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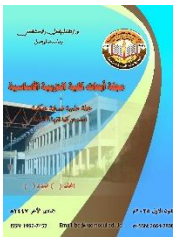

Determination of Chlamydia Trachomatis and Other Bacteria Infections in Infertile Women in Mosul

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Abstract

Chlamydia trachomatis is the most common negative gram bacteria obligate intracellular bacteria that causes sexually transmitted diseases, Chlamydia trachomatis is, an obligate intracellular bacterium, that parasitizes eukaryotic cells. C. trachomatis shows a tropism for genital and conjunctival epithelia and ocular trachoma worldwide. It affects reproductive system in women and men. If infections aren't identified and treated, they might lead to pelvic inflammatory disorders and spread to fallopian tubes, Infections of CT are 80–90% asymptomatic, females have been found to have the greatest rates of CT infection. Present study was carried out to diagnose genital CT infection among reproductive-age women, attending Albatool Hospital from(July to September) 2022 by Immunochromatographic test rapid test (the immunochromatographic card test represents a fast and cheap method to detect Chlamydia). Out of those women, 100 were infertile against 25 . A total of 12(12%) of the cases were found positive for CT by ICT. 12 out of 100 infertile women were positive for CT and none of fertile women were positive for CT. Also, prevalence of common microorganisms in the vagina and cervical canals of infertile women was clarified. Numerous microorganisms, including Neisseria gonorrhoeae, Candida albicans, viruses, Trichomonas vaginalis, and aerobic and anaerobic bacteria, are responsible for common other infections in female genitalia. Evaluate finding of wet preparation and direct gram staining of the vaginal swabs in Chlamydia positive and negative cases. the association of Chlamydia infection and culture isolate has been studied.

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تحديد الكلاميديا التراخومية والالتهابات البكتيرية الأخرى لدى النساء المصابات بالعقم في الموصل

نادية محمد أمين شاكر محمد امين شاكر نور نبيل شاكر

مختبر الإمام في الموصل/ وزارة الصحة العراقية

المستخلص

المتدثرة التراخومية أو المتدثرة الحثرية Chlamydiae trachomatis (CT) هي البكتيريا الإجبارية السلبية الجرام الأكثر شيوعًا داخل الخلايا التي تسبب الأمراض المنقولة جنسياً، وكانت Chlamydiae trachomatis، على مستوى العالم، تسبب مشاكل صحية خطيرة وتمثل عبئًا على الصحة العامة. بكتيريا داخل الخلايا إلزامية، تتطفل على الخلايا حقيقية النواة. يظهر C. trachomatis توجهًا للظهارة التناسلية والملتحمة والتراخوما العينية في جميع أنحاء العالم. إنه يؤثر على الجهاز التناسلي عند النساء والرجال. إذا لم يتم تحديد العدوى ومعالجتها، فقد تنتشر إلى قناتي فالوب وتؤدي إلى أمراض التهابية في الحوض مثل التهاب عنق الرحم والتهاب بطانة الرحم والتهاب قناة فالوب. تكون عدوى CT بدون أعراض بنسبة 80–90٪ وقد وجد أن الإناث لديهن أعلى معدلات الإصابة بـ .CT أجريت الدراسة الحالية لتشخيص عدوى التصوير المقطعي المحوسب التناسلي بين النساء في سن الإنجاب، اللواتي يترددن على مستشفى البتول خلال الفترة من يوليو 2022 إلى سبتمبر 2022 عن طريق اختبار المناعة الكروماتوغرافية السريع (يمثل اختبار بطاقة المناعة الكروماتوغرافية طريقة سريعة ورخيصة للكشف عن الكلاميديا)، الدراسة. من بينهن 100 امرأة عقيمات و25 امرأة خصبة. تم العثور على إجمالي 12 (12٪) من الحالات إيجابية للتصوير المقطعي المحوسب عن طريق اختبار المناعة الكروماتوغرافية. كانت 12 من أصل 100 امرأة عقيمات إيجابية للتصوير المقطعي المحوسب ولم تكن أي من النساء الخصيبات إيجابية للتصوير المقطعي المحوسب. كما تم توضيح انتشار الكائنات الحية الدقيقة الشائعة في المهبل وقنوات عنق الرحم لدى النساء المصابات بالعقم. العديد من الكائنات الحية الدقيقة، بما في ذلك النيسرية البنية، والمبيضات البيضاء، والفيروسات، والتريكومونوس المهبلية، والبكتيريا الهوائية واللاهوائية، مسؤولة عن الالتهابات الأخرى الشائعة في الأعضاء التناسلية الأنثوية. تقييم نتائج التحضير الرطب والتلوين المباشر بصبغة جرام لمسحات المهبل في الحالات الإيجابية والسلبية للكلاميديا. تمت دراسة العلاقة بين عدوى الكلاميديا وعزل الثقافة.

الكلمات المفتاحية: الكلاميديا الحثرية؛ التهابات الأعضاء التناسلية الأنثوية؛ الأمراض المنقولة جنسياً (النيسرية البنية).

1. INTRODUCTION

The gram-negative bacteria Chlamydia trachomatis (CT) is the most common cause of sexually transmitted infections. Over four million new cases of CT are reported in US each year, with an estimated 152 million instances occurring globally (Grieshaber *et al.* 2022, Feodorova *et al.* 2022).

The most dangerous side effects are those that have a long-term impact on the female reproductive system, (Mohammed *et al.*.2017, Omer *et al.* 2020). Chlamydia trachomatis is the primary cause of sexually transmitted infections (STDs) worldwide (Feodorova et al. 2022). Cervical CT infection is the first cause of dysuria and vaginal discharge. Infections can progress to the fallopian tubes and cause pelvic inflammatory illnesses such as cervicitis, endometritis, and salpingitis if they are not detected and treated. (Fatholahzadeh *et al.*2012). Infections of CT are 80–90% asymptomatic (Wiring, A. E., Ness, R. B., Darville, T., Beigi, R. H., and Haggerty, C. L. 2020) among females have been found to have the greatest rates of CT infection, making them a reservoir for more transmission (Unemo M, *et al*. 2020 , Hasanabad *et al.* 2011).

Numerous microorganisms, including Neisseria gonorrhoeae, Candida albicans, viruses, Trichomonus vaginalis, and aerobic and anaerobic bacteria, are responsible for the common other infections in female genitalia. (Sturd *et al.* 2023 , Kimberly and William 2010). According to these organisms, pelvic infection, bacterial vaginosis, viral cervicitis, vulvovaginal candidiasis, and vaginal trochomoniasis can all occur. Varying combinations of vulvar discomfort, dyspareunia, dysuria, and increased and changed vaginal discharge are all symptoms of these vulvovaginal infections (Jury *et al.* , 2023, Kimberly and William 2010). Serious consequences from some vulvovaginal infections, like anaerobic PID and a higher likelihood of Immune Deficiency Virus (HIV) transmission through sexual contact, are possible (Hocking JS *et al.*,2023 , Holmes 2008). Viral infections such as human papillomavirus and herpes simplex may persist for life (Collingro *et al.*, 2020 , Campbell and Monga 2008).

