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Prevalence of *Chlamydia trachomatis* among women attending gynecology and infertility clinics in Gaza, Palestine

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Summary
Background: Chlamydia trachomatis is an obligate intracellular bacterium characterized by a biphasic developmental cycle of replication. The organism is recognized as one of the major causes of sexually transmissible human bacterial infection throughout the world. Since there have been no previous studies dealing with chlamydial diagnosis in Palestine, this study was conducted to determine the prevalence of *C. trachomatis* infection among women attending gynecology and infertility clinics.
Methods: Endocervical swabs were collected from 109 women, aged 18—52 years (median 29 years), attending gynecology and infertility clinics in Gaza. These specimens were processed using molecular (polymerase chain reaction, PCR) and enzyme immunoassay (EIA; IDEIA™ PCE Chlamydia) techniques.
Results: The results obtained show that the overall prevalence rate of *C. trachomatis* was 20.2%. The sensitivity was 73% for the EIA, 86% for the MOMP (major outer membrane protein gene)-based PCR, and 100% for the plasmid-based PCR. Meanwhile the specificity was 94% for the EIA, 98% for the plasmid-based PCR, and 100% for the MOMP-based PCR. In multivariate analysis, only cervical discharge was significantly associated with positivity for *C. trachomatis* (adjusted odds ratio 5.6, 95% confidence interval 2.0—15.5; $p = 0.001$).
Conclusions: The study revealed that a significant proportion of Palestinian women expressed evidence of exposure to *C. trachomatis*. Women with cervicitis are more likely to have been previously infected or exposed to Chlamydia infection. Furthermore, PCR proved to be superior and more efficient in the diagnosis of *C. trachomatis* than EIA.

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Introduction

Chlamydia trachomatis is an ubiquitous pathogen worldwide and causes ocular, urogenital, and respiratory infections in humans.[1] *C. trachomatis* infection of the lower genital tract is one of the most prevalent sexually transmitted diseases (STDs) in the world.[2] According to the World Health Organization (WHO), approximately 89 million new *C. trachomatis* infections occur annually worldwide.[3] It has been estimated that there are approximately 4 million new *C. trachomatis* infections each year in the USA.[4] Among women, the consequences of the disease include pelvic inflammatory disease, mucopurulent cervicitis, ectopic pregnancy, and infertility.[5] Symptoms of chlamydial infection in women include: abnormal genital discharge, painful or frequent urination, burning or itching in the vaginal area, redness, swelling or soreness of the vulva, and pain in the pelvis during sexual contact.[6]

Recently, nucleic acid amplification techniques, such as the polymerase chain reaction (PCR), with the potential to offer improved sensitivity for diagnosing *C. trachomatis* infections, have become available. These DNA amplification methods are reported to be more sensitive than cell culture techniques or conventional antigen detection tests, such as enzyme immunoassay (EIA).[7,8] However, despite the advent of DNA amplification technology, EIA tests are still widely used for the diagnosis of *C. trachomatis* in Palestine. Although molecular amplification analysis is increasingly used for confirmation testing, its use as a routine screening test for *C. trachomatis* is limited by the high cost for each test compared with current routine methods. The advent of improved sensitive immunoassays for detecting chlamydial lipopolysaccharide (LPS) might offer an opportunity for a lower cost test, while providing comparable sensitivity to molecular amplification methods.

The objectives of the present study were to determine the prevalence of *C. trachomatis* infection in women attending gynecology and infertility health centers in Gaza, Palestine, to identify factors that might be associated with developing chlamydial infection, and to evaluate the performance of the IDEIA^{TM} PCE Chlamydia kit (EIA) in comparison with the PCR technique.

Materials and methods

Study population

The sample size was calculated according to the formula suggested by Raosoft Inc.,[9] with a marginal error of 10%, confidence level of 95%, and response distribution of 50%. According to the medical records of Shifa Hospital and the Basma Infertility Center, the average number of patients in the previous 6 months was 4500. Therefore, the recommended sample size was 95. However, our study included 134 eligible participants over a period of 6 months from September 2005 to February 2006. Twenty-five participants were not enrolled (20 women refused to participate and five women participated but their endocervical specimens did not comply with standard collection techniques) and the remaining 109 constituted the study population.

