal discharge to urogenital tract irritation and edema to a higher risk of cervical cancer and unfavorable pregnancy outcomes[20&21]. Trichomonasis, a condition brought on by Trichomonas vaginalis, has significant implications for health, society, and the economy. Premature placental membrane rupture, early labor, and low birth weight are risks for pregnant women who have an infection [22].

Aim: isolation and identification of pathogenic microorganism which causes vaginitis in women in kirkuk city in iraq.

Methods

Specimen collection:

Through the period extending from December 2021 to April 2022, 160 specimens were collected from the Children Hospital in Al-Nasr neighborhood and in private outpatient clinics in Kirkuk.

Samples collection:

Hospital in Kirkuk city, Iraq, two vaginal swab from women of reproductive age Vaginal swab (vs.) taken via speculum and sterile swab from high vaginal area By assistance of clinicians the specialist doctors .

Sample processing :

sterile Lateral and posterior yaginal fornices were swabbed using sterile cotton-tipped applicators after Cusco's speculum was inserted. All of the women underwent clinical examinations and special questionnaire interviews. After being taken out of the vagina clinic, a constricted range (3.5–6) of pH strips are placed on the speculum for direct assessment of the color and pH of vaginal excretion. Two of high vaginal swabs were taken, the first swab was for microscopic examination the vaginal fluid was put in to (0.5)N.S to prepare wet preparation direct slide to detect the presence of clue cell by smear staining, then a drop of(10%) potassium hydroxide(KOH) was placed on a glass slide and immediately evaluated for the presence of a fishy odor (Wiff tast),and within five to ten minutes of the material being collected, a wet mount preparation was done at the patient's bedside to look for *Trichomonas vaginalis.*The second swab with transport media (amies) to keep it moist until taking to the laboratory to cultured aerobically on (Blood Agar, MacConkey Agar, Mannitol Salt Agar and Sabouraud Dextros Agar (SDA)), then incubated at (37 ° C) for a period of(24-48) hours. And for anaerobic, chocolate agar plates and diamond media, were kept at 35 to 37 °C for up to 48 hours in an incubator with 5% CO2. preparation and execution. Performance and preparation (Culture media) evaluations were carried out in accordance with the manufacturer's recommendations.

Identification of bacteria:

Isolated bacteria and yeast and parasite were recognized using traditional techniques like as (Gram stain, wet mount, colony morphology, germ tube test, motility, and biochemical assays, like catalase, coagulase, oxidase, Indole, citrate utilization, urease, Kligler iron, oxidase). Automated system (Marcy l'étoile, France; Vitek II, bio-Mérieux) and API 20 E (Analytical Profile Index System) .

pH of Vagina

By attaching a short piece of pH paper with a pH range of 1 to 14 to a pair of forceps and dipping it into the vaginal discharge in the lateral fornices, it was simple to measure the vaginal pH. Then, contrasted with the colors and associated pH values on a reference chart.

Whiff test

When a (10%) KOH is used to discharge and emit a foul smell as a helpful signal, the examination a whiff test is positive [23].

Microscopic tests

A wet amount approach is used in these tests to identify vaginal discharge. WBCs, bacteria, clue cells and *Trichomonasvaginalis* (Clue cells) are large vaginal epithelial cells coated in bacilli bacteria. Aerobic bacterial vaginosis is examined using the gram stain [24&25].

Candidiasis identification

By directly wetting the sample with 10% KOH, which kills the bulk of bacteria and clarifies the appearance of yeast cells or pseudohyphae, the presence of VC was verified. The sample was then cultured on Sabouraud's dextrose agar. For 48 hours, the plates were incubated at (37C) [26].Furthermore, Candida isolates were recognized by germ tube development to separate C. albicans from other non-Candida albicans species and by cultivating on chrom agar to categorize Candida spp., which distinguishes Candida species based on the distinct color that each one produces [27].