At the last, the most common conditions for which people go for gynecologists to seek treatment are genital tract infections. So, gynecologists can implement suitable anti-microbial therapy for treat these disorders if they have a thorough grasp of the pathophysiology of these diseases and a successful approach with their diagnosis.

The Aims of the Study

One of the reasons for the difficulties in controlling infections is diagnosis delay, so this study aimed to

1. Identification of Chlamydia Trachomatous in the cervix of infertile and fertile women and found if there is a correlation between chlamydia infection and women's infertility.
2. Completely identify the bacteria that were taken from the cervix as well as the vagina of the females who were being researched, and its correlation with chlamydial infections.

2. MATERIALS AND METHODS

This research's goals are to evaluate the diagnostic value of Rapid test for Chlamydia trachomatis detection and prevalence in endocervical swab samples from infertile Iraqi females from Mosul.

Specimens and Collection

125 women participated in this study. As a control group, 25 of them were fertile women, whereas 100 of them were infertile. These patients went to the Al-Batool Teaching Hospital in Mosul City's private gynecological clinic and the patients' clinic.

The samples were collected between June and August of 2022.

Swab collection

Three swabs were taken the first for the Chlamydia Rapid Test was performed utilizing the chlamydia cassette test. (ECO test /UK). Black, C.M. 1997, Second swab was for the detection of other bacterial species isolated from women's endocervics while the Third swab was used for direct wet smear and gram staining methods.

By sterilized disposable speculums patients were examined under fullilluminated conditions. Utilizing sterilized cotton swabs for microbiological study collected. Before the collection of the cervical swabs, the excess of mucus was removed by cotton ball.

The swab was inserted into the cervical canal until most of the tip was no longer visible. The swab was rotated for 10-15 seconds and placed in dry sterile transport tube,

The following steps were used to process the samples concurrently:

1. One cervical swab utilized for direct chlamydia detection
2. The second cervical swab for microbial diagnostic.
3. Third swab from vagina for direct smear.

Detection and Identification of Microorganism

Wet Preparation

The vaginal wet preparation was prepared by the addition of about 2-3 drops of normal saline were placed on a clean slide and the vaginal swab was shaken gently, and covered with a cover slip. The slide was examined under low and high power looking for PMN (pus cells), clue cells, bacteria, yeast's buds and motile Trichomonads.

Direct Gram's Staining

The vaginal and cervical, swabs were rolled on the center of a clean slide forming smear film about 1-2cm in diameter. They were dried, fixed, stained by gram's method and examined under 1000X microscope for gr (-) diplococci intracellular, gr (-) bacilli, gr (+) cocci, candida.

Chlamydia trachomatis detection test by chromatography test

1. Before testing, the test equipment, specimens, and chemicals were all allowed to come to room temperature using a chlamydia cassette test called the rapid immunochromatographic card.
2. Five whole drops of reagent A were added to the extraction tubes. The swabs were put in right away, squeezed to the tubes' bottom, turned ten times, and then left to stand for two minutes.
3. The extraction tubes were filled with seven whole drops of reagent B. After compressing the tubes' bottoms and rotating the swabs until the solution's color changed to a clear light blue—or yellow if the swab was bloody—they were left to stand for a minute.
4. The extraction tubes' tops were equipped with dropper tips. The test device's specimen well (S) received three whole drops of the extracted solution. 3. At ten minutes, the results were read. Positive findings were indicated by the presence of two red lines, one in the test region (T) and one in the control region (C), whilst negative results were shown by the appearance of a single line in the control region.

Culture:

Cervical swabs specimens were cultured immediately on chocolate agar, which was incubated at 5-10% CO_2. The specimens were cultured on other culture media at the same time or after 1-2 hours. They were cultured on blood agar, incubated aerobically. MacConkey's agar was also used and incubated aerobically. All plates were incubated at 37 C° for 24 hours with further 24 hours incubation if there was no growth. When potential harmful bacteria grew in the culture, the findings were reported as positive, when normal flora grew, the

findings were presented as negative (Lacto bacilli, Diphetheroids or Staphlococcus epidermisis) or growth doesn't exist.
The cultural growths were identified through the following steps:

Cultural characteristics identifying the colony morphology and changes in the media. Gram's staining of fixed smears prepared from the growth; Oxidase test a strip of filter paper was soaked with freshly-prepared 1% (tetramethyle para phenylen diamin hydrochloride) solution of the reagent and then at once used by rubbing a speck of culture on it with a wooden stick. A positive reaction was indicated by intense deep purple Colour, appearing within 5-10 seconds.

Statistical Analysis

The results of the measured CT detection variables and the percentage of variable bacterial culture results, parametric data were analyzed using Chi-Square test (Chi2) to extract the significance value .statistical analysis , to compare the significant differences between the group of infertile and fertile women.

3. RESULTS AND DISCUSSION

Detection of Chlamydia

Immunochromatographic card test

Twelve of the 100 infertile individuals, who were between the ages of 25 and 38, tested positive for chlamydia using a cassette test called the rapid immunochromatographic card test (Figure 4.1). In contrast, every fertile group 25 sample (control) tested negative for chlamydia (Table 4.1; Figure 4.2). Consequently, 12% of the infertile subjects had a chlamydia-positive test result.

Figure 4.1 Immunochromatographic Card Test for Chlamydia Detection

A. Two red lines at the T (test) and C (control) areas indicate a positive result.
B. A single line appears in the C region of the negative result.

Table 4.1 Chlamydia Positive Infection in Fertile and Infertile Cases

	No. (%)		Total number	P-Value
	Chlamydia positive	Chlamydia negative		
Infertile	12 (12%)	88	100	<0.0001
Fertile	0	25	25	**
Total	12 (9.7%)	113 (90.3%)	125	Significant

