



Detection of *Chlamydia trachomatis* in ectopic pregnant women using 16S rDNA genes in Mosul / Iraq

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Abstract

Three hundred forty (340) swabs were collected from the cervix to investigate the infection with *Chlamydia trachomatis*, of (158 women had cervicitis, 95 infertile, 75 were aborted, and 12 had an ectopic pregnancy), by the Obstetrics and gynecologist specialized in Mosul Hospital and Al-Batool Hospital, Mosul, Iraq, during the period (from August 2022 to February 2023). Their ages were ranged from (<15-45) years. Three swabs were taken from the top of the dry vagina, High Vaginal Swabs (H.V.S), with special dacron swabs, in a circular motion and in one direct direction, with scraping of the epithelial cells: The 1st.swab for staining with the Giemsa stain, the 2nd.for the immunological examination of the antigen, and the 3rd.for the molecular diagnosis by PCR technique to investigate the gene of 16S rDNA. The results showed that (128), with a rate of (37.6%), were suffering from clinical symptoms such as vaginal discharge, bleeding, and lower abdominal pain, and (212) were at a rate of (62.3%). The highest incidence of *Chlamydia trachomatis* was in the age group (24-34) years, at a rate of (63.0%), followed by the age group (15-24) years at a rate of (25.9%), and the lowest percentage was in the age group (35-44) years at a rate of (11.1%). Dry smears when stained with giemsa stain were showed the presence of it a ratio of (7.9) 340/27. Immunoprecipitation gave a rate of 340/21 (6.1%). Detection of 16S rDNA genes using PCR technique was indicated infection with it at rate of 340/11 (3.2), Most of women under study live in city at a percentage of 310 (91.1%). The percentage of Country side women were at (8.9%), and now positive results were recorded for them.

Keywords: *Chlamydia trachomatis*, Ectopic pregnancy, 16S rRNA

Introduction

Chlamydia trachomatis are Gram-negative obligate intracellular parasites. It belongs to class: Chlamydia, order Chlamydiales, from family Chlamydiaceae (Willey *et al.*, 2020) [21]. It has a unique reproduction life cycle. It starts with an elementary body (EB) adhering to the surface of the host cell. EBs are (0.2 to 0.6) μm in diameter (Willey *et al.*, 2020) [21], contain dense nuclear material and a rigid cell wall, and is an infectious agent. Host cells become infected with EBs by endocytosing them (Arif *et al.*, 2020) [6]. An EB is held in an endosome that evades fusion with lysosome. After this point, it begins binary fission to become a Reticulate Body (RB), which continues until the host cell dies. Reticulate bodies range in diameter from (0.5 to 1.5) μm, having more ribosomes than EBs and less dense nuclear material; their walls are also more flexible. After (20 to 25) hours, RBs differentiate into infectious EBs. The host cell lyses and releases EBs (48 to 72) hours after infection (Elwell *et al.*, 2016) [4]. Human pathogens known *Chlamydia trachomatis* serovars mostly produce genital and eye infections. Chlamydiae have an exterior cell wall that resembles gram-negative bacteria. Despite the lack of the strong outer membrane, the dense center of this bacteria is surrounded by a double-layer outer membrane. All members of the family share a lipopolysaccharide (LPS) in their cell walls. There is very little endotoxin action in LPS. MOMP, a significant outer membrane protein, which is specific to each species, is an essential structural element of the outer membrane (Arif *et al.*, 2022) [7]. *Chlamydia trachomatis* is the sexually transmitted pathogen that causes cervix inflammation, Pelvic Inflammatory Disease (PID), urethral inflammation, epididymal inflammation, LGV, and rectum

inflammation. When it infects pregnant women, it can also lead to fetal conjunctivitis and eosinophilic pneumonia (Workowski *et al.*, 2021) [22]. Without antibiotic treatment, long-term *C. trachomatis* infection of the female vaginal tract may result in chronic inflammatory diseases even though its infection is typically asymptomatic, may cause behind immunopathological tissue damage pelvic inflammatory diseases, and infertility (Lehr *et al.*, 2018). *C. trachomatis* isolates share a 7.5-kb virulence-associated plasmid (Comanducci *et al.*, 1990) [10]. Although its infections can be treated with antibiotics, no medication is sufficiently affordable to eradicate the pathogen, and no efficient vaccine has yet been created (Booth, 2020) [9].

