Investigation of trichomoniasis among Women from Basra Province, Iraq, using the PCR technique.

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Abstract:

An important cause of sexually transmitted diseases, *Trichomonas vaginalis* is a parasite that inhabits the vaginal cavity. Several ways (direct contact, or using unhealthy public toilets) can spread trichomoniasis to children, adults, and pregnant women. This study aims to compare and determine between (microscopic observation of motile parasite in wet - mount preparation, hematoxylin-eosin staining (H&E) and polymerase chain reaction technique (PCR). A case control study was conducted on 230 women aged from 20 to 60 years. They were divided into two groups (155 patients' groups and 75 control groups without infection symptoms) in the period from November 2020 to July 2021 at the main center for gynecology and obstetrics in the city. ″Basra Maternity Hospital″.Our study revealed that *T. vaginalis* were detected in 10 cases (38.5%),7 cases (26.9%) and 20 cases (76.9%) by wet smear preparation, hematoxylin-eosin-staining and PCR method respectively. The sequences of *T. vaginalis* in the present study have been recorded at GenBank (NCBI) with accession numbers OM925552, OM925553, and OM925554.This study found that PCR was most effective for detecting *T. vaginalis* infection. Therefore, PCR may represent the best option for definitive laboratory diagnosis for genital infections.

Keywords:

PCR technique, (H &E) stains, NCBI, trichomoniasis.

Introduction

An important cause of sexually transmitted diseases, *Trichomonas vaginalis* is a parasite that inhabits the vaginal cavity (1,2). Several ways (direct contact, or using unhealthy public toilets) can spread trichomoniasis to children, adults, and pregnant women. (3). Women suffering from this condition may experience cervical neoplasia, infection, adverse pregnancy outcomes, and infertility due to

atypical pelvic inflammatory disease. (4, 5). Men can suffer from non-gonococcal urethritis caused by it, which contributes to male factor infertility (6). Other symptoms, the patient also feels itching, burning, dysmenorrhea, and abdominal pain when urinating. (7) Displaying small spots of hemorrhages on the membrane (8). Since it is difficult to understand the disease, trichomoniasis initiates from simple symptoms but can development to acute illness, resulting in one or more health problems. Study objectives were to compare and determine between (microscopic observation of motile parasite in wet - mount preparation, hematoxylin-eosin staining and polymerase chain reaction (PCR).

The study participants

A case control study was conducted on 230 women aged from 20 to 60 years. They were divided into two groups (155 patients' groups and 75 control groups without infection symptoms) in the period from November 2020 to July 2021 at the main center for gynecology and obstetrics in the city. ″Basra Maternity Hospital. It excluded patients with vaginitis or cervicitis who were receiving treatment.

Laboratory methods:

Three different laboratory methods were used to examine swabs from the women. A wet mount was generated using the first swab after mixing with normal saline for direct microscopic examination. A further staining of hematoxylin-eosin was performed on the smears from the first swab after fixing them with 70% ethanol. [9,10]. To prepare the third sample for PCR, it was placed in 1 ml of sterile PBS and stored at -20°C.

Polymerase chain reaction: As part of the study, DNA was extracted from a vaginal smear using Promega kit (ReliaPrepTM Blood gDNA Miniprep System) and the concentration and purity of DNA were calculated using Nanodrop (Nano Drop thermo scientific 200/USA), with 260 – 280 nm ratio (11). Technique of The PCR and DNA sequencing methods were used to identify the Strains of parasites *T. vaginalis* spread in Basra Governorate, southern Iraq using the gene *(T. vaginalis* beta-tubulin gene) with a molecular weight (505 base pair) shown in a table (1), The primer used in this study was designed using the NCBI Gene Bank online database were synthesized at Bioneer, Korea.

***T. vaginalis* beta-tubulin (btub) gene, complete GenBank: L05468.**

Forward primer sequence:	**CACAACACCAACATACGGCG**

Reverse primer sequence:	**CTGGAACTGGGAGTCGACAC**

1. Results:

Results of the concentration and purity DNA of measurement

The findings of Nanodrop's DNA analysis show that the ratio 260/280 was 1.78– 1.90, It is located Within the normal range of 1.7-2.0 which was detected by Nanodrop's. Results of concentration and Purity of (*Trichomonas vaginalis*) in the ratio 260/280 show in the table (1).

Table (1): concentration and Purity of sample in the ratio 260/280.

