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Evaluation of RT-PCR in molecular diagnosis of Gardnerella vaginalis and Trichomonas vaginalis infection in comparison with other conventional method in Tikrit province

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KEY WORDS:
Gardnerella vaginalis , *Trichomonas vaginalis* ,RT-PCR , bacterial vaginalis, GTI .

ABSTRACT
Background: *Gardnerella vaginalis*, and *Trichomonas vaginalis* are the organisms most commonly associated with vaginitis; each can also be present in an asymptomatic state. *G. vaginalis* has been shown to be increased in patients with findings of a gray or homogenous discharge. While *T.vaginalis* is more prevalent in patients with symptoms, foamy gray or yellow discharge. *Gardnerella vaginalis i*s a bacterium that is commonly associated with bacterial vaginalis (BV). BV is a condition characterized by an overgrowth of certain bacteria in the vagina, disrupting the normal vaginal flora. **Aim of the study:** The aim of this study was isolation of *Trichomonas vaginalis* and *Gardnerella vaginalis* , and was to examine the prevalence and distribution of *G.vaginalis* in the vaginal ecosystem of pregnant women at two different stages of pregnancy (first and third trimester) . **Materials and methods:** From November 2023 to April 2024, 57 patient's urine samples and vagina swab were collected. In Tikrit city, pregnant women with infects the Genital tract infection (GTI) , visited Tikrit Teaching Hospital. The samples were divided into three parts , apart for microscopic examination (for *T.vaginalis*) ,part for culture (for *G.vaginalis*) and apart for RT-PCR (for *T.vaginalis* and *G.vaginalis*) . Result: present the percentage of samples positive on culture in vagina swap for *G.vaginalis* was 17(29.8%), including (15.8%)and (14%) isolated from (20-30) and (31-40) age and no result Growth in urine samples ,but the result in RT-PCR different between the types of samples (urine and vagina swab) were positive in urine samples is 43(75.4%) including 25 (43.8%)and 18 (31.6%) isolated from (20-30) and (31-40) age , and in vagina swab samples is 50(87.7%) including 30 (52.7%)and 18(35%) isolated from (20-30) and (31-40) age ,was no result for *T.vaginalis* . **Conclusion:** This study confirm that ages (20-30) are more affected than ages (31-40) due to sexual activity ,RT-PCR is more specific and sensitive way for diagnosis also the most common age for infection in (20-30).

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INTRODUCTION

Trichomoniasis is the most common sexually transmitted infection (STI) with an estimated annual incidence of 276.4 million cases globally and about 30 million cases in Africa[1]. According to the World Health Organisation (WHO) an estimated 276.4 million new cases of trichomoniasis occurred globally in 2008 making it more prevalent than *gonorrhea* and *Chlamydia*, however, this is predominantly in developing countries [2] *Trichomonas vaginalis*, and *Gardnerella vaginalis*, are responsible for 90% of vaginitis in Iran [3]. Trichomoniasis has been found to be associated with various health complications including pelvic inflammatory disease (PID), significant pregnancy complications, cervical cancer, prostatitis, infertility and the acquisition of human immunodeficiency virus (HIV)[4]. Both *Gardnerella vaginalis*, and *Trichomorns vaginalis* are the organisms most commonly associated with vaginitis; each can also be present in an asymptomatic state. *G .vaginalis* has been shown to be increased in patients with findings of a gray or homogenous discharge. While *T.vaginalis* is more prevalent in patients with symptoms, foamy gray or yellow discharge[5]. *Trichomonas vaginalis* is a flagellated protozoan parasite which has a simple life cycle without necessity an intermediate host, described by the presence of only trophozoite stage that infects the genital tract of the human female and male, and there is no cystic stage. Sexual contact is the most common method of transmission of the *T. vaginalis* between people [1]. *T. vaginalis* is also a common parasitic pathogen of sexually transmitted diseases (STDs)[6]. *G. vaginalis* are gram-negative to gram-variable, small, pleomorphic rods that are non motile and do not possess flagella, endospores, or typical capsules[7]. Bacterial vaginosis (BV) is the most prevalent cause of symptomatic vaginal discharge and is associated with complications of reproductive health, such as preterm birth and acquisition or transmission of sexually transmitted infections, including human immunodeficiency virus infection (STI) [8] *Gardnerella vaginalis* is a bacterium that is commonly associated with bacterial vaginalis (BV). BV is a condition characterized by an overgrowth of certain bacteria in the vagina, disrupting the normal vaginal flora. *Gardnerella vaginalis* is one of the major organisms involved in BV. Symptoms of BV include a thin, grayish-white vaginal discharge with a distinct "fishy" odor, vaginal itching, and irritation[9]. The diagnosis of *T. vaginalis* infection by RT-PCR is a sensitive and specific method that could be incorporated into a joint strategy for the screening of multiple STDs by using molecular amplification methods[10]. *G. vaginalis* are gram-negative to gram-