A total of eight Open Reading Frames (ORFs) have been identified in its plasmid, each (Pgp1–8) are expressed during infection. For plasmid maintenance, *pgp1*, *pgp2*, *pgp6*, and *pgp8* are crucial. *Pgp4* is a critical positive regulator of plasmid-encoded *Pgp3* and the chromosomal genes, including *GlgA*, *CT049-CT050*, and *CT142-CT144*. *Pgp5* is a negative regulator of *Pgp4*-regulated genes. *Pgp7* resembles integrases and is a homologue of *pgp8*. Two short antisense RNAs that are involved in plasmid maintenance are encoded by the plasmid (Gong *et al.*, 2013; Yang *et al.*, 2020) [13, 24]. A distinctive feature of Chlamydiae is their biphasic developmental cycle, which alternates between an infectious, extracellular, metabolically inactive EB and a non-infectious, intracellular, metabolically active RB (Moulder, 1991) [15]. Characterizing the makeup of the cervicovaginal microbiota in health and disease has greatly advanced over the past ten years thanks to high-throughput culture-free approaches based on the examination of 16S rDNA gene sequences (France *et al.*, 2020) [11].

The recognized gold standard for its detection for a long time was culture. McCoy cell lines require rather complicated and time-consuming cultivation procedures, as well as skilled laboratory technicians for accurate microscopy and follow-up staining (Sheetawy *et al.*, 2007)^[18]. Nucleic acid amplification testing NAAT is significantly more sensitive than the culture methods (Swain *et al.*, 2004)^[19]. So, the aim of this study is to identify *Chlamydia trachomatis* in women with EP by using Giemsa stain, immunoprecipitation and molecular detection techniques for 16S rDNA genes using PCR.

Materials and methods

1. Participants

Clinical samples were collected from (340) Women aged (15-<44) years admitted to the out-patient clinics of Gynecology and Obstetrics specialists, in Mosul Hospital and Al-Batool Hospital, Mosul/Iraq during the period (from August 2022 to February 2023). These females were diagnosed as had EP depending on the clinical features of Chlamydial infection, ultrasound findings, risk factors and the distinctive criteria of national guidelines for EP disease (Workowski *et al.*, 2008)^[23].

Detection of *Chlamydia trachomatis* Infection

Each patient received three plastic swabs, which were used to collect the specimens by inserting one of them into the

endocervical canal and vigorously rotating it against the wall of the canal to scrape off more endocervical cells. Swabs were carefully removed after that to prevent coming into touch with any vaginal fluids (Al-Jammally and Al-Taii, 2020)^[5]. The swabs were distributed as follows:

- The first was placed in a dry tube for staining by Giemsa stain, which is specific to it to detect inclusion bodies.
- The second was used for immunoprecipitation to detect it.
- The third swab was used for molecular detection methods and immersed in a simple tube containing 1 ml of either normal saline or Phosphate Buffered Saline (PBS) transport medium for the purpose of detecting 16S rDNA genes.

The specimen was transported to the laboratory of College of Sciences, University of Mosul, Mosul / Iraq.

DNA Extraction and PCR Protocol

Endocervical swabs were used in a DNA extraction process. It was carried out in accordance with the manufacturer's (Geneaid/USA) specified methods. The obtained DNA was kept at 2–8°C for later use and processing in order to be molecularly identified. Conventional PCR primers were used to detect the (16S rDNA) gene using the conditions given in Tables (1) and (2).

Table 1: Primers and Product Size of Genes

Genes	Primer length	Sequence	Size of gene(bp)	References
16S-rRNA-F	20	CGGAGCAATTCGECAA	567	Designed using pick primer program within NCBI
16S-rRNA-R	20	AAGGGGAACACAAAAGGGGTT		

Table 2: Program and conditions in PCR for 16S rDNA genes

No:	Stage	Temperature (°C)	Time (min)	Cycle
1.	Initial denaturation	95	6	35
2.	denaturation	95	45 sec	
3.	Annealing*	53	1	
4.	Extension	72	1	
5.	Final Extension	72	5	

*Determine the temperature using primer3 plus program

Agarose gel electrophoresis was used to confirm PCR amplification (Sambrook and Russell, 2006)^[7]. For 50 minutes, the electric current was allowed to flow at 70 volts. DNA bands were observed using a UV trans-illuminator, and a digital camera was used to take pictures of the gel (Vendramini *et al.*, 2011)^[20].

Statistical Analysis

C. trachomatis frequency distribution was shown as a percentage and histogram.

Results

Out of the total (340) women, only 27/340 (7.9%) infected women were diagnosed by Giemsa staining and microscopy as positive, as shown in Fig. (1), It was based on the presence of inclusion bodies stained in smears from the endocervix which appeared circular oval in shape within the cytoplasm of the epithelial cell, as shown in Fig.(2) while the remaining (313/340) were negative.

