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The Valuable Microbiological Role of Vaginal and Cervical Swabs in The Management of Persistent and Recurrent Reproductive Tract Infections (RTIs)

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ABSTRACT

Background: Reproductive tract infections (RTIs) are a frequent medical condition that can lead to serious health issues such as infertility and newborn infections if it is not treated promptly.

Objective: This study aimed to determine the risk factors, demography, causative pathogens, and prevalence of RTI in women who are reproductively aged.

Subjects and methods: A prospective study was conducted among women who attended Maternal-Child Health Clinics. Vaginal/cervical swabs were examined, and relevant data were retrieved and then statistically analyzed. 295 women were included in the study with a median age of 25.2 years.

Results: At the time of the study, 84% of the patients were married. Overall, 42% of cases had *Trichomonas vaginalis*, 23% had *Neisseria gonorrhea*, 18% had bacterial vaginosis, 9% had *Candida* infection, and 22% of cases with symptoms had normal flora growth. Although the true frequency of mixed vaginal infections may be higher, only 15.6% of cases were reported to have them. Married women frequently had RTIs, and many of them also used IUDs.

Conclusion: In Middle Eastern nations, RTIs are highly prevalent among married women. *Neisseria gonorrhea* and *Trichomonas vaginalis* were the most frequently found bacteria in the current study. It has been shown that there was a poor correlation between the symptoms and certain types of laboratory-confirmed infections. This underlines the significance of laboratory testing as a key diagnostic tool for accurate RTI diagnosis and highlights the difficulty of diagnosing RTIs based solely on clinical judgment.

Keywords: Vaginal infection, Trichomonas, Genital tract, Reproductive tract infection (RTI).

INTRODUCTION

In the Third World, reproductive tract infections (RTIs) are a common medical issue. In addition to negatively impacting baby survival and health, their complications can seriously jeopardize women's health, het married life, fertility, reproductivity, and the efficacy of family planning programs if an early diagnosis and appropriate therapy are not received [(1 - 3)]**.** Research on the prevalence of RTIs in Middle Eastern countries has been hampered by low participation rates, and little is known about its rates, which exacerbates the issue and makes it worse. Women frequently suffer from RTIs in silence, and young married women in particular have a high prevalence of RTIs but rarely seek treatment due to numerous ethical and social issues [(4)]**.**

Microorganisms are attributed to two main groups of vaginal diseases: vaginal flora overgrowth and sexually transmitted infections (STIs). Vulvovaginal candidiasis (VC) and bacterial vaginosis (BV) are the most prevalent endogenous infections, but the three most common STIs are Chlamydia trachomatis (CT), Neisseria gonorrhoea (NG), and trichomonas vaginalis (TV) [(5 - 7)]**.**

Acute morbidity from RTIs can result in pelvic inflammatory disorders (PIDs), infertility, persistent pelvic discomfort, spontaneous miscarriage, stillbirth, low birth weight, and neonatal infections, among other consequences [(5, 8)]. Due to the numerous similarities in the presentations, a clinical diagnosis made solely based on symptoms described by the patient is nonspecific [(9)]. For better care of such instances, particularly when dealing with recurrent or persistent problems to reduce their subsequent complications, laboratory diagnosis is very crucial. The benefits of routinely using microbiological swabs among women who visit Maternal-Child Health and Family Planning (MCH/FP) Clinics complaining of medical symptoms related to RTIs are a topic of controversy. Some studies support the routine use of this procedure, while others show little benefit [(10)]. But, in general terms, it is recommended that clinical judgment should be used in conjunction with microbiological swab results to determine the most effective course of treatment [(11)].

Our study aimed to determine the laboratory-based prevalence of symptomatic lower genital tract infections among women who attended MCH/FP clinics and to determine the causative microorganisms, patients' symptomatology, demographics, and risk factors. In addition, evaluation of the importance of routine clinical implementation of vaginal/cervical swabs among those complaining women aiming to provide better effective reproductive health care for those patients.

Methods:

This study was a prospective study that was conducted during the period from November 2020 to November 2022. Two hundred and ninety-five patients were enrolled from the Gynecology Department of Ain Shams University Hospitals.