Trichomoniasis identification

Jerky movement of the organisms in a wet direct mount allowed investigators to recognize T. vaginalis infections. Giemsa and Leishman stains were used to create dried smears as well. Using compound microscopy at 40 and 100 times magnification, all slides were inspected [28].

Results& Discussion

160 vaginal swabs 106 (66.25%) was healthy women and 54(33.75%) swabs from infected women, the age from (15-50)years. 36-45 20(12.5%) was the maximum rate of infection, The lowest percentage was between the age range (15-25) (4.375%). this result agreement with [36].

Table1. The age distribution among healthy women and infected with genital tract infection

Table 1. Table title. Table captions should always be positioned *above* the tables.

Age group	Number of tested sample	Healthy women	Infected women
15-25	23(14.375%)	16(10%)	7(4.375%)
26-35	65(40.625%)	49(30.625%)	16(10%)
36-45	51(31.875%)	31(19.375%)	20(12.5%)
45 above	21(13.125%)	10(6.25%)	11(6.875%)
total	160	106(66.25%)	54(33.75%)

The number of pregnant 19(11.875%), were non pregnant women 141(88.125%). Number of diabetic women9(5.625%), were hypertension women16(10%), women with anemia 3(1.875%), women have ulcer 1(0.625%), polycystic ovaries1(0.625%) and abortion 1(0.625%).The study revealed that from 54 infected women the women with (illiterate) and (elemntary) education, wasof the top percentage 39(72.22%), whereas low rate of infection, was noticed in top educational level 15 (27.77%). In urban residence the infection rate, 33(61.11%) was significantly higher than, infection rate in rural 21(38.88%), were women with regular menstrual cycle 28(51.85%),and irregular menstrual cycle 19(35.18%), menopause 4(7.40%), sterility1(1.85%), Removal of the uterus 2(3.70%). In this study, alkaline PH samples were enrolled. The PH of vaginal discharge differs naturally during the menstrual cycle. When the PH level is less than 4.5, this indicates the absence of vaginosis. But when the PH level is greater than 4.5, it indicates the increased presence of pathogenic bacteria [31]. After the swab culture on the many culture, pure pathogenic isolates were characterized by (colonies morphology, diameter and shapes ,gramstain, and hemolytic reactions on blood agarplates). Identification of bacterial genus or species,was done by routine laboratory biochemical examinations suchas (oxidase, catalase, bacitracin, coagulase optochin), for gram-positive bacteria, for Gram-negative bacteria (indole production, gas production, H_2S production, urease utilization tests, citrate, motility, and fermentation of various carbohydrates, as well as the(Analytic Profile Index API) was used to confirm the results for the biochemical identification and by vitek-2systeum). The cultivated samples appeared 54 of total samples as positive results for bacterial and fungal growth that cultured on optimal culture media such as Blood agar, Mannitol salt agar, Chocolate agar, Sabouraud dextrose agar and Macconkey agar aerobicaly and anaerobicaly. The results,of the diagnosis showed differences, in the (numbers, types and proportions) of bacteria isolated. The results, reveal that from examined swabs of viagina, (50 isolates) were gram positive and 54 isolate gram negative. The main bacteria isolate was Escherichia coli and St.aureus. Escherichia coli made up 15(27.77%) represented the was the highest percentage of gram-negative isolates. This result converged with[38]. Staphylococcus aureus 16(32%), were isolated Which was closed to a study by[Farhan and Yaseen in Al Ramady city] [39], [40]. then *P.aeruginosa* 12 isolates,*Staph. Saprophyticus*9 isolate, *Enterobacter cloacae* 8 isolate ,*Staph. Epidermidis* ,*Staphylococcus haemolyticus* and *Klebsiella pneumoniae* 7 isolate, *Staphylococcus lentus* 4 isolate, *Klebsiella oxytoca 3 isolates, Streptococcus agalactia, citrobacter spp, Serratia marcesens*and *Pseudomonas putida* 2 isolate, Staphylococcus vitulinus,*Micrococcus luteus, Enterococcus fecalis, Gemella sanguini , Kocuria kristinae , Proteus mirabilis, G. viginals and Aeromonus spp*1 isolate,as shown in table 2.