The subjects were married, non-pregnant women, aged between 18 and 52 years (mean age 30.0 $\pm$ 7.5 years, median age 29 years). A questionnaire for the collection of information on age, level of education, contraceptive methods, symptoms, and infertility status was completed. Patients who received antibiotics were excluded. The cases were selected according to the Centers for Disease Control and Prevention (CDC) and Wessex Sexual Health Network guidelines for the management of *C. trachomatis* genital infection, where the patients complained of infertility, cervicitis, pelvic inflammatory disease, deep dyspareunia, inflamed or friable cervix, contact bleeding, dysuria, and urethral syndrome.[10,11]

Specimen collection

The Helsinki Committee, Shifa Hospital, and the Basma Infertility Center administrations gave permission and ethical approval for the current study. Following an explanation of the importance and objectives of the study, each patient signed a consent form.

Endocervical swab specimens were collected from the 109 participants. For each participant, a specialist gynecologist collected two Dacron-tipped swabs individually. Swabs for PCR were placed in phosphate buffered saline (PBS) and those for EIA testing were placed in special transport medium (IDEIA Chlamydia collection kit, S600730). The swab specimens were collected randomly and not in an ordered fashion. Collection was undertaken according to CDC guidelines and as indicated by the test manufacturer.[10]

Detection of the lipopolysaccharide epitope of *Chlamydia trachomatis*

C. trachomatis antigen was detected by enzyme-linked immunosorbent assay (IDEIA^{TM} PCE Chlamydia; Dako Ltd, UK). The Chlamydia microplate EIA utilizes a genus-specific monoclonal antibody for the direct detection of chlamydial organisms in human urogenital specimens.

Specimen preparation and processing by EIA

Endocervical swabs were placed in a boiling water bath for 15 min before EIA processing. Two hundred microliters of each heat-treated specimen were added to microwells and 200 μl of each positive and negative control were added to separate microwells. The negative control was prepared in triplicate with each batch to find the mean absorbance value. Specimens were incubated for 90 min with shaking at 15–30 °C, after the addition of 50 μl of conjugates. Specimens were washed four times with working washing buffer, then 100 μl each of amplifier A and amplifier B were added to each specimen. After incubation for 30 min with shaking at 15–30 °C, 100 μl of stop solution was added. The absorbance of each specimen was read at 490 nm.

Calculation and interpretation of the results

The cut-off value was calculated by adding 0.05 to the mean of the three negative control absorbance values. A result was considered positive when the optical density (OD) obtained was greater than the cut-off value plus 0.015, negative when the OD obtained was less than the cut-off value minus 0.015, and equivocal when the OD obtained was equal to the cut-off value plus or minus 0.015. The cut-off of the results was equal to 0.23 (0.18 + 0.05), hence positive specimens were defined

as any yielding an OD_{490} of >0.245 and negative specimens an OD_{490} of <0.215.

Detection of chlamydial DNA by PCR

Swab specimens were collected and stored at $-20\,^{\circ}C$; before processing, these were left at room temperature for at least one hour. Specimens were then well rotated and transferred to a clean 1.5 ml Eppendorf tube; 200 μl of PBS were added and the tube closed and centrifuged at high speed (13 000 rpm) for 30 min. The resulting supernatant was removed by a micropipette to maintain the pellet.

DNA extraction

Total DNA was extracted from the pellet by using a QIAmp DNA Mini Kit with a bacterial DNA extraction protocol. The pellet was resuspended in 180 μl of buffer ATL (QIAGEN) with 20 μl of proteinase K and then incubated at 56 °C with occasional vortexing until the pellet was completely lysed, which took 30 min. After lysis of the sample, 200 μl of buffer AL was added to the sample and the mixture was incubated for 10 min at 70 °C. The mixture was then combined with 200 μl of absolute ethanol and mixed by pulse-vortexing for 15 s. The mixture was applied to a spin column, which holds a silica gel membrane, and spun for 1 min at 6000 × *g*. The spin column was washed with 500 μl of buffer AW2 by centrifugation at 20 000 × *g* for 3 min. The DNA bound on a membrane was eluted by centrifugation with 50 μl of buffer AE after incubation for 5 min at room temperature. The resulting DNA extracts were stored at $-20\,^{\circ}C$ until PCR assessment.