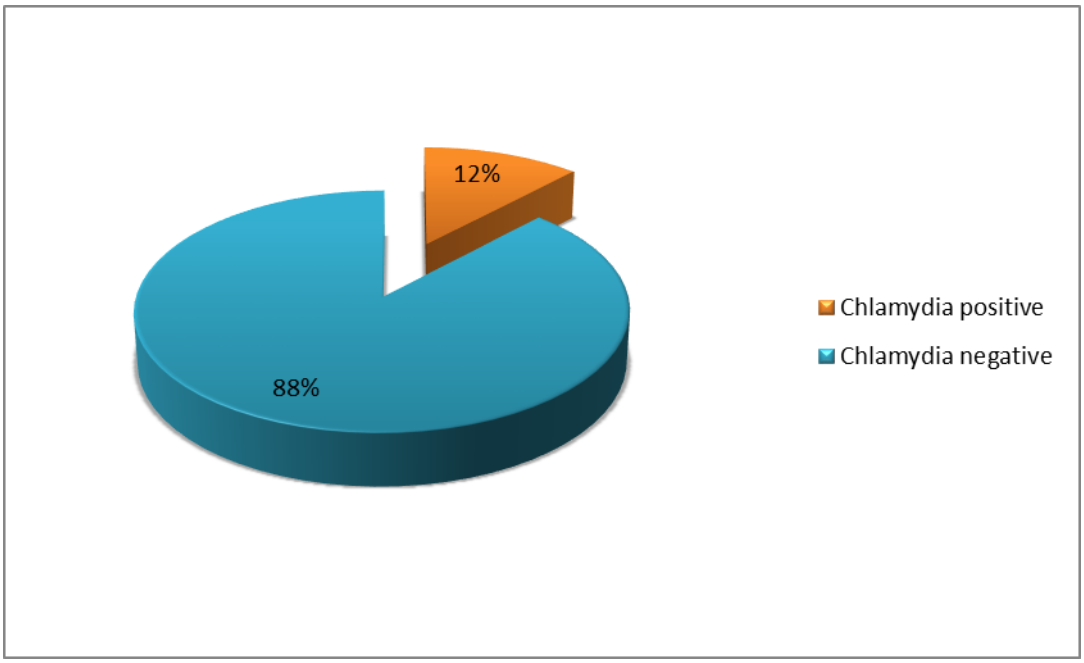


Figure 4.2 Chlamydia Positive Infection in Fertile and Infertile Cases

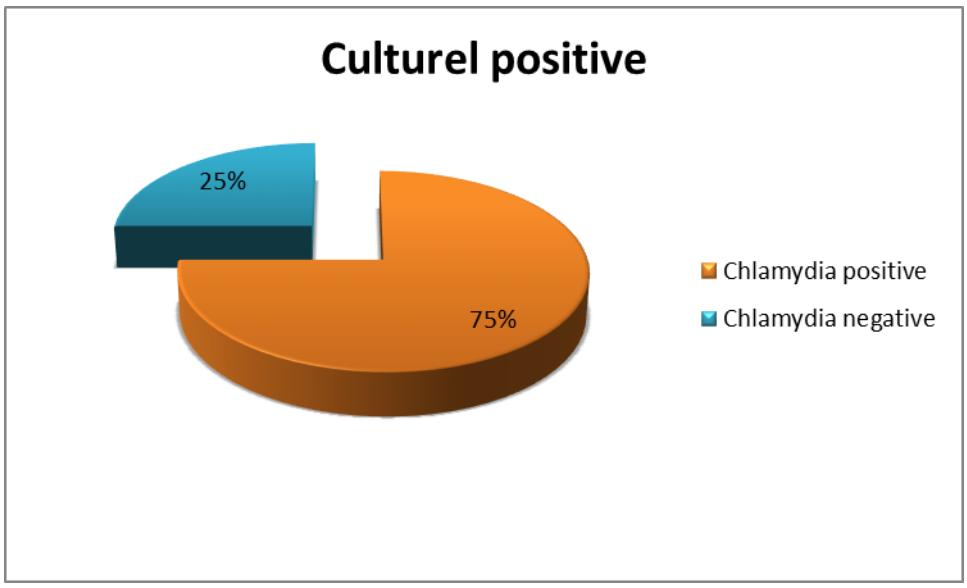
Infertile people in the study had a 12% prevalence of chlamydia, while fertile people had a 0% prevalence. Previous studies have found significant variation in the prevalence of chlamydia in both patient groups. Chlamydia was shown to be present in (3.4–44)% of infertile individuals (Peng et al. 2020).
While in the fertile groups (5 to 14.5)% (Moller et al. 2010, Mertz et al. 2010). These variations in the spread of chlamydia between the various studies may be related to social or geographical variation as well as test type utilized in the detection of chlamydia. So, chlamydia infection is expected in approximately 1/5 to 1/6 of adult married women, whether or not they are infertile. Such a common prevalence should be carefully monitored to prevent serious sequelae like PID or infertility. Previously, it was reported that chlamydia could be a significant cause of tubal infertility (Patton et al. 2010).

Bacterial Growth Culture

Among 12 infertile chlamydia positive cases, 9 (75.0)% on ordinary culture media (culture positive) had growth of bacteria and 3 (25%), (culture positive and negative, Table 4.2 and Figure 4.3) cultured on Blood agar, MacConky agar, Nutrient agar.

Table 4.2 Positive and Negative Bacterial Culture Growth in Infertile

Bacterial culture	No. (%)		P-value
	Chlamydia positive	Chlamydia negative	
Positive	9 (75.0%)	72 (81.8)	P≤ 0.00 ** Significant
Negative	3 (25.0%)	16 (18.2)	
Total	12	88	



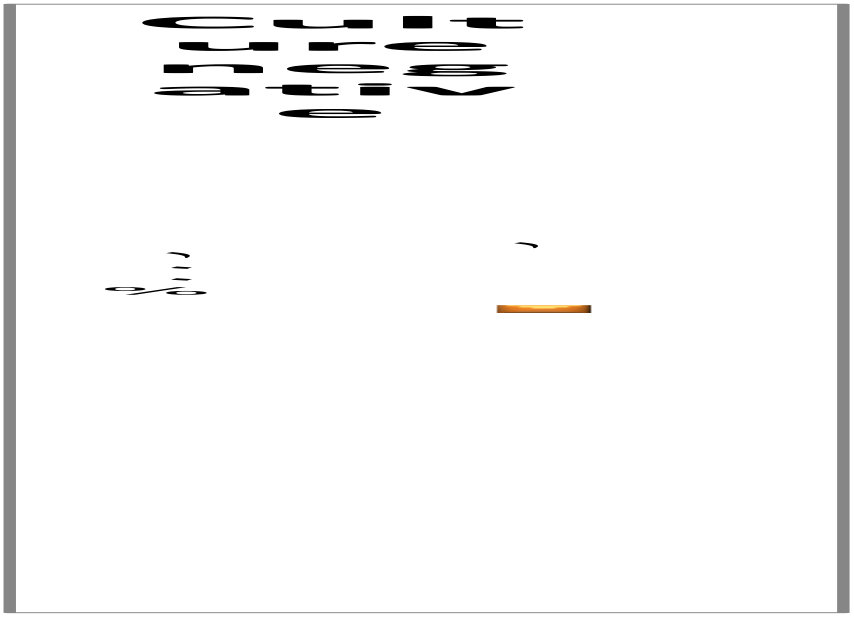


Figure 4.3 Positive and Negative Bacterial Culture Growth in Infertile

Only 5 of the 25 fertile control chlamydia-negative subjects had cervical and vaginal swab cultures that were positive (Table4.3 and Figure 4.4).