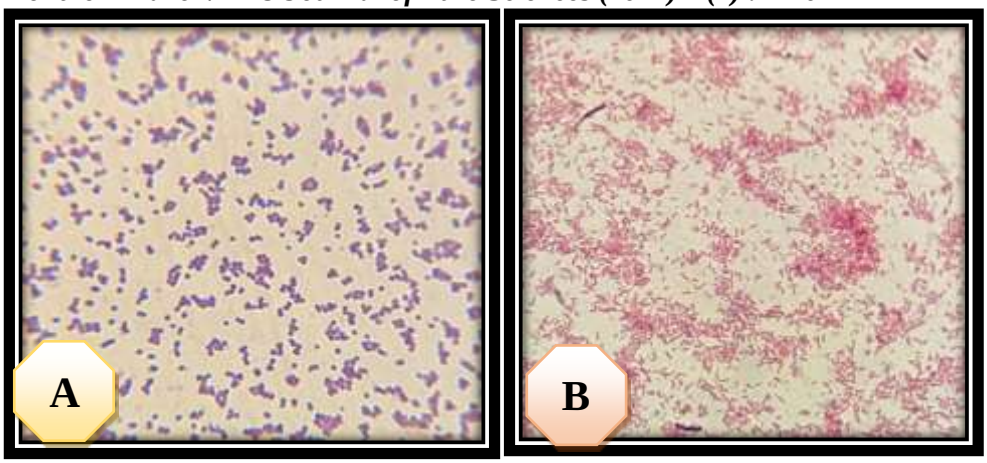
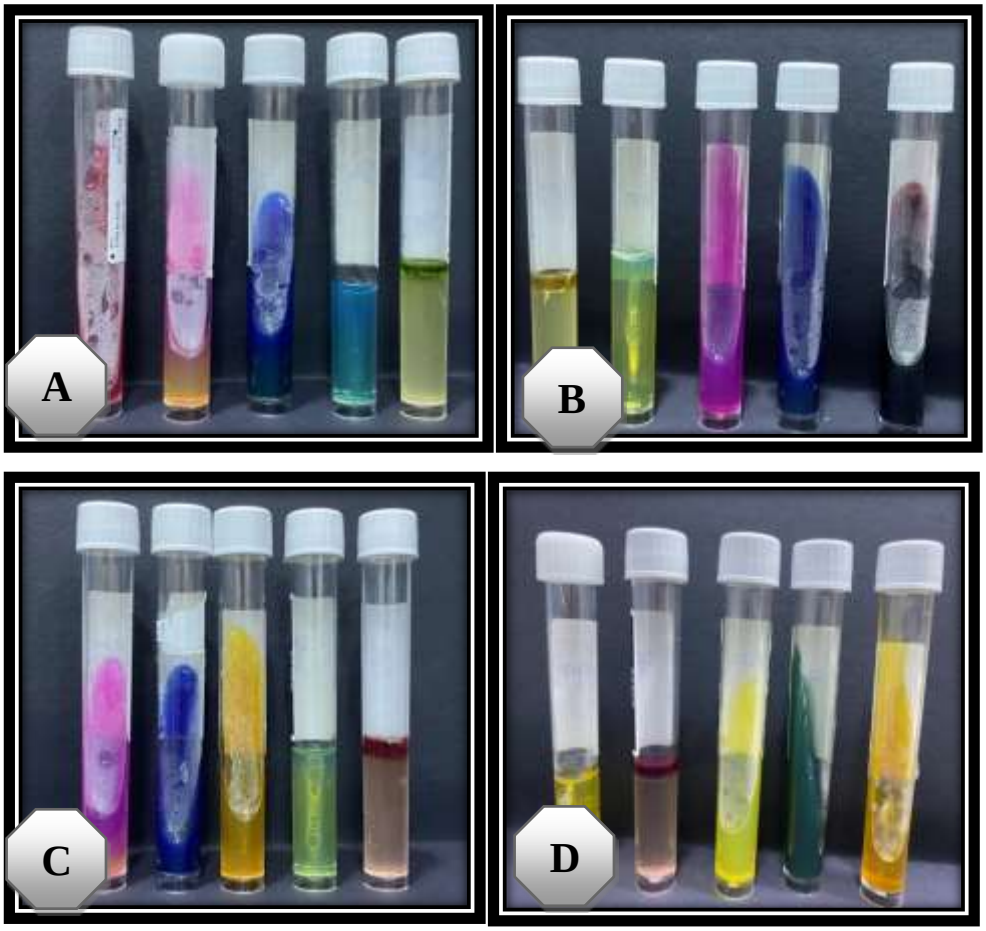