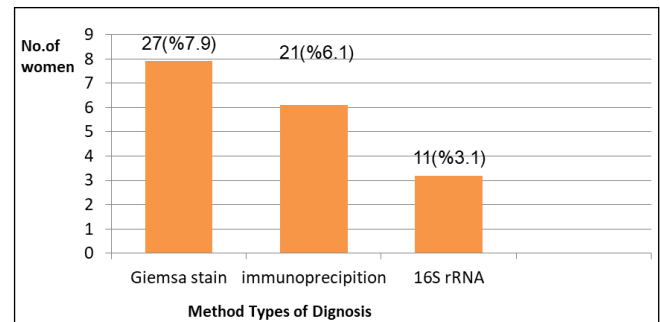


Fig 1: Numbers and Frequency of *C. trachomatis* infection using Giemsa, Immunoprecipitation and PCR for Diagnosing Methods 16S rRNA

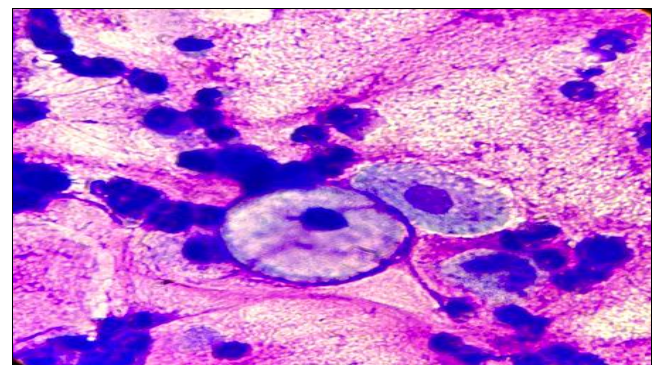


Fig 2: *Chlamydia trachomatis* Inclusion Bodies Staining with giemsa Stain

Detection of *C. trachomatis* using immunoprecipitation as antigen in (340) symptomatic and asymptomatic cases, 21 (6.17%) infected women were indicate presence of *Chlamydia* infection, wheras the remaining (319/340) were negative. It is noticed that out of 11/340 (3.17%) of women showed positive results for the genes using conventional PCR. In fected women were strongly linked to tubal EP:11

positive cases out of 27 tubal EP is 5.7 times more common (Rashid *et al.*, 2022) [16], as shown in Fig.(1). Primers for 16S rDNA genes were designed for the first time in the current study and were specialized for diagnosis *C. trachomatis*, all of them yielded approximate results 11/340 (3.2%) at molecular weight(567) bp, as shown in Fig.(1),and Fig. (3).

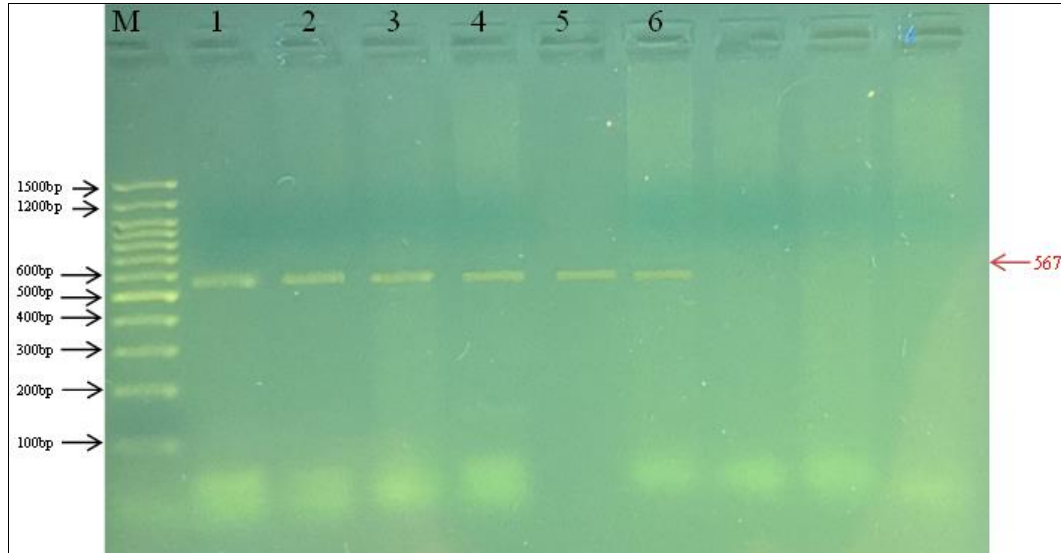


Fig 3: 16S rDNA genes PCR products at 567 bp for *C. trachomatis* in Endocervical Swabs among patients with Ectopic Pregnancy Disease. Using 2% Agarose gel electrophoresis at 70 volt for 50 min The 16S rRNA bands visualized under U.V light after staining with ethidium bromide. L: 1500 bp ladder; positive results lane (1-6)