Table 4.3 Positive and negative Bacterial culture growth in fertile

Bacterial culture	**No. (%)**		**P-value**
	Chlamydia positive	**Chlamydia negative**	
Positive	0 (0%(	5 (20)	0.15 Not Significant
Negative	0 (0%)	20 (80)	
Total	0	25	

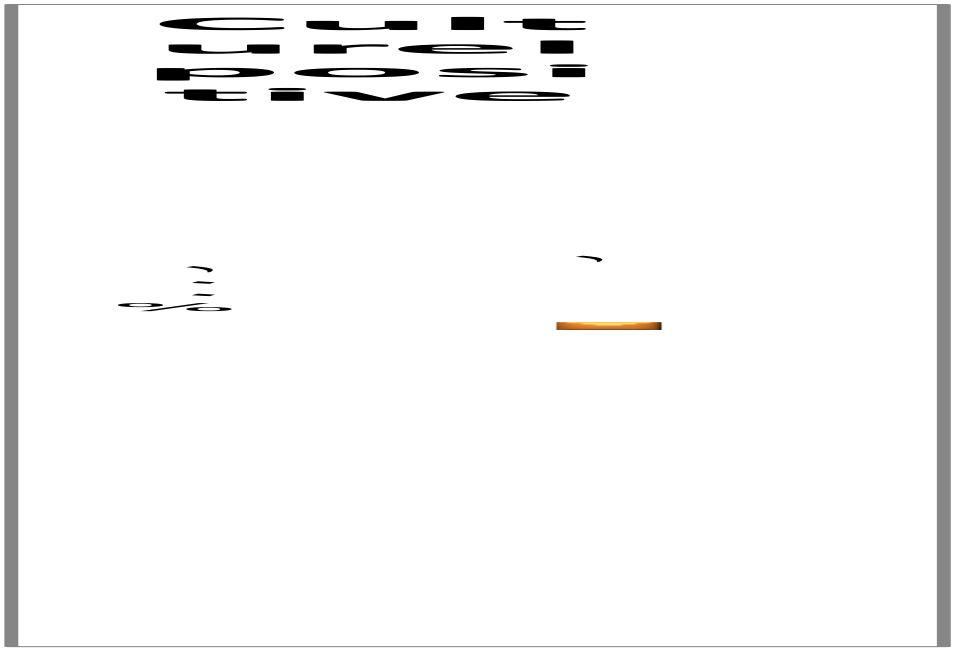


Figure 4.4 Positive and negative Bacterial culture growth in fertile

In the nine culture-positive instances, the isolated microorganisms from endocervical and vaginal swabs were identical. Out of these 9, 4 had only one growth, and the other five had many growths. The isolated single growth microorganisms were *E.coli*. (Figure 4.5), *staph aureus* and *streptococcus* group D, while the cases with mixed.

Growths isolated bacteria were *E.coli* together with *staph-aureus*. or *streptococcus* group (D) The bacteria recovered from the infertile chlamydia positive cases (Table 4.4) and (figure 4.6) were *E.coli,(*13–15%). *E. coli* can affect both female and male fertility. (Cools *et al*. 2017) *staphylococcus aureus* . *streptococcus* group D , Neisseria gonorrhaeo (NG), 2.3% of studied cases (Afrasiabi *et al.* 2014). The isolation of *E.coli* in association with chlamydia is in agreement with the study of Witken *et al*. (2010). Chlamydia exposure decreased from 3/12 of culture-negative (no growth)patients with 9/12 of culture-positive patients with presence of bacterial cervicitis. These findings might be explained on the basis that the presence of other bacteria in the cervix may interfere with chlamydia's ability to colonize the region. Such an impact might result from non-chlamydial microorganisms producing lactic acid, bacterial toxins, or enzymes.

Figure 4.5 Growth of gram negative bacteria *E.coli* on MacConky agar

Chlamydia and gonorrhea are sexually transmitted diseases. The occurrence of both diseases together with or without other infections is a matter of debate. In the current study the two cases of gonorrhea were found in chlamydia negative patient. However, a previous study reported the co-existence of both types of infection in 0.03% of their patients (Witken *et al*. 2010). Social and environmental differences between the various communities could play a role in these discrepancies.

Table 4.4 The percentages of the isolated microorganisms in chlamydia positive cases

Isolates Type	Infertile (culture positive) N = 32/12	
	Bacterial No. n=32	% From isolate n=32
Streptococcus – non – hemolysis	1	4.34
E. coli	2	8.69
Staph – aureus	10	4.34
Staph – epidermidis	0	0
Streptococus – α – haemolytic	0	0
Streptococus – β – haemolytic	0	0
Neissena gonorrhoeae	3	13.04
Candida albicans	3	13.04
Gardnerella vaginalis	9	39.13
Trichomunas vaginalis	4	17.39

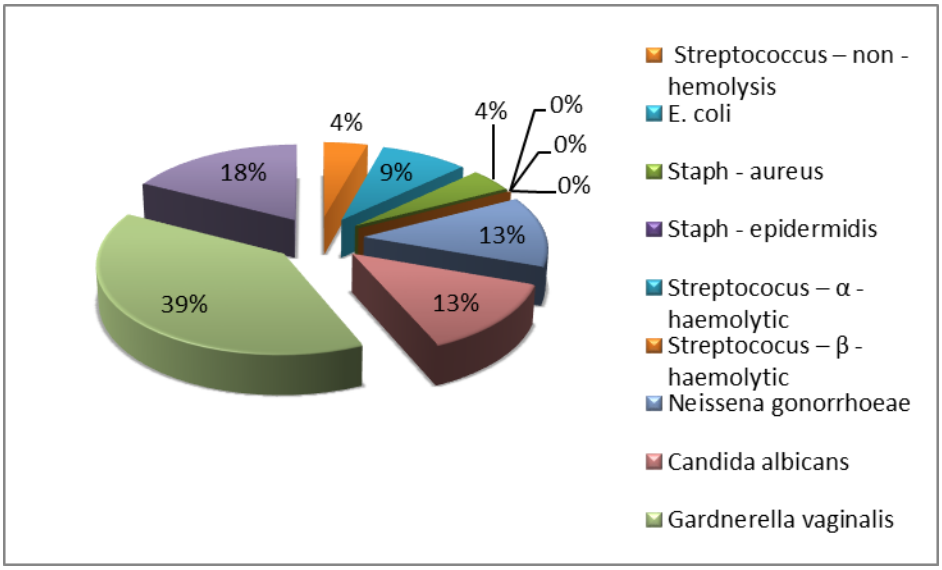


Figure 4.6 The percentages of the isolated bacteria in cases where chlamydia was detected

The cervicalswab (Figure 4.7) and vaginal swab(Figure 4.8) among the 88 infertile chlamydia negative cases showed that 72 (81.8%) other bacteria were isolates of culture positive and 16 (18.2%) were appeared culture negative "No pathogen isolated". Similar results were reported in a previous study. These results are in keeping with those of (Witken *et al.* 2010).