variable, small, pleomorphic rods that are non-motile and do not possess flagella, endospores, or typical capsules[11].

G vaginalis has been shown to be increased in patients with findings of a gray or homogenous discharge, *Gardnerella vaginalis* is a facultative anaerobe and uses carbohydrates as its major energy source, this microorganism has a fermentative metabolism and acetic acid is a major product [12].

The diagnosis of *G.vaginalis* and *T.vaginalis* infection by RT-PCR is a sensitive and specific method that could be incorporated into a joint strategy for the screening of multiple STDs by using molecular amplification methods [13] ,This study aims to study the effect of sampling on the results as well as the most accurate diagnostic method in Tikrit Teaching Hospital .

MATERIAL

Collection of samples

Through the period from November 2023 to April 2024, 57 clinical samples of urine and vagina swab were collected from patients who Pregnant women with Genital tract infection(GTI) attending to Tikrit teaching Hospital, in Tikrit city . By using sterile equipment's and media, then samples were transferred to the lab for isolation and identification of *Gardnerella vaginalis* and *Trichomonas vaginalis* .

A total of 57 vagina swab and urine samples isolates were obtained from Tikrit teaching hospitals in Tikrit city. The initial identification of *Trichomonas vaginalis* of General urine examination and wet mount microscopy , bacteria *Gardnerella vaginalis* growth on Columbia blood agar, the colonies appear smooth, small colonies (hear pin) , The *G. vaginalis* is a fastidious organism that needs an enriched medium for culture; in this study, Columbia agar that was supplemented with antibiotics and blood was used to promote the growth of this organism. [11] Growth on Columbia agar medium this medium was used for the isolation of *G.vaginalis* from clinical specimens, it consists of :

- Columbia blood agar base.
- Human blood 5 %.
- *G.vaginalis* selective supplement.

Preparation of *G.vaginalis* selective supplement It was made by dissolving one vial of antibiotic (2 mg Gentamicin sulphate, 15 mg Nalidixic acid, 1 mg Amphotericin B) in 2 ml distilled
Water.

Detection *G.vaginalis* and *Trichomonas vaginalis* from vagina swab and urine samples by RT-PCR .

RESULTS

The collected samples from Pregnant women at two different stages of pregnancy (first and third trimester).were distributed into two groups according to age and type of samples urine and vagina swab samples . As shown in Table (1).

Table 1: Demographic distribution of pregnant women according to the age .

Age	Total
20-30	36 (63.2%)
31-40	21 (36.8%)
Total	57 (100%)

Isolation and diagnosis of *G.vaginalis and T.vaginalis* .

The present study showed that *Gardnerella vaginalis* growth on Columbia blood agar isolation from vagina swab but from urine no growth in culture , the colonies appear smooth, small colonies (hear pin) , The *G. vaginalis* is a fastidious organism that needs an enriched medium for culture Figure (1). The characteristic of G.vaginalis Gram stain and lactose fermentation on MacConkey agar, hemolysis on Colombia blood agar, catalase , indole tests , sucrose , Methyl red test ,Voges Proskauer ,inulin and Mannitol is variable but oxidase , citrate , urea hydrolysis and sorbtol is negative [13] .

Trichomonas vaginalis is a flagellated protozoan parasite which has a simple life cycle without necessity an intermediate host, described by the presence of only trophozoite stage that infects the genital tract of the human .