Inclusion Criteria: Women who attended Maternal, Child Health, and Family Planning (MCH/FP) Clinics

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complaining from RTI symptoms in whom microbiological vaginal/cervical cultures were performed, with available medical data upon their question and reviewing their electronic medical records.

Exclusion criteria: Women who attended MCH/FP Clinics complaining from symptoms other than RTI symptoms and those without available microbiological vaginal/cervical cultures results.

Sample Size: 295 women who attended MCH/FP clinics complaining of RTI symptoms.

Study Procedures:
Enrolled patients were subjected to the following: Detailed history taking (through patients' questioner and assessing medical records) and clinical examination including pelvic and speculum examinations. Provided vaginal and/or cervical swabs were subjected to full microbiological examination.
Data were analyzed for the following variables: demographic data, microbiological profile, complication, and the RTI risk factors for each patient.

Microbiology examination:
Pelvic and speculum examinations were done, and vaginal/cervical swabs were examined for 295 women with signs and symptoms of RTI. Two swabs were collected from most of the participants. Microbiology examination of the samples was done as follows.

- A macroscopic examination of the swabs with documentation of the color and nature of the discharge was done **(figure 1).**
- Then, microscopic examination of one of the two swabs by performing wet mount preparation to examine for *trichomonas*, monilial budding, or clue cells (A wet mount smear was prepared by the addition of one drop of saline on the microscopic slide and rolling of the swab surfaces over it then covered by a cover slide and examined by the expert microbiologist, different findings were reported and documented).
- Gram-stained smears for both pus cells and microorganisms. Identification were performed and reported.
- Then, the second swab was cultured on blood, MacConkey, and chocolate agar plates and incubated at 37 °C for 72 hours. The plates were checked daily for any significant growth and further microbiological workup was done according to each pathogen identification and according to standard operating procedures (SOPs) of our laboratory department. Different morphological growths of the isolated pathogens were identified and reported.

Ethical approval: The study underwent a thorough evaluation and received permission from The Research Ethical Committee, Faculty of Medicine, Ain Shams University (reference number. FM ASU R 350/2023). The study followed the code of ethics of the World Medical Association (Declaration of Helsinki) for studies that included humans.

Statistical Analysis: Statistical analysis was done on a personal computer using IBM, SPSS statistical version of 21 (IBM, Corp, Armonk, NY, USA). Data were collected, tabulated then analyzed using appropriate statistical tests. The D'Agostino-Pearson test was used to test the normality of numerical data distribution. Numerical data were presented as mean and standard deviation (if normally distributed) or as median and interquartile range (if skewed). Categorical data was presented as numbers and percentages or as ratios. P value was considered significant when its value was less than 0.05.