In Table 4.5, 72 (81.8%) patients with culture positive, CT negative cases had evidence of growth. In 72/88 (%) of the patients, the cervical and vaginal swabs revealed comparable cultural findings.

The 72 culture-positive cases resulted in a total of 74 microorganism isolates. 34 patients (45.9%) had isolated bacterial growths, as opposed to 24 (32.4%) who had mixed growths. Isolated bacteria frequency is displayed in (Table 5) Gardnerella veginalis was the most frequent isolate 18 (18/74), followed by *candid albicans* 14 (14/74) *Streptococcus group D* 10 (10/74), *Trichomonas vaginalis* 10 (10/74). It is crucial to look at the existence of Chlamydia with different pathogenic or opportunistic bacteria. The relationship of chlamydia infection with a negative bacterial culture has been researched with the current study. In situations where women were infertile, this link was shown to be statistically significant, but not in cases where women were fertile. Despite being insignificant, cultures that were positive with bacteria in the fertile and infertile individuals without rule out the potential of the infection to chlamydia. This considerable correlation can be explained by the fact that chlamydial infection was mostly to blame for the symptoms in the cases when cultures were negative, Moreover, the most frequent isolate in these cases was *Gardnerella vaginalis* 18/74 (Smith *et al*. 2010, Adad *et al.* 2009). This finding is in

agreement with other studies (Elbhar *et al.* 2010, Egwari *et al.* 2009). However, it disagrees with study of ALQuaiz *et al.* (2010) in which *Gardnerella vaginalis* was isolated in higher rate (24%), the *Non-heamolytic streptococcus* was the commonest gram-positive bacterium isolated. This is in accordance with *E.coli* was identified in 3 of cases and this result goes with previous works (Elbhar *et al.* 2010). However, the latter study showed the same rate (4.1%) of isolation of *B-haemolytic Streptococcus. Neisseria gonorrhoea* infection was found in only 3/74 case (1.42%). This result concurs with previous studies of ALQuaiz (2010), Wathne *et al.* (2000). Among the fertile (control) cases only 5/25 were culture positive for bacteria, while 20/25 of these were culture negative. These results showed that chlamydia positive among infertile cases were more than fertile (control) cases (12%). Finally, it was concluded that the similarity and differences in the rate of isolation of these bacteria in the various studies might be due to the difference in the group selection of patients or to environmental and social variations.

The other less frequent isolated bacteria were *Staphylococcus aureus* 6 (6/74), *B-heamolytic streptococcus* 4 (4/74) (%), *% Heamolytic streptococcus* 3 (3/74).

Only 5 of the 25 fertile control chlamydia-negative subjects had cervical and vaginal swab cultures that were positive (Table 4.5 and Figure 4.9). The isolated microorganisms in these 5 cases were *candida albicans* 2 (2/5) (5%), *staph aureus* 2 (2/5) (5%) *Gardanerella vaginalis* 1/5 (5%) (Figure 4.10).

Infertile patients had a statistically significant correlation between Chlamydia infection and a negative bacterial culture ($p > 0$), whereas fertile cases did not ($p > 0$).

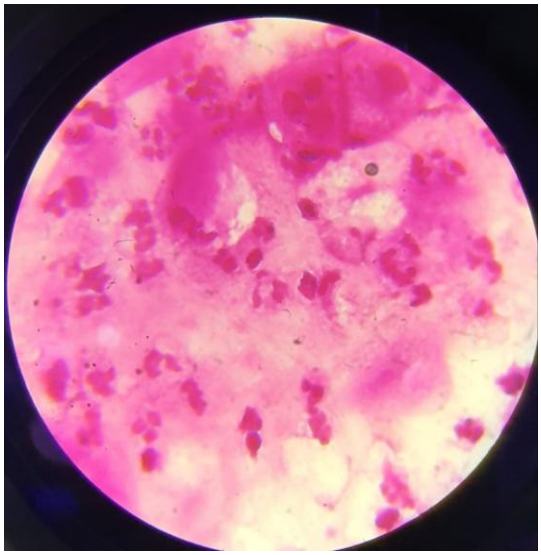


Figure 4.7 Direct gram stain of cervical swab

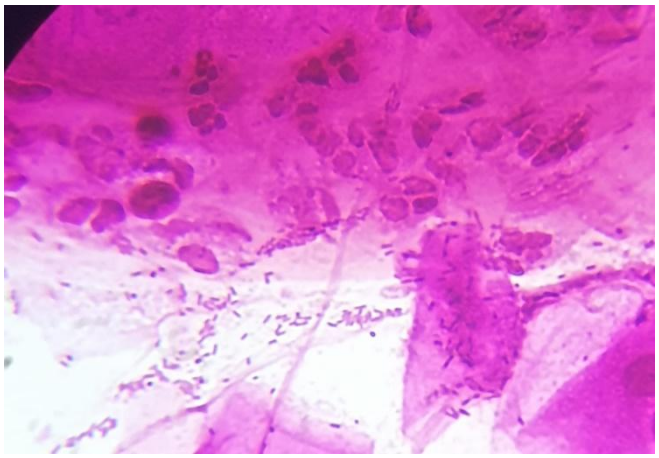


Figure 4.8 Direct gram stain of vaginal swab

Table 4.5 Chlamydia's Isolated Bacteria and Their Prevalence Negative Cases in Fertile and Infertile

Isolates Type	Chlamydia negative			
	Infertile		Fertile	
	Isolates No. n=74	% From isolates n=88	% Isolates No. n=25	
E.coli	3	4.05	0	
Non-hemolytic streptococcus Group "D"	10	13.51	0	
Gardnerlla varginalis	18	24.32	1	
Staphylococcus aureus	6	8.10	2	
Staphylococcus epidermidis	3	4.05	0	
α-hemolytic streptococcus	3	4.05	0	
β-hemolytic streptococcus	4	5.40	0	
Neisseria gonorrhoea	3	4.05	0	
Trichomones varginalis	10	13.51	0	
Candida albumins	14	18.91	2	