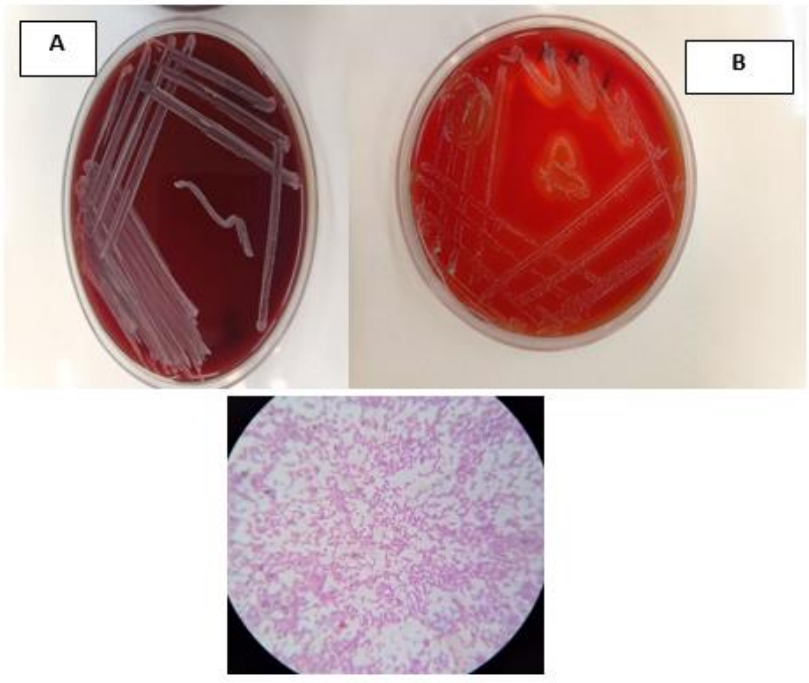

Figure 1: *Gardnerella vaginalis* growth on Columbia blood agar, the colonies appear smooth, small colonies (hear pin) . (A); A. non hemolysis colonies , B. β-Blood hemolysis colonies , on Columbia blood agar after incubation at 37° C for 24 hrs.(c): colonies microscopically diagnosis .

Table (2): Distribution of culture growth of *G.vaginales* isolation according to the age

Age	No. positive	No. negative	Total
20-30	9 (15.8%)	27 (47.4%)	36 (63.2%)
31-40	8 (14%)	13 (22.8%)	21(36.8%)
Total	17 (29.8 %)	40 (70.2%)	57 (100%)

Results RT-PCR

MOLECULAR DIAGNOSIS

DNA Extraction

kit (DNA extraction kit (Macrogen / Korea)) was used in DNA extraction from all frozen high

vaginal swabs and urine .

Detection of Specific Gene Markers By RT-PCR

The primer and probes was used to detection primer listed in Table (4)

Reaction mixture contained 5µl of 2×Taq Man qPCR Master Mix , 0.5µl of forward primer, 0.5µl of reverse primer, 5µl of extracted DNA and 12µl of nuclease free water. These RT-PCR Master mix reaction component placed in standard PCR tubes containing the PCR PreMix as lyophilized materials containing all other components needed to PCR reaction such as (2×Taq Man qPCR Master Mix).

Table (3) RT-PCR reaction system:

Reagent	50µl	Final Conc
2×Taq Man qPCR MasterMix	25µl	1×
Forward Primer, 10µM	1µl	0.2µM[1)]
Reverse Primer, 10µM	1µl	0.2µM[1)]
Probe, 10 µM	1µl	0.2µM[2)]
DNA template 2µl[3)]	2µl[3)]	
50×Low ROX or High ROX(optional)[4)]	1µl	1×
ddH_2O	up to 50µl	

Table (4) Primers and probes.

Name	Probe		Primer sequence (5′-3′)	Annealing	Product	Length	Tm
G.vaginalis	FAM	F	TGGTGTTTGGTGGTTTGAGA	58	226	20	52.4C^0
		R	TCCTGTCTACCAAGGCATCC				
T.vaginalis	TAMRA	F	CAGGGAGAATCACCTCCAGA	58	234	20	52.4C^0
		R	CTTGGCAGGATCAACCAAGT				

F =Forward \ R =Revers

Table (5): Distribution of *G.vaginales* isolates from Vagina swab according to the age by RT-PCR .