PCR

The extracted DNAs were subjected to PCR with primers specific for plasmid and the major outer membrane protein (MOMP) gene. The primers (flanking a region of 364 bp) used to target the plasmid of *C. trachomatis* were F, 5′-GCAAGATATCGAGTATGCGTTGTTAGG-3′ and R, 5′-TTCATTGTACTCATTAAACGAGCGG-3′ (Maxim Biotech Inc., USA). Oligonucleotide primers (flanking a region of 182 bp) used for MOMP gene amplification were F, 5′-AACTCAAAACCCTCTCATTCTCAA-3′ and R, 5′-AAACGTTCGTCCCAGGAAGAAGCC-3′ (Maxim Biotech Inc.). The primers (flanking a region of 209 bp) used for the human β-globin PCR were F, 5′-ACACAACTGTGTTCACTAGC-3′ and R, 5′-GAAACCCAAGAGTCTTCTCT-3′ (Operon Biotechnologies, Germany).[12,13] *The β-globin primers were used as the internal control for PCR amplification, for the detection of any inhibitory* specimens. In brief, 5 μl of DNA extracts were processed in a 25-μl reaction volume using optimized detection kits for both plasmid and MOMP-based PCRs (Maxim Biotech Inc.).

Master mix preparation

Two hundred and fifty microliters of each of the pre-mixed primers were added to each tube of optimized PCR buffer, aliquoted, and stored at $-20\,^{\circ}C$. The master mix (20 μl per PCR assay) and Taq DNA polymerase (0.2 μl per PCR assay) components were premixed in sufficient quantity for daily needs, and this working master mix was dispensed into an individual reaction PCR tube before adding the specimen or control DNA (5.0 μl per PCR assay). The final volume was made up to 25 μl with nuclease free water when specimen or control cDNA used was less than 5.0 μl. The positive controls used were both the cDNA included in the kit and LGV type II DNA control. These showed the same results.

PCR reaction profiles

Amplifications were carried out in a Mastercycler (Eppendorf). The first cycle, consisting of a 3-min denaturation at 94 °C, was followed by 35 cycles each of 1 min at 94 °C, 1 min at 56 °C, and 1 min, at 72 °C, with a final extension for 10 min at 72 °C (for plasmid PCR). The same reaction profile was used for MOMP DNA, except that the annealing temperature was set at 59 °C.

Analysis of amplified DNA

Five microliters of amplified specimen were subjected to electrophoresis in an agarose gel 3%, containing 0.5 μg/ml ethidium bromide. A band of 364 bp indicated a positive result for cryptic plasmid, while a band of 182 bp indicated a positive result for MOMP. In addition, a band of 209 bp demonstrated that the specimen was positive for the human β-globin gene.

Interpretation of results

The results were interpreted according to the criteria suggested in previous studies.[14,15] Concordance of at least two of the three methods used (EIA and plasmid- and MOMP-based PCRs) was used for differentiating positive results from negative ones. A false-positive for any test was defined in the case of a positive result for that test and a negative result for the other two tests. Similarly, a false-negative for a test was defined in the case of a negative result for that test and a positive result for the other two tests.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 13 for windows. For normally distributed data, means and standard deviations were calculated. Chi-square and Fisher's exact tests were applied to assess differences in proportions. The odds ratio (OR) and its respective 95% confidence interval (CI) were calculated to assess the magnitude of association between correlates. Variables found to be significant by univariate analysis at p values of <0.10 were selected for inclusion in multivariate modeling. Non-conditional multiple logistic regression was used to model the association between *C. trachomatis* positivity and selected correlates adjusted for confounding factors identified in the univariate analysis. A p-value of <0.05 was considered statistically significant. Discordance between EIA and PCR was assessed by the McNemar test, while concordance of results was verified by the Kappa statistic value.

Results

This study is the first study in Palestine that has focused on the detection of *C. trachomatis* in endocervical swab specimens. The subjects were married, non-pregnant women aged between 18 and 52 years (mean age 30.0 ± 7.5 years, median

Table 1 Comparison between the plasmid- and MOMP-based PCR and the EIA test results.