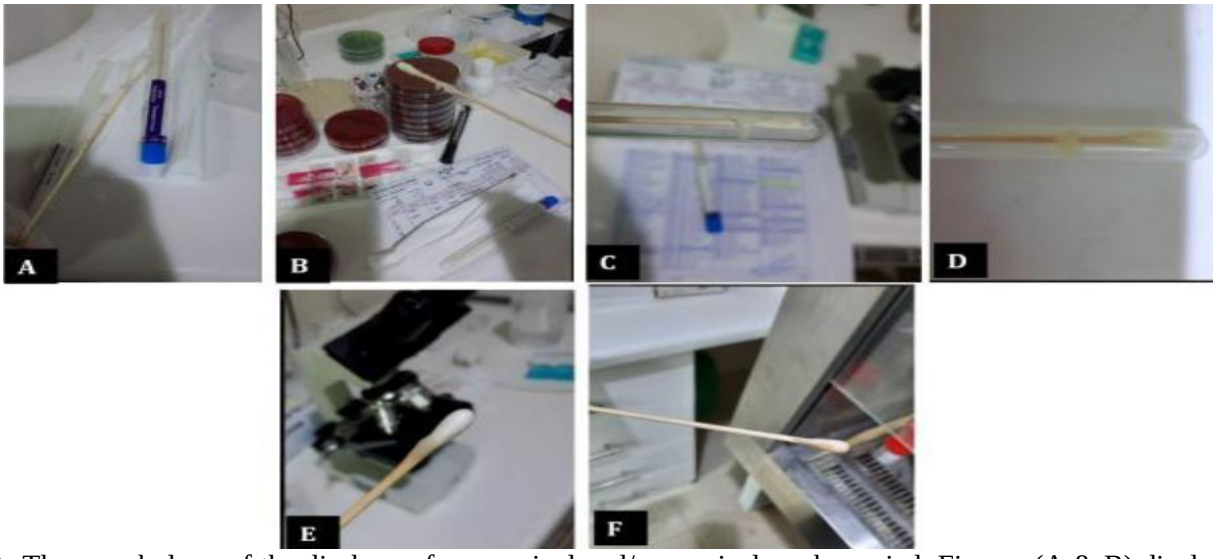

Figure (1): The morphology of the discharge from vaginal and/or cervical swabs varied. Figures (A & B) displayed a white, chalky discharge that was indicative of a candida infection. A significant amount of a deep yellow discharge, typically seen in cases of severe bacterial infections, was seen in Figure (1D). While figure (C) displayed an abundance of light-yellow discharge indicative of a trichomonas infection. Figure (1E) depicts a tiny amount of grey discharge associated with a bacterial vaginosis case. Figure (F) displayed a bloody-tinged discharge that was indicative of various infections, particularly severe cases.

RESULTS

Two hundred and ninety-five patients were enrolled from MCH/FP clinics. They were complaining from RTI manifestations. Regarding the demographic data of the studied group, their ages ranged from 15 to 55 years, most of them were adult-aged females with a median age of 25.2 years**.** Regarding the demographic data and its correlation with the prevalence of RTI; RTI was more prevalent in age categories 20 - 39 as 217 cases (73.5%) were documented among those groups of patients (Table 1 illustrated the demographic characteristics of the studied patients).

Table (1): Demographic characteristics of the studied group of patients

	Characteristics	Number (%)
Age (years)	Mean (±SD)	25.2 ±7.47
	Age (years)	
	⩽24	103 (34.6%)
	25-34	140 (47.5%)
	⩾35	52 (17.6 %)
Education	None/basic literacy	36 (11.8%)
	Primary school (1-6 years)	79 (26.8%)
	Secondary school (7-13 years)	80 (27.1%)
	University/Professional (>13 years)	100 (33.8%)
Marital status	Never married	22 (7.5%)
	Currently married	248 (84%)
	Widowed/divorced	23 (7.7%)
	Missing value	2 (NA)
Parity	Median (range)	2.0 (0; 10)
Abortion/ stillbirth (<12 months)	Yes	22 (7.6%)
	No	273 (92.5%)

84 % of included patients were married at the time of the study.

Using contraception is a well-known risk factor for RTI; (65%) of the participants were using contraception methods; 53% of them used an intrauterine device (IUD), 25% used hormonal contraceptive pills, and 22% of them used condoms. Sexual intercourse in the past 3 months is an additional risk factor for RTI as 195 of the studied cases were documented as positive (66.1%) while the remaining cases (100) were negative (33.8%).

From a microbiological point of view, the results of the microbiological culture were available for all selected cases, microbial isolates were documented in (230/295) (78 %), while the remaining patients showed growth of normal flora (65/295) (22%).

The overall prevalence of *Trichomonas vaginalis* was 42 %, *Neisseria gonorrhea* 23 %, bacterial vaginosis 18 %, and *Candida* spp. was 9 % **(figure 2).**

Mixed vaginal infections were documented in 15.6 % of cases, while the true incidence of mixed infections may be higher than that recorded by our study.