Sample	Concentration	Purity
1	57.2	1.90
2	45.6	1.86
3	38.5	1.77
4	58.1	1.94
5	45.7	1.88
6	48.2	1.87
7	39.7	1.84
8	53.5	1.91
9	44.2	1.85
10	48.7	1.88

Demographic data

Results revealed that *T. vaginalis* were detected in 10 cases (38.5%),7 cases (26.9%) and 20 cases (76.9%) by wet smear preparation, hematoxylin-eosin-staining and PCR respectively.

Diagnosis	**No. of positive**	**%**
Wet preparation	10	(38.5%),

H&E stain	7	(26.9%)
PCR	20	(76.9%)

Amplifying PCR

The results of the current study shown the amplification process of DNA extracted from vaginal secretion samples after electrophoresis in the agarose gel, under this study with the primer beta-tubulin gene of *T. vaginalis* at a molecular weight of 505 bp. Figure (1) the sample A, C, D and F show the positive results, while B, E and G show the negative result, L is DNA Ladder to compare results.

Molecular identification of Trichomoniasis etiological agent among women.

A PCR product of 505 bp was successfully amplified using the mentioned primer set in the section of materials and methods from 30 samples randomly selected from the four samples enrolled in this study (Figure 1).

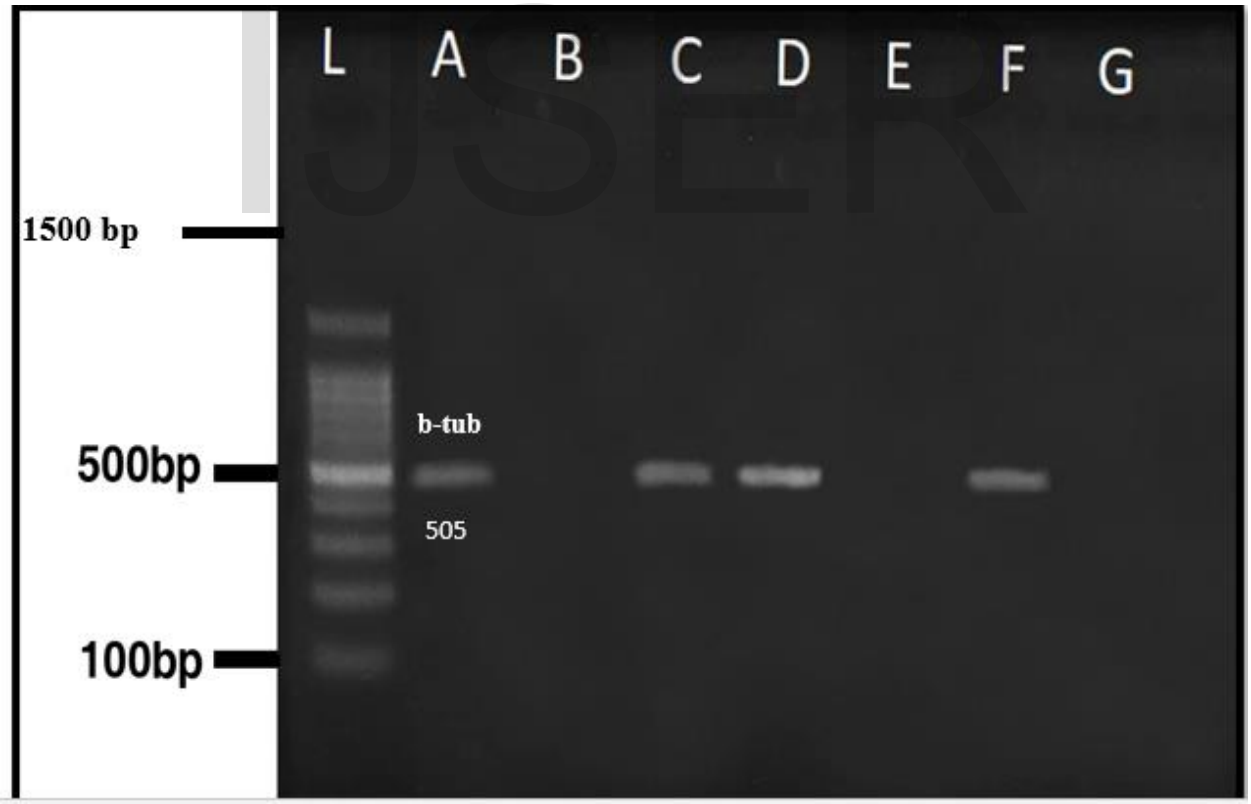

Figure (1): Agarose gel electrophoresis (1.5%) showing the PCR product of beta-tubulin gene, partial cds from 10 samples randomly selected. Lane L DNA ladder.