Age	No. positive	No. negative	Total
20-30	30 (52.7%)	6(10.5%)	36 (63.2%)
31-40	20 (35%)	1 (1.8%)	21 (36.8%)
Total	50 (87.7%)	7 (12.3%)	57 (100%)

Table (6): Distribution of *G.vaginales* isolates from Urine according to the age by RT-PCR .

Age	No. positive	No. negative	Total
20-30	25 (43.8%)	11 (19.4%)	36 (63.2%)
31-40	18 (31.6%)	3 (5.2%)	21 (36.8%)
Total	43 (75.4%)	14 (24.6%)	57 (100%)

Table (7): Distribution of culture growth of *G.vaginales* and RT-PC

	Culture Growth	Number	Mean ±SD.
Pregnant	Positive	17	24930 ± 4.680a
	Negative	40	22.236 ± 4.664 a
	P-value		N.S 0.310

*The some letters mean no differences between them **.**

Table (8): Distribution of *Gardnerella vaginalis* according to the age diagnosis by RT-PCR

	Age	**Number**	**Mean ± SD**
Pregnant	20 -30	36	23.265 ± 3.854 ab
	31 -40	21	23.200 ±5.830 ab
Total	-	57	23.076 ±4.752 a
	P-value		*0.058

*The some letters mean no differences between them .

Detection of *T.vaginalis* from vagina swab and urine by microscope and RT-PCR according to the age (21-40) in 57 pregnant woman . No result for *T.vaginalis* .

DISCUSSION

In healthy women of reproductive age, the vaginal microbiome is characterized by low bacterial diversity, often dominated by different species of the genus Lactobacillus [14][15]. However, when a profound change in bacterial profiles occurs, a dysbiogenic condition called bacterial vaginosis (BV) emerges.) may arise. BV is characterized by depletion of lactobacilli, combined with the growth of a polymicrobial community of anaerobic species [16] Among these species, *Gardnerella vaginalis* (*GV*) is thought to play a major role in the pathogenesis of bacterial vaginosis [17]. *GV* has a great ability to form a biofilm that serves as a scaffold on which other anaerobic species, such as *Atopobium vaginae* and *Prevotella* spp., can To stick to it later [18][19].

Although increased levels of BV-associated bacteria in the vaginal microbiome can negatively impact maternal and neonatal health, assessing the distribution of the *GV* clade during pregnancy could deepen the pathogenesis of dysbiosis and open the way to new strategies for preventing obstetric complications (eg, preterm birth) [20][21].

the aim of this study was to examine the prevalence and distribution of *GV* in the vaginal ecosystem of pregnant women at two different stages of pregnancy (first and third trimester).

The samples were analysed to determine the prevalence of *G.vaginalis* isolates causing Genital tract infections (GTIs) in pregnant women , and diagnosed by culture and RT-PCR ,compare results between urine and vagina swab samples ,results were different between samples.

Many samples were taken in the third trimester of pregnancy because the patient refused to give samples in the first and second trimester .

The results were of culture from vagina swab (29.8%) ,but on result of growth culture from urine , and RT-PCR in urine samples (75.4%) , in

vagina swab samples (87.7%) . The current study apparently showed that age plays a non- significant role in infection. The present results showed high percentage of infection with *G.vaginalis* was within the age group of 20-30 years (15.8%) and rate occur in age group 31-40 years, (14%) . This is due to being the most sexually active age group and hormones reach their highest levels, as well as this age group considered as childbearing age .