	EIA		McNemar *p*-value	Kappa κ-value
	Positive	Negative		
Plasmid PCR			0.79	0.6
Positive	15	8		
Negative	6	80		
MOMP PCR			0.79	0.57
Positive	13	6		
Negative	8	82		

EIA, enzyme immunoassay; MOMP, major outer membrane protein.

Table 2 Interpretation of results in accordance to the criteria.

Results	Assays		
	EIA	Plasmid PCR	MOMP PCR
True-positive	16	21	19
True-negative	82	85	87
False-positive	5	2	0
False-negative	6	1 [a]	3
Sensitivity	73	100	86
Specificity	94	98	100
Positive predictive value	76	92	100
Negative predictive value	93	100	98
Efficiency	90	98	97

EIA, enzyme immunoassay; MOMP, major outer membrane protein.
[a] One false-negative specimen by plasmid PCR was positive by MOMP PCR. It was included in the true-positive category when measuring plasmid PCR performance.

age 29 years). Among the women who participated, 25% were under 24 years of age, 50% were under 29, and 75% were under 36.

Correlation between the EIA and plasmid- and MOMP-based PCRs

The plasmid PCR detected 23 positive specimens. It detected 15 specimens that were positive by EIA (Table 1); the remaining eight positive specimens were negative by EIA. Six specimens that were positive by EIA were identified as negative by plasmid PCR. Hence, a total of 14 discrepant results was found. There were also 14 discrepant results between the EIA and the MOMP-based PCR assay. The degree of agreement is based on interpretation of values of the Kappa statistic as described previously in the literature, in which $0.41 < \kappa < 0.60$ is 'moderate' agreement. Statistical analysis by Kappa test revealed moderate agreement between the EIA and the plasmid PCR ($\kappa = 0.6$) and between the EIA and the MOMP PCR ($\kappa = 0.57$). No statistically significant differences were shown by McNemar test ($p = 0.79$) (Table 1).

MOMP-PCR results

Nineteen specimens were found to be positive by the MOMP PCR; hence the prevalence by MOMP was 17.4%. There were six discrepant specimens between the plasmid and MOMP PCRs; of these six, one specimen was positive by MOMP PCR and negative by plasmid PCR. The remaining five specimens were negative by MOMP PCR and positive by plasmid PCR.

Interpretation of the assays in accordance with the criteria

According to the criteria described previously, the results of EIA, plasmid-based PCR, and MOMP-based PCR were analyzed and are summarized in Table 2. By applying the criteria, there were 22 truly infected women, giving a chlamydial infection prevalence rate of 20.2% (22/109).

Characteristics and factors associated with developing chlamydial infection

The prominent symptoms and signs correlated with chlamydial infection were purulent cervical discharge, contact bleeding, and infertility as determined by univariate logistic regression (Table 3). However, in multivariate logistic regression analysis, cervicitis was the only independent factor significantly associated with chlamydial infection (Table 4). Although a higher rate of chlamydial infection was found in secondary infertility (33.3%), this was not statistically significant (Table 5).

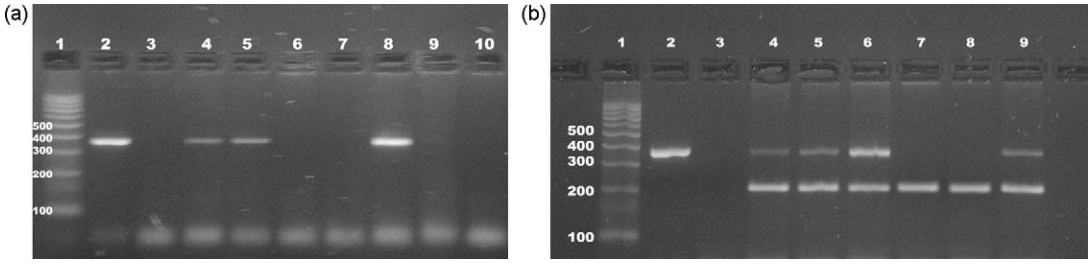

Figure 1 (a) PCR amplification product for cryptic plasmid. Lane 1: molecular weight ladder (100 bp); lane 2: positive control; lane 3: negative control; lanes 4, 5, 8: positive specimen PCR products (364 bp); lanes 6, 7, 9, 10: negative specimens. (b) PCR amplification product for cryptic plasmid, with internal control. Lane 1: molecular weight ladder (100 bp); lane 2: positive control; lane 3: negative control; lanes 4, 5, 6, 9: positive specimen PCR products (364 bp); lanes 7, 8: negative specimens. The internal control was detected for all specimens (209 bp).