Lanes 1-10: PCR product of beta-tubulin gene, partial cds of 505 bp from 10 randomly selected samples.

The molecular identification using beta-tubulin gene, partial cds succeeded to confirm the identity of the etiological agent of Trichomoniasis in women as T. vaginalis. The four randomly selected samples out of fifty samples proved to carry T. vaginalis with a frequency of occurrence of 97-100%. Moreover, the three nucleotide sequences of the ten strains of T. vaginalis were deposited in GenBank under the following accession numbers: The three strains were nominated as T. vaginalis isolate Sq1, Sq3 and Sq7 beta-tubulin gene, partial cds (OM925552.1, OM925553.1and OM925554.1.

The phylogenetic tree constructed by MEGA11.1 Sequence is presented in Figure (2). The three isolates of *T. vaginalis* identified in this study were distally related to each other. Moreover, these isolates were distally related to some of beta-tubulin gene, partial cds sequences of *T. vaginalis* retrieved from GenBank as representative examples of *T. vaginalis* genotypes.

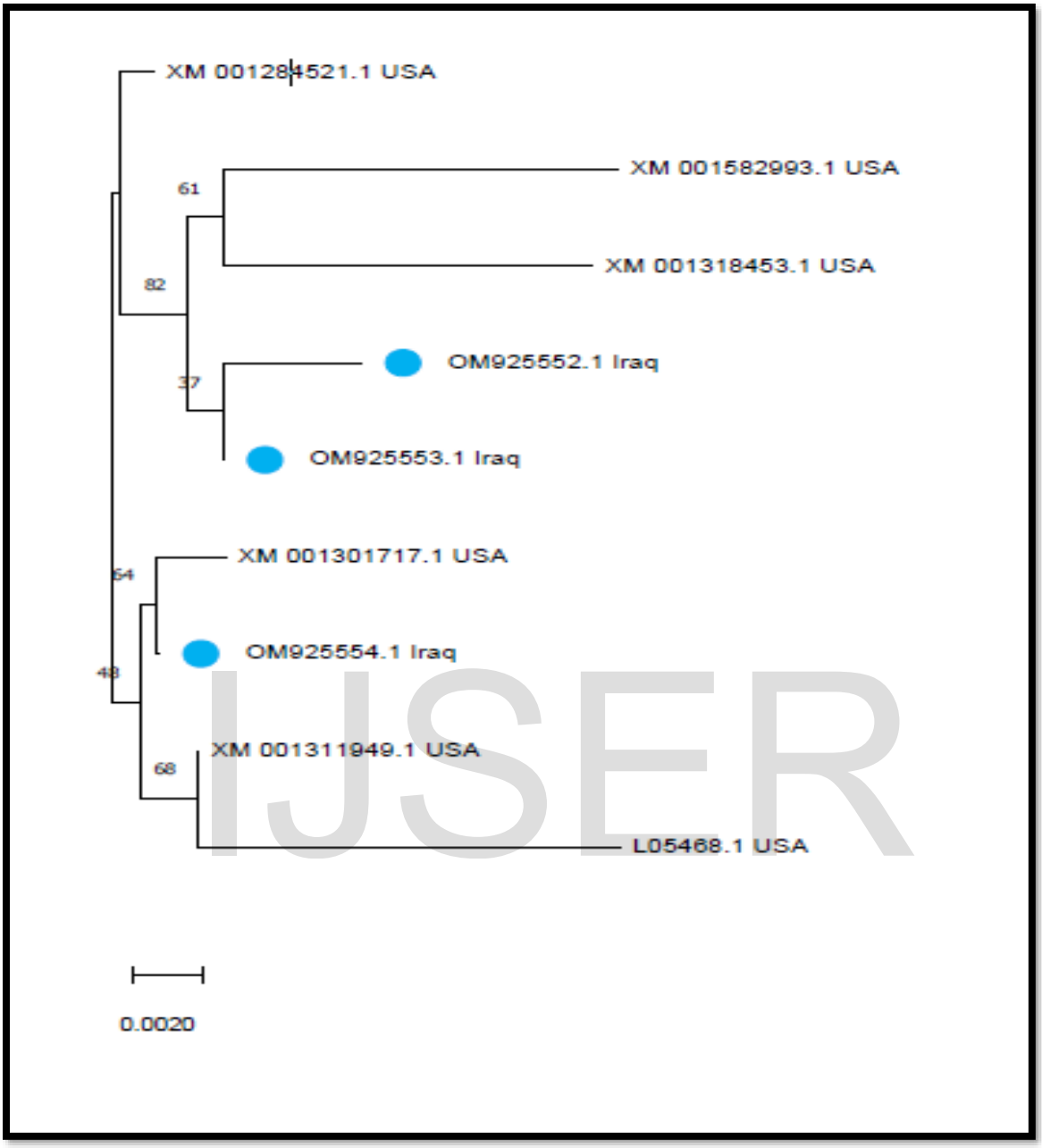

Figure (2): Neighbor-Joining phylogenetic tree constructed by MEGA11.1 Sequence Viewer 8.0 showing the genetic relatedness among the three of T. vaginalis identified in this study (of T. vaginalis isolate Sq1, Sq3 and Sq7 beta-tubulin gene, partial cds (OM925552.1, OM925553.1and OM925554) and other beta-tubulin gene, partial cds nucleotide sequences of T. vaginalis retrieved from GenBank. Numbers on branches indicate to the bootstrapping values.

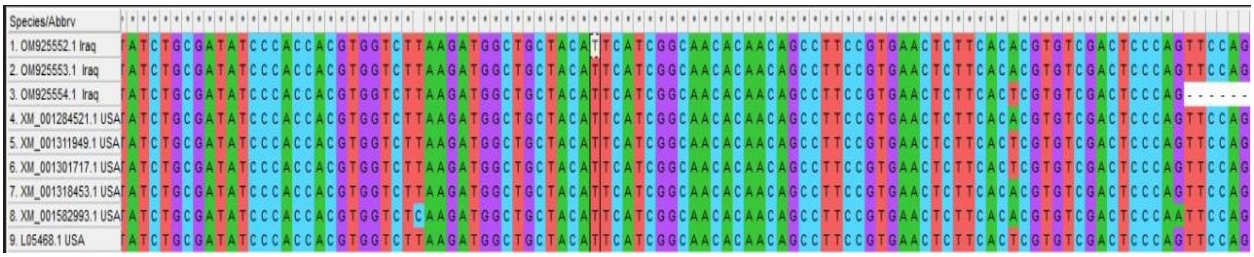

Figure (3): Multiple sequence alignment, constructed by MEGA 11.1 Sequence viewer 8.0 program, of three local sequences of Trichomoniasis and six NCBI sequences. The degree of conservation of each nucleotide base among the whole sequences was displayed in the figure as star above columns.

Discussion:

A study conducted in Basrah governorate investigated the diagnosis of the disease in women. In the current study hematoxylin-eosin stain detected the fewest number of infections with *T. vaginalis* 7(26.9%). Staining of hematoxylins-eosin have better sensitivities but are more labor-intensive also take time during processing of the stain and are moderately expensive (17). The next step was to use a wet mount preparation, which is the most common method of testing in today's laboratories; 10 of them (38.5%) were positive for trichomoniasis infection (18). Nevertheless, this method is inaccurate and has low sensitivity when compared to other methods, resulting in the parasite losing movement quickly if exposed to any delay before this test (19). Therefore, alternative methods should be investigated that can provide more specific results at a lower cost and length of time (20). Thus, molecular methods are used to identify parasites in research samples. The techniques are new and have been developed for detecting any infections such as trichomoniasis (21,22). In PCR, a molecule of nucleic acid DNA can be amplified for a million copies for one target cell (23). A PCR detection of *T. vaginalis* identified 20 (76.9%) positive results in the study. PCR show as a highly sensitive and specific (100% and 90.9%) method to detect *T. vaginalis* than direct microscopy which is able to number of samples that fail to detect by direct microscopic examination and the reason of this result related to the PCR requires only DNA, also from viable or non-viable organisms, at a concentration of as low as one organism for each reaction (24) so this result is acceptable in every study presented (25) and (26).

Conclusions: This study found that PCR was most effective for detecting *T. vaginalis* infection. Therefore, PCR may represent the best option for definitive laboratory diagnosis for genital infections.

Clearance for ethical conduct: Health and higher education and the scientific research ministries in Iraq approve the Research Ethical Committee's conduct of scientific research.

Interest Conflicts: Conflicts of interest are not declared by the authors.

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