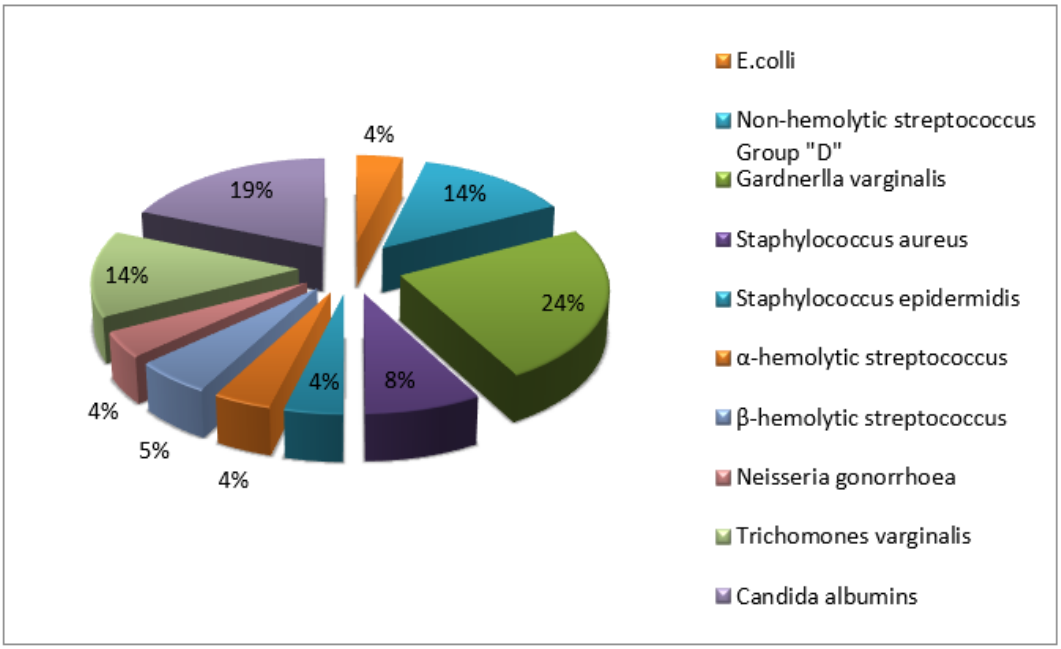


Figure 4.9 The percentages of isolated bacteria in chlamydia-free instances

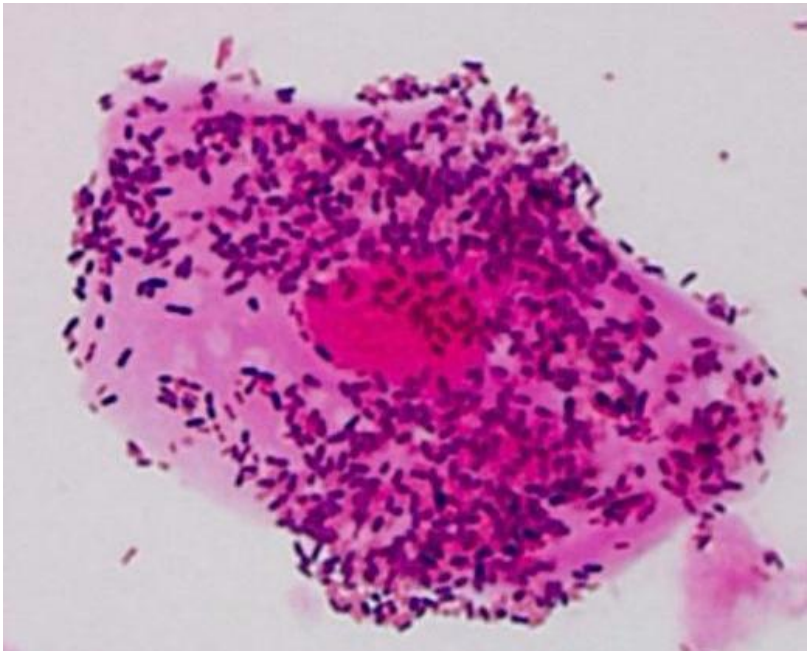


Figure 4.10 Vaginal swab showing *Gardnerlla varginalis* by gram staining

Direct Microscopical Examination of Vaginal Swabs:

Wet Preparation Microscopic Examination of Positive and Negative CT:

Both infertile and fertile cases of vaginal swabs were examined under a microscope after being prepared moist. These results are displayed in (Table 4.6). Through the 12 infertile cases that positive with chlamydia, the PolyMorpho-

Nuclear Cells (Figure 4.11), 7 of them (59%) cases were < 10 were detected in microscopic high power filed (40) hpf and 5 (41%) count was > 10 hpf. The results of the wet preparation also showed that 5 (41%) of the cases had clue cells and 7 (59%) did not. In addition, only 8 (66.7%) patients had Trichomonas vaginalis infection, whereas 4 (33.3%) instances did. 5 (41%) of the patients exhibited Candida budding cells, but none were seen in the remaining 7 (59%) individuals. Infertile and fertile chlamydia positive patients were found to have clue cells in 5/12 and 5/25 of the cases, respectively, according to the wet preparation findings. Comparable findings were reported by (Majeroni *et al.* 2009) and (Hakakha *et al.* 2010). Beside this work was consistent also with previous works of (Thejls *et al,*2009, Majeroni *et al.* 2009) work on the wet preparation that showed 1 (20%) patient with clue cells and 4 (80%) cases had none. Also, 1 (20%) case was infected with *Trichomonas* (Figure 4.13), and 4 (80%) were not. Two (40%) of the patients had Candida budding cells, while three (60%) did not. The results of the wet preparation were assessed for the presence of chlamydia. These researchers investigated the relationship between chlamydia and elevated PMN counts (>10/hpf) in infertile chlamydia positive patients, and they found that it was statistically significant (p=0.001).

It's possible that a bacterial infection in the fertile cases other than chlamydia boosted the PMN counts in the wet preparation, explaining the discrepancy in the connection between the presence of higher PMN count and chlamydia in infertile and fertile cases. Chlamydia and Trichomonas vaginalis co-infections were discovered in 2/25 of fertile patients and 4/12 of infertile patients. A previous work of the infection rate among women is 3.1% in the United States (Wiringa *et al*. 2020.) and 4.7% in the Mediterranean (Gerland 2010) had demonstrated such existence of infections. Through the chlamydia that negative cases, *Trichomonas vaginalis* was found in 7/88 in infertile cases, whilst in the fertile it was detected in 5/25 patients. (AL- Malla 2010) reported a higher rate of 12.5%. According to these findings, chlamydial infections are more likely to occur in both fertile and infertile people who have trichomonal infections (i.e. similar to clue cells).

The presence of PMN in infertile cases does not support the possibility of chlamydia infection. This was on the contrary to the culture negative result of these patients, which increases the possibility of this infection. However, among the fertile cases, the increased numbers of PMN enhanced significantly the possibility of pathogenic bacteria presence.