Figure1.Gram stain (A)gram positive ,(B) gram negative



Figuer2. Biochemical test for bacteria:(A) Pseudomonas aeruginosa, (B) Proteus mirabilis, (C) Klebsiella pneumoniae, (D)E.coli.

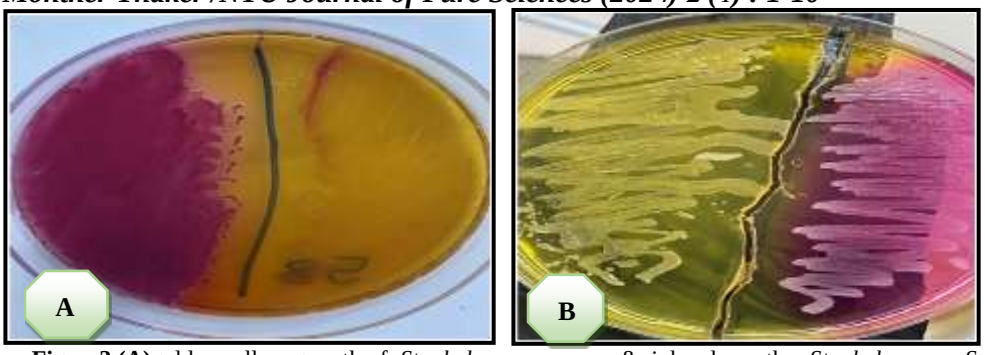


Figure3.(A)golden yellow growth of *Staphylococcus aureus*&pink color anther *Staphylococcus Spp.* on mannitol salt agar**.** (B)MacConkey agar pink color:lactose fermenting, colorless :non lactose fermenting