Table 3 Prevalence of *Chlamydia trachomatis* and risk factors among participants.

Characteristics	No. tested	*C. trachomatis* positive	%	*p*-Value	Crude OR	95% CI
Cervicitis						
Yes	34	14	41.2	**0.0002**	5.9	2.15–15.97
No	75	8	10.7			
Contact bleeding						
Yes	13	6	46.2	**0.023**[a]	4.3	1.27–14.45
No	96	16	16.7			
Infertility						
Yes	49	14	28.6	**0.049**	2.6	0.99–6.85
No	60	8	13.3			
Low abdominal pain						
Yes	25	8	32	0.094	2.35	0.85–6.51
No	84	14	16.7			
Low back pain						
Yes	27	6	22.2	0.76	1.2	0.41–3.40
No	82	16	19.5			
Dysuria						
Yes	21	7	33.3	0.13[a]	2.4	0.84–7.05
No	88	15	15			
Pruritis						
Yes	24	6	25	0.57[a]	1.4	0.49–4.2
No	85	16	18.8			
Dyspareunia						
Yes	28	9	32.1	0.067	2.5	0.92–6.7
No	81	13	16.0			
Menstruation						
Irregular	22	7	31.8	0.14[a]	2.24	0.78–6.44
Regular	87	15	17.2			
Age group (years)						
18–24	30	3	10	0.103	0.35	0.096–1.29
25–31	38	9	23.7	0.5	1.38	0.53–3.62
32–38	25	7	28	0.27	1.79	0.63–5.0
39–45	13	2	15.4	0.65	0.69	0.14–3.37
Education level						
Elementary	21	7	33.3	0.128[a]	2.43	0.84–7.051
Preparatory	19	4	21.1	1.0[a]	1.067	0.32–3.60
Secondary	45	9	20	0.97	0.98	0.38–2.54
Higher education	24	2	8.3	0.15[a]	0.29	0.064–1.37
Residence						
Rural	31	7	22.6	0.69	1.22	0.44–3.37
Urban	78	15	19.2			

OR, odds ratio; CI, confidence interval.
[a] Fisher's exact test.

PCR amplification

The amplified products for both plasmid- and MOMP-based PCR assays are shown in Figures 1 and 2. Figure 1(a) shows the amplified products for the plasmid-based PCR assay (364 bp). Figure 1(b) shows the amplified products for the plasmid-based PCR assay with internal control for the β-globin gene (209 bp). Figure 2 shows the amplified products for the MOMP-based PCR assay (182 bp).

Discussion

C. trachomatis is one of the most common causes of STDs. It is the most common cause of cervicitis and urethritis, and their sequelae (pelvic inflammatory disease, chronic pelvic pain, tubal factor infertility, and reactive arthritis).[16] Chlamydial infections are primarily an issue of women's healthcare since the manifestations and consequences are more damaging to the reproductive health in women than in

Table 4 Multivariate logistic regression analysis of factors associated with *Chlamydia trachomatis* infection.

Characteristic	Crude OR (95% CI)	Adjusted OR (95% CI)	*p*-Value
Cervicitis	5.9 (2.15—15.97)	5.6 (2.0—15.5)	**0.001**
Contact bleeding	4.3 (1.27—14.45)	1.28 (0.29—5.69)	0.74
Infertility	2.6 (0.99—6.85)	2.39 (0.85—6.7)	0.079

OR, odds ratio; CI, confidence interval.

Table 5 Chlamydial infection in relation to type of infertility.

Type of infertility	Chlamydial infection		*p*-Value
	True positive (%)	True negative (%)	
Primary	8 (25.8)	23 (74.2)	0.36
Secondary	6 (33.3)	12 (66.7)	0.13[a]
None	8 (13.3)	52 (86.7)	-
Total	22 (20.2)	87 (79.8)	

[a] Fisher's exact test, 2-tailed.

men.[17] Most urogenital *C. trachomatis* infections are initially asymptomatic but may subsequently cause considerable long-term morbidity. Consequently, accurate diagnosis of *C. trachomatis* infection requires the use of specific laboratory techniques.