A poor correlation between the symptoms and certain types of laboratory-confirmed infections existed. *Trichomonas, Candida spp., and Neisseria gonorrhea* were all identified by comprehensive interpretation of all the findings together including wet mount, gram-stained smear results, morphologic characteristics, and microbiological culture results **(figure 3, 4 & 5).**

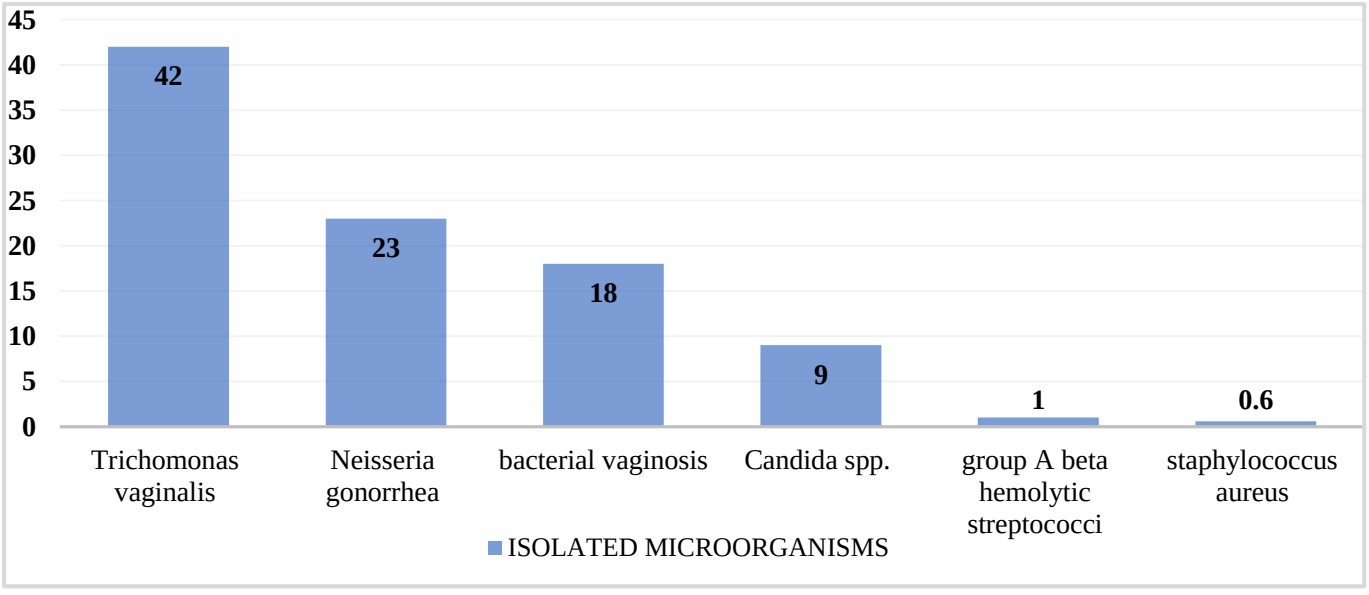

Figure (2): Percentage of isolated microorganisms from RTI patients

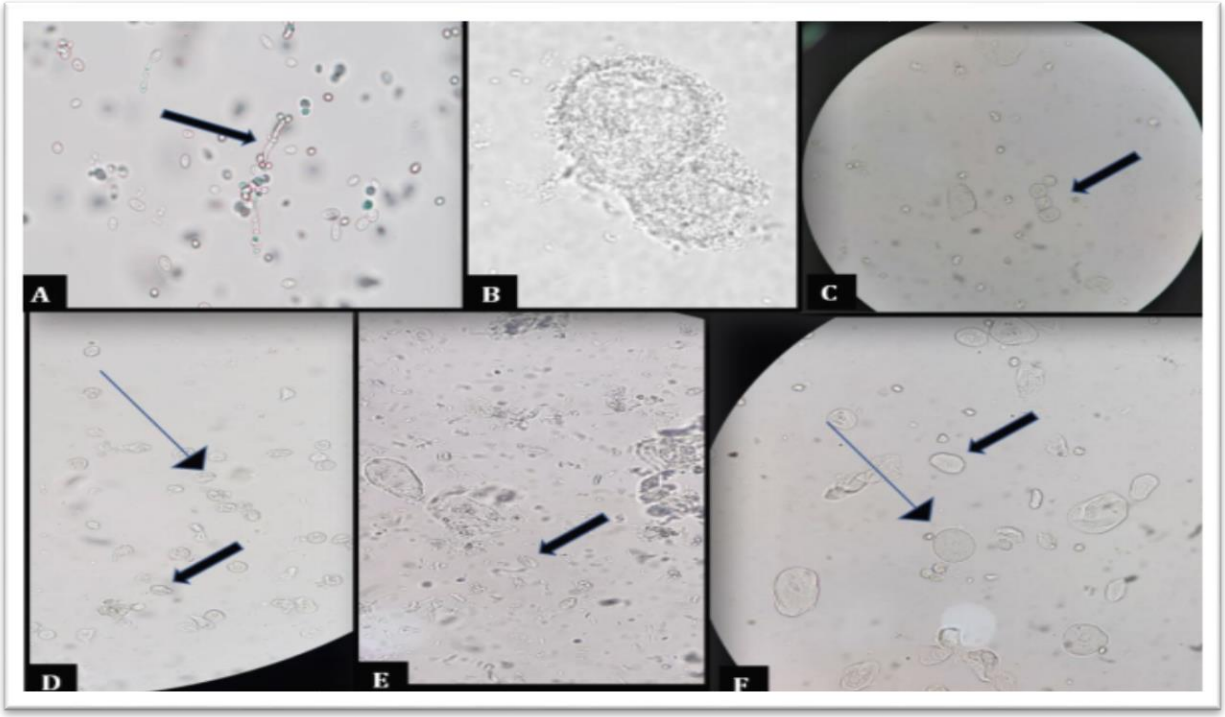

Figure (3): Figure (A)**:** Wet mount prepared smear from vaginal swab showing budding yeast cells and pseudohyphae (arrow) denoting candida infection, figure (B): Epithelial cells studded with a heavy number of small bacilli (clue cells) denoting bacterial vaginosis, figure (C): Trichomonas vaginalis appears as rounded cells characterized by its movement under the microscope (arrow points to 4 cells adjacent to each other denoting its replication by binary fission), figure (D): *Trichomonas vaginalis* appears as oblong cells (thin arrow) or pear-shaped cell (thick arrow), figure (E): *Trichomonas vaginalis* appears as oval shape with central bright line denoting its axostyle, figure (F): another morphology of *Trichomonas vaginalis* showing rounded large cells characterized by its movement (thin arrow), the surrounding undulating membrane can be evident in some of them (thick arrow).