Table.3- Indicate that 11 (6.9%) of the latter cases belong to the (158) symptomatic infected women and 16 (18.7%) cases belong to the (182) for asymptomatic one. The highest frequency among the age women (24-34) years was 17/27 (63%), wheras 7/27 (25.9%) for age group (15-24) years, and 3/8 (11.1%) for age group (35-44) years. The frequency was about (75%) above age (20) years as compared with (25%) at age (20) years and below, as in Fig. (4).

Table 3: Numbers and percentages of *Chlamydia trachomatis* infection detected in symptomatic and asymptomatic Cases.

Infected women with <i>C.trachomatis</i>	No. (%) of infection		Total
	positive	negative	
Symptomatic	11 (6.9)	147 (43.2)	158
Asymptomatic	16 (8.8)	166 (48.8)	182
Total	(7.9)27	(92.1)313	340

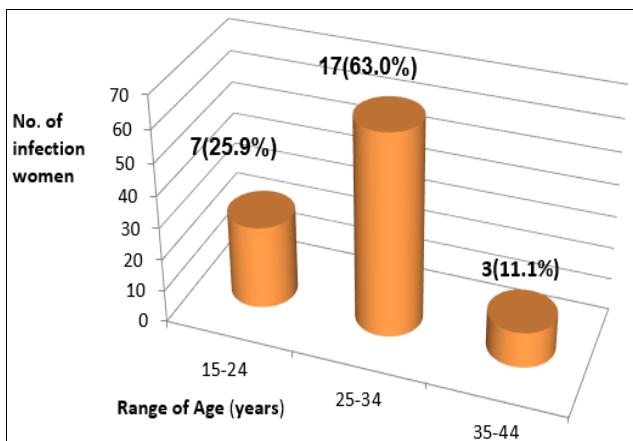


Fig 4: Number and Frequency of *Chlamydia trachomatis* in Endocervical Swabs of Women Depending on age.

Discussion

Chlamydia is generally difficult to diagnose due to challenges in its cultivation and direct detection utilizing specialized staining techniques (Berman *et al.*, 1993) [8]. As a result, a *Chlamydia* infection may experience long-term consequences, such as EP.

Evaluated of the diagnostic accuracy of the three method: direct microscopy, immunoprecipitation, and molecular methods among women suffering from symptoms and asymptoms, The gynecologist made the EP diagnosis in cases that also had risk factors for this illness, cryptic plasmid by PCR technique was targeted. Immunoprecipitationa was used for detecting chlamydial antigen as quick and useful method and undetected it at (7.9%) while giemsa staining, was at (6.1%) and PCR at (3.2%). Various studies were carried out in the Arabic World in Iraqi, it was (3.4%) (Al-Jammally and Al-Taii, 2020) [5], while in Qatar, it was (5.3%) (Al-Thani *et al.*, 2013) [2], while in Jordan, it was (3.9-5%) (Al Ramahi *et al.*, 2008). Furthermore, according to two distinct studies, the prevalence of *Chlamydia trachomatis* in Iran was (8.3%) and (22.0%), respectively. (Chabuck *et al.*, 2016) [3], in the United Arab Emirates, it was (2.6%) (Ghazal-Aswad *et al.*, 2004) [12]. This difference in frequency was related to sample size, participant age, the population investigated with diverse socioeconomic variables, as well as various study procedures (Chabuck *et al.*, 2016) [3].

Conclusions

Ectopic pregnancy women had prior *Chlamydia trachomatis* infection than are with an uncomplicated intrauterine pregnancy in terms of molecular evidence. A sensitive and specific way for identifying *Chlamydia trachomatis* in females is the use of the 16S rDNA genes. The low

incidence of chlamydial infection in patients from Iraq who also had EP may be attributable to Islamic and Arabic customs and ethics. The immunoprecipitation for *Chlamydia trachomatis* antigen represented a rapid, informative, and not expensive method for the detection of *Chlamydia* infection.

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