This study was performed to determine the prevalence of *C. trachomatis* infection among women attending gynecology and infertility centers in Gaza. This is the first study using EIA and PCR assays for the detection of *C. trachomatis* in endocervical swab specimens in Palestine. The overall prevalence of *C. trachomatis* in the study population was 20.2%. These results show that the prevalence rate of *C. trachomatis* is higher in Gaza than that reported in some neighboring countries.[18—20] However, other studies from neighboring and developing countries have shown higher prevalence rates than ours.[6,21,22]

The wide variation of Chlamydia prevalence in different studies could be due to several factors, such as study population (i.e., selection of high-risk groups), hygiene levels, socioeconomic status, and different techniques employed.

Correlation between the EIA/plasmid-based PCR and the EIA/MOMP-based PCR assays revealed a moderate agreement as determined by the Kappa statistic (0.6 and 0.57, respectively). Furthermore, the difference was not statistically significant ($p = 0.79$). However, the results showed that the IDEIA™ PCE test in comparison with PCR has lower

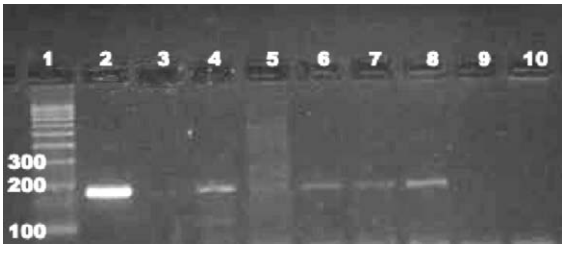

Figure 2 PCR amplification product for MOMP. Lane 1: molecular weight ladder (100 bp); lane 2: positive control; lane 3: negative control; lanes 4, 6, 7, 8: positive specimen PCR products for MOMP (182 bp); lanes 9, 10: negative specimens.

sensitivity (73%) and specificity (94%). The lower specificity could be explained by the presence of false-positive results, which could be attributed to some cross-reaction with other infectious components such as Gram-negative bacterial LPS. This explanation is supported by other recently conducted studies, which have shown that EIA has low sensitivity and specificity when compared to nucleic acid amplification tests.[23] In addition, the LPS-based EIA tests detect all three chlamydial species and, therefore, are not species-specific.

The sensitivity of the plasmid-based PCR was 100% and that for the MOMP-based PCR was 86%. A possible explanation for discrepancies in the results of our study between the plasmid- and MOMP-based PCRs might be a difference in the target used for the amplification. The PCR assay that we used to detect *C. trachomatis* is based on a cryptic plasmid primer, which has been proved to be a more sensitive approach than that used for the MOMP primer-based PCR,[24] because plasmids are present in elementary bodies in multiple copies. This may explain why five specimens were positive only by plasmid-based PCR but not by the MOMP-based PCR.

In addition, the specificity of the plasmid-based PCR was 98% and that for the MOMP-based PCR was 100%. This difference could be explained by the presence of two false-positive results in the plasmid-based PCR. Although these were recorded as false-positive for analytical purposes, this could also be explained by the slightly lower sensitivity of the MOMP-based PCR, and also it is unlikely that *C. trachomatis* infection would be excluded in such cases of symptomatic patients. The remaining specimen, which was found positive in both MOMP-based PCR and EIA, could contain a plasmid-free variant of *C. trachomatis*. This is also supported by other studies that have shown the possibility of encountering plasmid-free strains.[25,26]

Univariate analysis showed that cervicitis, contact bleeding, and infertility were significantly ($p < 0.05$) associated with chlamydial infection. Other symptomatic factors did not reach statistical significance with chlamydial infection, including low abdominal pain, low back pain, dysuria, dyspareunia, pruritis, and irregular menstruation. The age groups 25—31 years and 32—38 years were those with the

highest rate of infection. This finding is inconsistent with those of other studies that have shown a decline in the prevalence rate after 25 years of age.[27,28] Furthermore, women with an elementary education level showed the highest rate of chlamydial infection, while residency in a rural area revealed higher infection than residency in an urban area. However, in multivariate logistic regression analysis, cervicitis was the only independent factor associated with chlamydial infection.