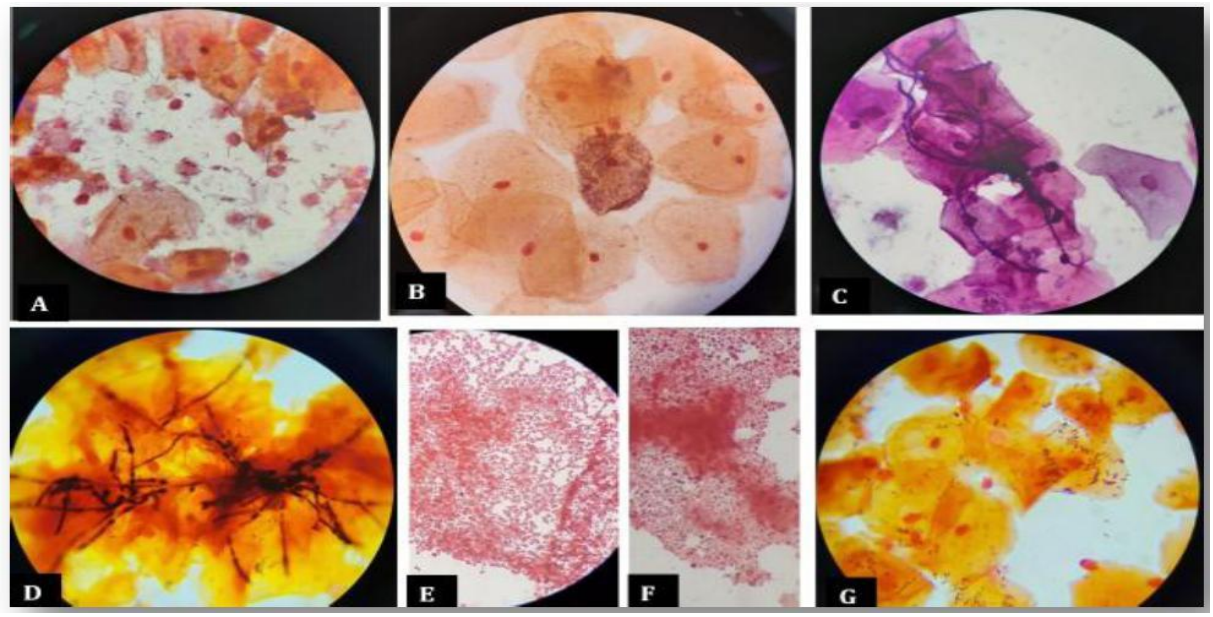

Figure (4): Fig (A): Gram stained smear showing a high load of pear-shaped homogenous gram-negative stained cells (denoting *trichomonas vaginalis* infection), figure (B): Many epithelial cells with single clue cell evident in the center of the film, figure (C): Gram-positive hyphae over the epithelial cells in the background of the film (denoting *candid*a infection), Figure (D): Heavy *candida* infection characterized by hyphae and budding yeast cells evident in the film, figure (E): Gram stained smear from the culture plate showing gram-negative cocci denoting *Neisseria gonorrhea*, figure (F): A higher magnificent of the same previous slide to see the characterized diplococci kidney shape morphology of *NG* and figure (G): Three oblong gram-negative cells denoting *trichomonas vaginalis* (from resolved case under treatment came for follow up purpose).

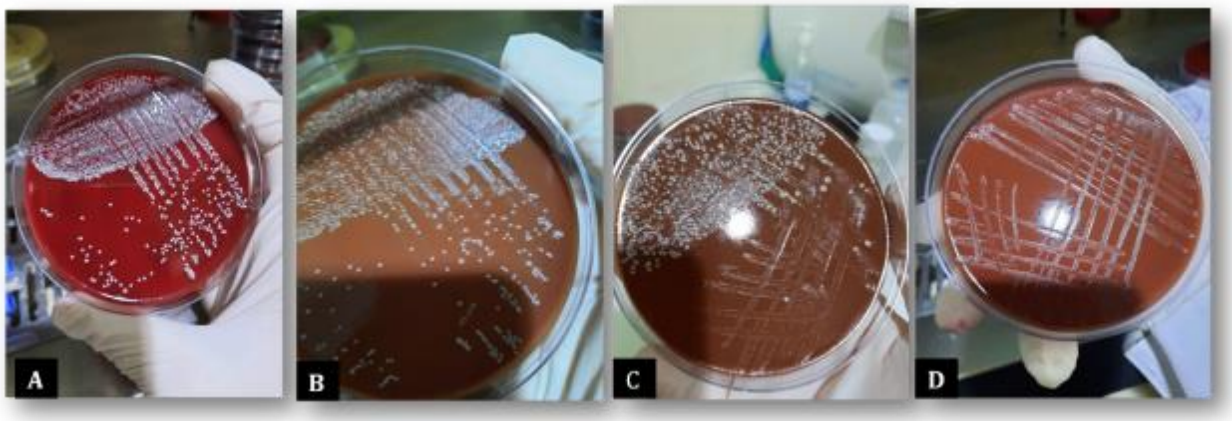

Figure (5): Figure (A): Blood agar showing pure heavy growth of white, creamy colored colonies (confirmed to be *candida*), figure (B): Chocolate agar showed pure heavy growth of rough white colonies with Foot-like extensions from the margin (confirmed also to be *candida* spp.), figure (C): Chocolate agar showed predominant heavy growth of minute translucent colonies (confirmed to be *Neisseria gonorrhea*) with moderate growth of white colonies (confirmed to *be diphtheroid* spp. considered as a part of normal vaginal flora) and figure (D): Subculture on chocolate agar to make *Neisseria gonorrhea* colonies separate colonies before performing antimicrobial sensitivity testing (AST) for the growth. Table (2) demonstrated the predictors of lower genital tract infection among symptomatic adult women of reproductive age, significant values are reported regarding younger age groups; age < 24 years, low level of education (education < 7 years), and sexual intercourse in the past 3 months.