Among the 88 infertile chlamydia negative of cases, the <10 hpf of PMN counts were found in 59 (67 %) cases and of ≥ 10 / hpf in 29 (33%) cases. Also, the wet preparation revealed that 26 (29 %) patients had clue cells and 62 (71%)

did not have them. Trichomonas infection was detected in 7 (8 %) cases, while in 81 (92%) patients the infection was negative. Also, that wet preparation in infertile chlamydia negative cases revealed that presence of clue cells in 26/88 of cases. A similar result was recorded in a previous work of (Causi *et al.* 2002), while others reported a higher rate (24%) of clue cells. Among the fertile chalmydia negative cases the clue cells were found in 4/20 of cases (Causi *et al.* 2002). Had reported similar results. These results revealed that clue cells were detected in a rate higher (12/30 infertile and 1/5 fertile) in chlamydia cases than in non-chlamydia ones (9.9% in infertile and 3.1% in fertile). This finding supports the assumption that the presence of clue cells enhances the possibility of chlamydia infection in both infertile and fertile cases.

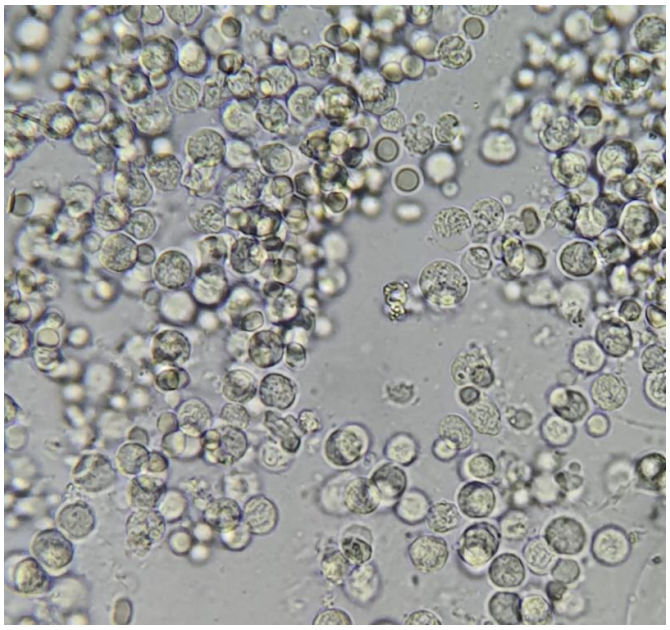


Figure 4.11 The polymorpho-nuclear cell (Pus cell) by wet preparation

Figure 4.12 Candida albicans from vaginal swab

19 (21.5%) of the cases exhibited Candida budding cells, while 69 (78.5%) of the patients had none.

In the 25 fertile chlamydia negative cases (control), the <10/ hpf of PMN counts were found in 18 (72%) patients and > 10/hpf in 7 (28%) patients. Furthermore, 5 (20%) cases had clue cells and 20 (80%) patients did not show them. Moreover, 2 (8%) and 23 (92%) of cases showed *Trichomonas* and Candida budding cells respectively.

Infertile chlamydia positive cases had significantly higher of PMN counts (> 10 hpf) of wet preparation of vaginal swabs than infertile chlamydia negative cases, according to statistics (P 0.000). However, this link was statistically significant (P=0.023) among the fertile instances. In moist of preparation of chlamydia positive patients, candida budding cells were found in 5/12 of infertile patients and of 2/25 of fertile patients. The same results were reported by (Moller *et al.* 2010). Moreover, among the infertile chlamydia negative cases Candida budding cells were found in 19/88 of cases. Comparable findings were shown by Adad *et al.* (2009). In the fertile chlamydia negative cases Candida budding cells were present in 2/25 of cases and Geraldo *et al*. (2010) reported similar results. These findings suggest that candidal infections in infertile patients were more common in non-chlamydial than in chlamydial cases. However, in the fertile cases candida infection were found more in chlamydia positive patients with negative ones.

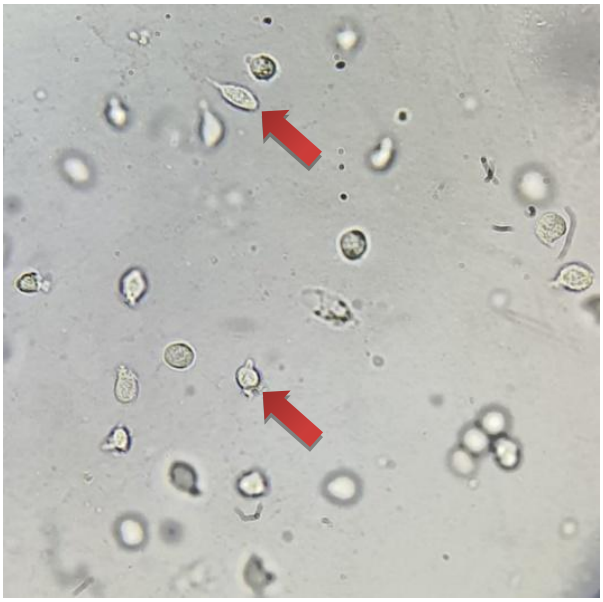


Figure 4.13 Trichomonas vaginalis by wet smear from vaginal swab

Table 4.6 Microscopically Finding of moist Preparations in Infertile and Fertile Chlamydia Positive and Negative Cases

Wet preparation items	No. (%)				P-value
	Chlamydia positive		Chlamydia negative		
	Infertile n=12	Fertile n=0	Infertile n=88	Fertile n=25	
PMN <10 ≥ 10	**7 (59)** **5(41)**		**59 (67)** **29 (33)**	**18 (72)** **7 (28)**	0.000 Sgnificant
P	0.001 significant		0.003 significant		
Clue cells Positive Negative	**5 (41)** **7 (59)**		**26(29)** **62(71)**	**5 (20)** **20 (80)**	0.023 Sgnificant
P					
Trichomonas Positive Negative	**4 (33.3)** **8 (66.7)**		**7 (8)** **81 (92)**	**2 (8)** **23 (92)**	0.003 Sgnificant
P	0.025 significant		0.03 significant		
Candida Positive Negative	**5 (41)** **7 (59)**		**19(21.5)** **69(78.5)**	**2 (8)** **23 (92)**	0.005 Sgnificant
P	0.001 Sgnificant		0.003 Sgnificant		

Direct Gram's Staining (DGS):

Gram staining was performed for vaginal and cervical swabs in order to detect directly and rapidly the presence of bacteria as well as PMN cells. Subsequently, a comparison between the results of direct gram staining and culture was done for both infertile and fertile cases in the light of the presence or absence of chlamydia.

The DGS of vaginal swabs in the 12 infertile chlamydia cases revealed positive results for gram-negative bacilli (possible pathogenic bacteria) in 1(8 %) cases, while 7 (58%) patients showed positive results for possible normal flora gram-positive bacilli *(Lactobacilli* and *Diphtheroids).* Gram-positive cocci. in 2 (17%) , gram-positive cocobacilli in 1(8%) , gram-negative cocci in 1 (9%) (Figure 4.14).