Infertility is an emerging health problem in many countries of the world including Palestine. The increase appears to coincide with the growing role played by *C. trachomatis* as a sexually transmitted disease. In our study, *C. trachomatis* infection was found in 28.6% (14/49) of the infertile women, which is quite high and surprising. This could be attributed to previous infection with *C. trachomatis*. The prevalence of *C. trachomatis* infection was more common in women with secondary infertility. This finding has been reported by other investigators.[29] However, statistical analysis revealed no significant association with chlamydial infection ($p = 0.13$).

To the best of our knowledge, this is the first study from Palestine that provides current data regarding the frequency of genital *C. trachomatis* infection in married Palestinian women suffering from gynecological problems and infertility, and may be particularly helpful to physicians treating such patients in Palestine, where the quality of women's healthcare requires drastic improvement.

In conclusion, the prevalence of *C. trachomatis* in Gaza is considerable. Women with cervicitis are more likely to have been previously infected or exposed to chlamydial infection. Furthermore, plasmid- and MOMP-based PCR assays are superior and more efficient for the detection of *C. trachomatis* infection than EIA (IDEIA™ PCE Chlamydia kit).

We recommend the introduction of a PCR technique as the routine test for diagnosis of chlamydial infection in clinical laboratories. Further studies on a larger sample size of the general population are required, to more accurately determine the prevalence of *C. trachomatis*, to further identify risk factors associated with chlamydial infection, and to identify the prevalent *C. trachomatis* strains, which could be useful for epidemiology and for the identification of both mixed and re-infected patient cases.

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References

1. Yamazaki T, Hagiwara T, Kishimoto T, Sasaki N, Takahashi S, Ishihara O, et al. Distribution of *Chlamydia trachomatis* serovars among female prostitutes and non-prostitutes in Thailand, and non-prostitutes in Japan during the mid-90s. *Jpn J Infect Dis* 2005;**58**:211—3.
2. Gerbase AC, Rowley JT, Heymann DH, Berkley SF, Piot P. Global prevalence and incidence estimates of selected curable STDs. *Sex Transm Infect* 1998;**74**:S12—6.
3. Rowe PJ. *Reproductive tract infections. Annual Technical Report. Human Reproduction Programme*. Geneva: World Health Organization; 1998 . p. 186—90. Available at: http://www.who.int/reproductive-health/publications/HRP_ATRs/1998/rti.pdf (accessed September 2008)..
4. Chlamydia trachomatis infections: policy guidelines for prevention and control. Centers for Disease Control, Prevention. *MMWR* 1995;**34**:53S—74S.
5. Paavonen J, Eggert-Kruse W. *Chlamydia trachomatis*: impact on human reproduction. *Hum Reprod Update* 1999;**5**:433—47.
6. Mohamed N, Sharaf T. Evaluation of different techniques in diagnosing chlamydial endocervical infection among Egyptian females. *Egyptian J Hosp Med* 2001;**2**:138—47.
7. Loeffelholz MJ, Lewinski CA, Silver SR, Purohit AP, Herman SA, Buonagurio DA, et al. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. *J Clin Microbiol* 1992;**30**:2847—51.
8. Mania-Pramanik J, Donde UM, Maitra A. Use of polymerase chain reaction (PCR) for detection of *Chlamydia trachomatis* infection in cervical swab samples. *Indian J Dermatol Venereol Leprol* 2001;**67**:246—50.
9. Sample size calculator. Raosoft, Inc.; 2004. Available at: http://www.raosoft.com/samplesize.html. (accessed September 2008).
10. Recommendations for the prevention and management of *Chlamydia trachomatis* infections. Centers for Disease Control, Prevention. *MMWR Recomm Rep* 1993;**42**:1—39.
11. Wessex Sexual Health Network. Guidelines for the management of *Chlamydia trachomatis* genital infection. NHS document version 1. London, UK: Health Protection Agency, Department of Health; 2005. Available at: http://www.bashh.org/documents/888/888.pdf (accessed September 2008).
12. Lan J, van den Brule AJ, Hemrika DJ, Risse EK, Walboomers JM, Schipper ME, et al. *Chlamydia trachomatis* and ectopic pregnancy: retrospective analysis of salpingectomy specimens, endometrial biopsies, and cervical smears. *J Clin Pathol* 1995;**48**:815—9.
13. Lan J, Melgers I, Meijer CJ, Walboomers JM, Roosendaal R, Burger C, et al. Prevalence and serovar distribution of asymptomatic cervical *Chlamydia trachomatis* infections as determined by highly sensitive PCR. *J Clin Microbiol* 1995;**33**:3194—7.
14. Gaydos CA, Crotchfelt KA, Shah N, Tennant M, Quinn TC, Gaydos JC, et al. Evaluation of dry and wet transported intravaginal swabs in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in female soldiers by PCR. *J Clin Microbiol* 2002;**40**:758—61.
15. Jalal H, Stephen H, Al-Suwaine A, Sonnex C, Carne C. The superiority of polymerase chain reaction over an amplified enzyme immunoassay for the detection of genital chlamydial infections. *Sex Transm Infect* 2006;**82**:37—40.
16. Millman K, Black CM, Johnson RE, Stamm WE, Jones RB, Hook EW, et al. Population-based genetic and evolutionary analysis of *Chlamydia trachomatis* urogenital strain variation in the United States. *J Bacteriol* 2004;**186**:2457—65.
17. Jeffrey FP. Genital chlamydial infections. *N Engl J Med* 2003;**349**:2424—30.
18. Awwad ZM, Al-Amarat AA, Shehabi AA. Prevalence of genital chlamydial infection in symptomatic and asymptomatic Jordanian patients. *Int J Infect Dis* 2003;**7**:206—9.
19. Bamberger ES, Siegler E, Makler-Shiran E, Patel MV, Steinberg JM, Gershtein R, et al. *Chlamydia trachomatis* infections in female soldiers. *Israel Emerg Infect Dis* 2003;**9**:1344—6.
20. Ghazal-Aswad S, Badrinath P, Osman N, Abdul-Khaliq S, McIlvenny S, Sidky I. Prevalence of *Chlamydia trachomatis* infection