Table (2): Predictors of lower genital tract infection among symptomatic adult women of reproductive age

characteristics	OR	95% CI	*P* VALUE
Sexual intercourse in the past 3 months	2.1	1.249-3.569	0.005
Age <24 years	2.1	1.073-4.185	0.031
Low level of education (less than 7 years).	1.6	1.000-2.775	0.050

Abbreviations: CI, confidence interval; OR, odds ratio.

DISCUSSION

RTI is a prevalent medical condition that regularly affects women who visit gynecological clinics. If left untreated, it can have serious medical repercussions, such as infertility and newborn meningitis. The majority of symptomatic women had not sought treatment until after a protracted period of complaint; the reasons given included the belief that their symptoms were typical, the false belief in our society that infections of the reproductive tract are stigma, the lack of a skilled female provider in the closest health care facility in certain situations, lack of privacy, distance from home, and financial aspects should also be put into consideration [(12)].

This study's primary aims were to determine the laboratory-based prevalence of symptomatic lower genital tract infections among women and to identify the most common microorganisms and risk factors linked to them.

The age of patients, social status, and sexual behavior of the populace all influence the occurrence of lower RTIs, which varies globally. Therefore, gathering local data on the prevalence of RTIs and related risk factors is crucial to enhancing the management and prevention of these diseases. These data are also helpful in assessing the efficacy of the subsequent interventions [(5, 7, 8)].

RTIs are common in young married women in Middle Eastern nations, although they hardly ever seek therapy. Outreach and education are required to lessen the stigma, humiliation, and ignorance around RTIs. Women's low rates of treatment for these diseases seem to be significantly influenced by their poor social status, particularly among young women. For improved patient treatment, suitable preventive initiatives based on Islamic principles should be put into place.

Trichomonas vaginalis reproduces by binary fission and is found in the male urethra and prostate, as well as the female lower genital tract. The parasite's sole known host is humans, to whom it is primarily transferred through sexual activity. It does not fare well in the outside world. [(13)]. A significant number of women in research studies with *Trichomonas vaginalis* infection or bacterial vaginosis describe tingling, itching, burning, or other nonspecific complaints but do not report abnormal discharge.

Van Der Pol *et al.* [(14)] reported that the most common documented symptom of *Trichomonas vaginalis* infected patients was abnormal discharge; patients described it as yellow, frothy, and odorous. However, in certain cases, this may not always be the case, and pain during coitus or urination was also a common symptom. The most common sign of bacterial vaginosis was an unusual discharge, which was usually thin, whitish-grey, and smelled like fish. There have also been reports of burning or itching in the vagina

during urination. With comparable outcomes, our current study confirms these documented clinical symptoms.

Memish *et al.* [15] found that the most common STIs reported in the Middle East with regard to RTI prevalence were HIV, trichomoniasis, and nongonococcal urethritis. Because STIs are socially stigmatized, the overall number of reported cases based on STI surveillance may be significantly understated. The authors suggested that suitable preventive initiatives that uphold Islamic principles be put into place.

Regarding yeast infections, the majority of affected individuals reported erythema, vulvar discomfort, itching, and a thick, white discharge that resembled curd. In addition, pain during urination and coitus was frequently mentioned. **Van Der Pol.** [16] reported that *Candida Albicans* cultured from 10% to 20% of women with no indication of disease as *Candida* is one of the vaginal flora**.** In the current study, the result of each case was correlated with the patient's symptoms and only the predominant *candida* growth was considered as an attributable pathogen.

Because the presentations of the various causative pathogens are so similar, clinical diagnosis based solely on patient-reported symptoms is nonspecific [7]. Therefore, laboratory diagnosis is essential as part of routinely examining the profile of women who complain of RTI clinical manifestations to obtain an accurate diagnosis and timely management.