The results of this study were comparable with the results obtained by,Al-Sultany,2012 [25] found the frequency of *G. vaginalis* was (27.5%) from the culture in Babylon city, Gatea *et al.*,2020 [26] found that the isolation rate of *G.vaginalis* was (25.33%) obtained from miscarriage and non-miscarriage vaginosis women in Al-Hillah city by utilizing the culture method , Al-Dhalmi ,2013[22] found *G. vaginalis* at a rate of (10%) in the city of Al- Kufa and, Al-Muk and Hasony.,2001 [24] , found that the *G.vaginalis* isolates in a prevalence rate of (7.7%) were detected in Basrah city , while more than the results obtained by other researchers in Iraq, Al-Alwani .,2008 [23], , who conducted research in the city of Al-Ramadi, the frequency among the culture was 27 %, but less than the result abtained by Nisha *et al* ,2019 [27]who found that 83.3% of the isolates of *G. vaginalis* came from women with BV , Ali et al ., 2017 [28] who reported that *G. vaginalis* was isolated from (50%) of infection cases in Baghdad city, This variation may be result from the geographic distribution, sample type, number of tested samples and uptake of the antibiotic . The difference in the prevalence sexually transmitted pathogens could be caused by a number of reasons; including environmental and socio- economic factors, accurate an gynecologist takes a sample from a patient, the method of diagnosis, the number of tested samples, the type of samples, and cultural factors Ali *et al* .,2017 [28]. The current results showed that women in the (20-30) and (31-40) age groups were more susceptible to infection, this finding may be related to the fact that most women have high rates of reproduction at these ages because reproductive activity is more active at these ages than at other ages, with hormonal changes acting as the primary causative factor and sexual activity. Yuk et al .,2021[29] found the percentage of patients who tested positive for *G. vaginalis* was highest in the age group of (40-49) years old (37.8 %), followed closely by the age groups of (< 19) and (50-59) years old.

The results of *T.vaginalis* is (0%) no result ,not compatible with everyone , results of Nasir *et al* .,2022 [30], who indicated that the highest frequency of positive samples were detected in subjects 20-30 years of age (13.2 %), and Taher *et al* .,2018 [31], who found high prevalence rate (20.3%)

of *T. vaginalis* infected women occur in 25-35 years age group in compared with other age group. Also the present results not similar to Nsagha *et al* .,2015 [32] who showed higher infection rate in age group 26-40 years (59.0%). The
infection rate in present study was not agreed with the percentage mentioned in the report of the World Health Organization, where it was found that 3 million cases of *T. vaginalis* in the United States of America, including 2 million cases of *T. vaginalis* from the age of (15-24) years, with a percentage (66.62%).

This result also compared with some other studies Tompkins *et al* .,2020 [33] which showed that the prevalence of *T. vaginalis* infection did not differ by age category . found high rate of *T. vaginalis* infection occur 17-26 years age group, reaching 86%. While Sutton *et al* .,2007 [34], showed a higher rate (2.8 %) of infection among young women with the age of 18–25 years and above in the population. However, the parasite infected women in all reproductive-age and the prevalence of the infection is unknown Sutton *et al* .,2007 [34].

. These results not similar to the results off AL-Khalidy and Al-Abodi ,2020 [35], who found that the spread of the *T. vaginalis* infection in women with delayed pregnancy in Al-Qadisiyah
province was (25.5%). But the present results less prevalence than the results of Al-Tikrity and Al-Badry ,2014 [36], who detected that the Trichomoniasis was (7.2%) in women who revisions the tack care of family unit in AL-Liqaa hospital in Baghdad, and Tahher *et al* .,2018 [31], who found 22 (14.3%) of examined women were infected with *T. vaginalis*. Also this rate is less than that obtained by an earlier study off Al-Saeed ,2011 [37], which showed 5.4%
infection rate among 425 women in Dohuk province, north Iraq. However, the
present findings was lower than infection rate recorded by study of Jarallah ,2013 [38] for women who live in marsh villages of Al-Basrah governorate south of Iraq as researcher noticed that the total prevalence of Trichomoniasis infection was (57.85%), and Al-Hussuny ,2015 [39] who showed the prevalence of Trichomoniasis infection was (53.0%) through examination 120 vaginal samples for Al-batool educational hospital in Baquba city /Diyala governorate. Higher rate of infection was recorded in Baghdad (85.5%) Sahebet al .,2016 [40]. The disparity in infection rates of *T. vaginalis* among different studies are likely due to some influences, including sample size, variation in selection of the enrolled study population, diagnostic method used, the skill of the investigator, living circumstances, socio-economic

conditions, and immunological standings.

Moreover, *T. vaginalis* infection depends on many other factors, including age,

sexual activity, association of other STIs, and period of menstrual cycle Abdul-Jabbar and Al-Warid .,2021[41].

CONCLUSION

Moleculer diagnosis was more accurate than culture in diagnosis of *G.vaginalis* and *T.vaginalis* .

The swab samples is more accurate than urine samples for culture and RT-PCR

Were ages(20-30) more affected than other ages

CONFLICT OF INTEREST

Non.

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Non.

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