among women in a Middle Eastern community. *BMC Womens Health* 2004;**4**:3.
21. George JA, Panchatcharam TS, Paramasivam R, Balasubramanian S, Chakrapani V, Murugan G. Evaluation of diagnostic efficacy of PCR methods for *Chlamydia trachomatis* infection in genital and urine specimens of symptomatic men and women in India. *Jpn J Infect Dis* 2003;**56**:88—92.
22. Santos C, Teixeira F, Vicente A, Astolfi-Filho S. Detection of *Chlamydia trachomatis* in endocervical smears of sexually active women in Manaus-AM, Brazil, by PCR. *Braz J Infect Dis* 2003;**7**: 91—5.
23. Horner P, Skidmore S, Herring A, Sell J, Paul I, Caul O, et al. Enhanced enzyme immunoassay with negative-gray-zone testing compared to a single nucleic acid amplification technique for community-based chlamydial screening of Men. *J Clin Microbiol* 2005;**43**:2065—9.
24. Mahony JB, Luinstra KE, Sellors JW, Chernesky MA. Comparison of plasmid- and chromosome-based polymerase chain reaction assays for detecting *Chlamydia trachomatis* nucleic acids. *J Clin Microbiol* 1993;**31**:1753—8.
25. Stothard DR, Williams JA, Van Der Pol B, Jones RB. Identification of a *Chlamydia trachomatis* serovar E urogenital isolate which lacks the cryptic plasmid. *Infect Immun* 1998;**66**:6010—3.
26. An Q, Radcliffe G, Vassallo R, Buxton D, O'Brien WJ, Pelletier DA, et al. Infection with a plasmid-free variant Chlamydia related to *Chlamydia trachomatis* identified by using multiple assays for nucleic acid detection. *J Clin Microbiol* 1992;**30**:2814—21.
27. Centers for Disease Control and Prevention. Chlamydia screening among sexually active young female enrollees of health plans, United States, 1999—2001. MMWR Morb Mortal Wkly Rep 2004; 53:983—5.
28. Peipert JF. Clinical practice. Genital chlamydial infections. *N Engl J Med* 2003;**349**:2424—30.
29. Shi XB, Liu FY, Zhang HW. Study of *Chlamydia trachomatis* infection on cervical secretion of women with early pregnancy and secondary infertility. *HunanYi Ke Da Xue Xue Bao* 2001;**26**:169—70.