The wet preparation for microscopic inspection is the most widely used diagnostic assay in the world for laboratory diagnosis of vaginal disorders. However, the sensitivity and specificity of microscopic examination are generally questioned. Factors that decrease motility, such as time factor, can decrease the probability of observation and consequently the sensitivity of clue cells, motile trichomonads, or yeast (buds or pseudohyphae), which should be taken into consideration and treated accordingly. In saline preparations kept at room temperature, trichomonads lose their motility rapidly within ten minutes, up to twenty percent of the organisms become non-motile [10]**.**

The presence of inflammation may also confound microscopic findings; pus cells and trichomonads are similar in size, thus the presence of inflammatory discharge may make it difficult to see *Trichomonas vaginalis* and examine the sample properly, particularly in a preparation that is not quickly analyzed. The results of our current study revealed that *Neisseria gonorrhoea* (NG) and *Trichomonas vaginalis* (TV) are the most common organisms isolated from the studied population. In contrast, **Ezeh *et al.*** [6] reported that endogenous infections such as vulvovaginal candidiasis and bacterial vaginosis (BV) are the most frequent RTIs.

The current study found that 42% of participants had *Trichomonas* infection, 23% had *Neisseria gonorrhea*, 18% had bacterial vaginosis, and 9% had *Candida* spp. While the true frequency of mixed infections may be higher than that reported by our study, mixed vaginal infections were reported in 15.6 % of cases. The findings of this study are consistent with **Van Der Pol.** [16], who found that in most communities, *trichomonas* infection is more common than *Chlamydia trachomatis* and *Neisseria gonorrhoea* combined. The study reported significant values for the following predictors of lower genital tract infection among symptomatic adult women of reproductive age: age < 24 years, low level of education < 7 years, and sexual intercourse in the previous 3 months. An additional risk factor for RTI acquisition is the use of IUDs as a method of contraception.

The detection of *Neisseria gonorrhoea*, *Chlamydia trachomatis*, and *Trichomonas vaginalis* infections using a single-use, quick, point-of-care PCR test demonstrated excellent sensitivity and specificity and may mark a significant advancement in the development of rapid diagnostics for sexually transmitted infections and other infectious diseases [17 - 19].

Several types of literature have reported that vaginal swabs were stable for up to 7 days at room temperature, and that *trichomonas vaginalis* PCR performed better diagnostic performance than routine diagnostics using microscopy for women and culture for men ($P > 0.05$). The assay also performed well for all sample types tested. *Trichomonas vaginalis* PCR diagnosis was made possible by incorporating samples and reagents from the *Chlamydia trachomatis* and *Neisseria gonorrhoea* PCR assay into standard clinical testing [19]. The financial concerns still need to be considered in regular practical situations, though. Furthermore, it is highly recommended that both men and women use a point-of-care (POC) diagnostic to establish universal rapid screening and surveillance for the most common non-viral STI caused by *Trichomonas vaginalis* and other commonly claimed microorganisms [20]. This will improve the diagnosis of RTIs with fast results. However, its diagnostic sensitivity and specificity in comparison with the actual number of infected cases will remain questionable.

CONCLUSION

RTIs are common in married women in Middle Eastern nations, and the prevalence of the bacteria varies by region based on patient age, socioeconomic status, and sexual behavior of the population. The most frequent pathogen linked to RTIs in the current study was *Trichomonas* infection. Less often diagnosed and reported infections were *Neisseria gonorrhea*, bacterial vaginosis, and *Candida* infections. The study identified 15.6% of cases with mixed vaginal infections. Nevertheless, the true rate of mixed infections may be greater. RTIs are common among married women of whom many use IUDs. It was evident that there was no correlation between the symptoms and certain types of laboratory-confirmed infections. This underlines the

difficulty in accurately diagnosing RTIs-based solely on clinical judgment and emphasizes the critical role that laboratory testing plays in reducing the number of misdiagnoses that lead to persistent or recurrent infections and the ensuing medical complications. For improved patient treatment, suitable preventive programs based on Islamic principles had to be put into consideration.

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