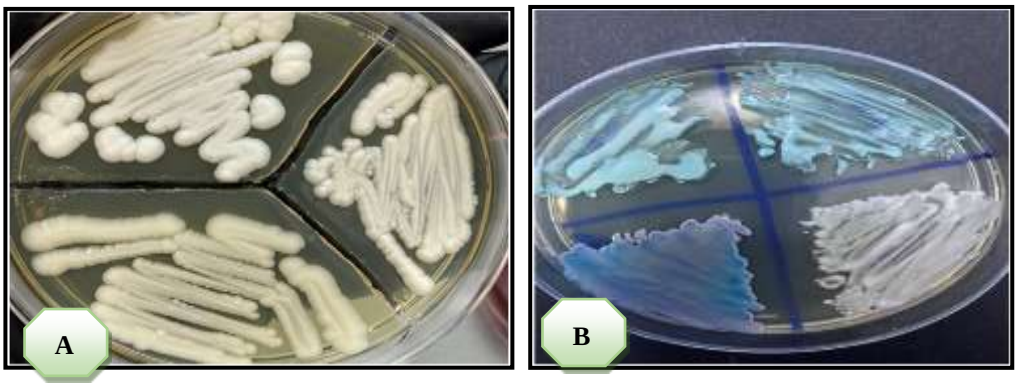


Figure4.Growth of Candida on(A) sabroued agar (B) chrom agar

In this study, identification of Candida clinical isolates was approved by the conventional mycological techniques, which contained: Microscopic characteristic of Candida spp., Cultural Characteristics, Germ tube formation, and Identification of Candida spp. by Candida Chrom Agar Medium and using (Vitek 2 compact system). Among candida isolates C.albicans 23(13.12%), Although, C. albicans is considered as a main causative agent of vaginal candidiasis and a main vaginal mycoflora [35,36&37],supports our result which had revealed that C. albicans with 53.64% was he chief causative agent of vulvovaginal candidiasis. Among 160 vulvovaginitis patients, a total of 8 (5%) instances of trichomoniasis were identified using direct wet mounts and dried smears stained with Giemsa stains. In this study, trichomoniasis prevalence (5%) was lower than that of the other vaginal illnesses. However, this prevalence was closely to the recorded by [38,39&40]. whereas it was lower than that of [41&42]. These discrepancies might be caused by changes in the sample size, diagnostic methods, personal and environmental cleanliness, socioeconomic status, research population, and sexual activity [43&44].

Figure 5. Trichomonas vaginalis by Direct wet mount .

In the current study, the prevalence of mixed bacterial infections with Candida spp. was examined, and it was shown that C. albicans co-occurred with S. aureus, S. epidermidis, S. saprophyticus, E. coli, and Klebsiella pneumoniae, but that no mixed bacterial infections with P. aeruginosa were found. Competition for nutrients, contamination from other places, or co-existence as secondary infections can all contribute to the coexistence of bacteria and candida. It was discovered that Saureus infections were the most common, which was consistent with the theory put forth by [29&45] that C.albicans encourage the growth of Staphylococci. Other microorganisms, such as E. coli and Klebsiella pneumoniae, had a lower incidence of co-infection with Candida. Our research demonstrated that neither a P. aeruginosa infection nor a combination infection existed. The above findings were also reported by [46], who established an inhibitory impact caused by P. aeruginosa product, such as pyocin pigment on C.albicans and proposed that, this inhibitory effect might be the cause of the absence of combination with P. aeruginosa..The results of the current research revealed that the percentages of infection with yeast were 28.2%, while the mixed infections between bacteria and yeast were 17.5%.The current results agreement with the study carried, out by [47].

Table 2. Numbers and Percentages of Isolated according to isolated species group

Pathogenic species	No. of isolate	Percentage (%)
Gram positive bacteria		
1. Staphylococcus aureus	16	32
2. Staph. Saprophyticus	9	18
3. Staph. epidermidis	7	14
4. Staphylococcus haemolyticus	7	14
5. Staphylococcus lentus	4	8
6. Streptococcus agalactia	2	4
7.Staphylococcus vitulinus	1	2
8.Micrococcus luteus	1	2
9.Enterococcus fecalis	1	2
10.Gemella sanguini	1	2
11.Kocuria kristinae	1	2
Gram negative bacteria		
1. Escherichia coli	15	27.777
2. Pseudomonas aeruginosa	12	22.222
3. Enterobacter cloacae	8	14.814
4. Klebsiella pneumoniae	7	12.962
5. Klebsiella oxytoca	3	5.555
6. citrobacter spp	2	3.703
7. Serratia marcesens	2	3.703
8. Pseudomonas putida	2	3.703
9. Proteus mirabilis	1	1.851
10.Aeromonus spp	1	1.851

11. Gardnerella vaginalis	1	1.851
Candida spp.		
C. albicans	21	13.12
C.tropicalis	1	0.625
C.krusei	1	0.625
Trichomonas vaginalis	8	5
Total	135	100
Mix infection	28	17.5

Conclusions

A sizable proportion of the women who participated in the current study were infected, primarily with bacteria, by one of the three etiological agents causing vulvovaginitis. For these patients, routine vaginal discharge culture must be carried out, and the medication susceptibility of bacterial isolates must be established. To minimize this disease, comprehensive healthcare education is required. It will take further research involving a large number of affected women to spotlight this serious condition that is treatable.

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