The 2 cases with positive results for gram-negative bacilli by DGS were identified bacteriologically to be *E.coli.* In all 7 patients with *Lactobacilli* (with or without *Diphtheriods)* the culture results confirmed their presence. However, gram-negative bacilli *(E.coli)* in 1 out- of 12 patients were detected by culture only. Finally, the 2 cases with gram-positive cocci results by DGS were also detected by culture identified bacteriologically to be staph-aureus and

streptococcus group D. The overall agreement between the results of DGS and culture was found in 12/12 cases, therefore, the consistency between these two tests was (100%).

The DGS of vaginal swabs in infertile chlamydia-positive cases revealed positive results for normal flora *Lactobacilli* (with or without *Diphtheroids)* in 7/12 and 6/25 respectively. The culture results confirmed the presence of these bacteria in all cases. (Hillier *et al.* 2010). Reported similar results of those lactobacilli found in 75 % of patients with chlamydia infection. The DGS in the remaining of our chlamydia-positive cases in both groups of patients either showed negative results for bacteria or positive findings for gram-negative bacilli. The consistency between the findings of the DGS and culture results were 1/12 for infertile and 6/25 in fertile. These results may be explained on the basis that chlamydia usually does not cause disturbance in the presence of *Lactobacilli.* Therefore, most patients revealed positive results for these bacteria in the DGS and culture. However, the concomitant infection as bacterial vaginosis and *Trichomonas vaginalis* may affect the presence of this normal flora (Table 4.7).

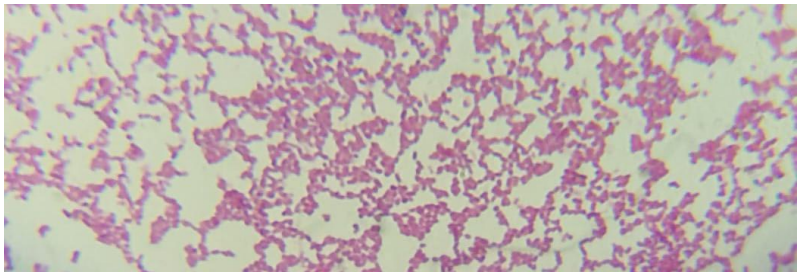
The association of chlamydia and increased PMN counts of > 10/ hpf in DGS was studied. Significant statistical associations were found in infertile and fertile groups of patients. These findings are supported by a previous report of (Theweseen *et al.* 2010) that demonstrated similar significant associations ($p<0.013$ in infertile and $P<0.00$ in fertile cases).

Table 4.7 Results of Direct Gram's Staining in Infertile and Fertile Chlamydia Positive and Negative Cases

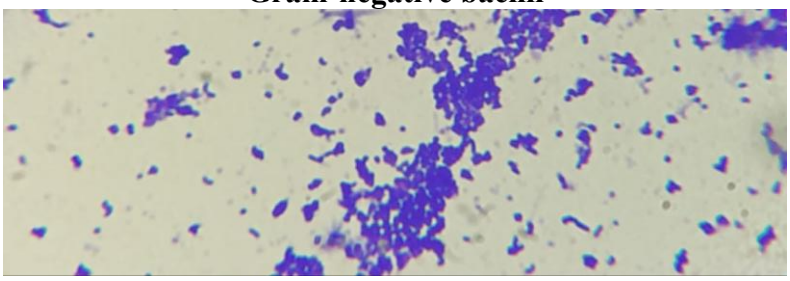
Type of bacteria	Numbers			
	Infertile (n=100)		Fertile (n=25)	
	Chlamydia positive (n=12)	Chlamydia negative (n=88)	Chlamydia positive (n=0)	Chlamydia negative (n=25)
Gram negative bacilli	1 (8)	9 (15)	00	6 (24)
Gram positive cocci	2 (17)	17 (28.5)	00	13 (52)
Gram positive coccobacilli	1 (8)	15 (25)	00	00

Gram negative cocci ▪	1 (9)	00	00	00
Gram positive bacilli ▪▪	7 (58)	19 (31.5)	00	6 (24)
P-value	0.0013 significant		P=0.00**	

▪ Neisseria gonorrhea, ▪▪ Lactobacilli and Diphtheroids



Gram-negative bacilli



Gram-positive cocci

Figure 4.14 Gram-negative bacilli and gram positive cocci

In the 88 infertile chlamydia negative cases, the direct gram's-stained vaginal swabs revealed positive results for possible pathogenic bacteria in 60 cases. These bacteria were gram-negative bacilli in 9 (15%) cases, gram-positive cocci in 17 (28.50%) gram negative coccobacilli in 15 (25%) Possible normal flora *(Lactobacilli* and *Diphtheroids)* were recognized in 19 (31.5%) patients (Table 4.7).

All the 60 cases with positive results for possible pathogenic bacteria were culture positive and the culture results confirmed their presence. Among the 10 with gram-negative bacilli the bacteria isolated in cultures were *E.coli* (10). Among those with gram-positive cocci the isolated bacteria were *Non-hemolytic streptococcus* (10), *%-hemolytic streptococcus* (2), and *B-hemolytic streptococcus* (3). In all cases with gram-positive coccobacilli the isolated bacterium was *Gardnerella vaginalis*.

In the 19 patients with positive results for *Lactobacilli* (with or without *Diphtheroids*) the culture results had also confirmed their presence. However, these samples showed also gram-negative bacilli in 9/88 and gram-positive cocci in 20/88 patients by culture. The gram-negative bacilli were *E.coli* (9). The gram-positive cocci were *Non-hemolytic streptococcus* (10), □*-hemolytic streptococcus* (3), *Staphylococcus aureus* (6), and *B-hemolytic streptococcus* (3). The bacteria isolated in the latter cases were *Gardnerella vaginalis* (18). The overall agreement between the DGS and culture was 88/88 (100%).

The 25 fertile chlamydia-negative cases showed non-pathogenic bacteria in 20/25 (80%) patients, which were *Lactobacilli* and *diphtheroids.* Furthermore, the culture results of the vaginal swabs confirmed the presence of bacteria detected by gram staining in all 5 positive cases. Meanwhile. The remaining 5/25 (25%) patients revealed by culture the presence of *E.coli* in 1 cases, and 2/25(52%) *Staph. aureus*, 2/25 Candida albicans. The DGS of vaginal swabs showed PMN counts of < 10 cells/ hpf in 17 cases (Table 7). The DGS in the infertile chlamydia-negative cases revealed that 60/88 had possible pathogenic bacteria, and 19/88 had *Lactobacilli* (with or without *Diphtheroids)*. These results were compared to those of the culture. The outcome, of this comparison, indicated that the agreement between culture and DGS was 55.4%. This finding is in accepting with the study of (Ison *et al.* 1982). On the other hand, the culture result fully confirmed the finding of DGS and was consistent with the findings of a previous work conducted by (Mijatovic *et al.* 2010).

4. CONCLUSIONS:

1. Results of this study revealed a significant correlation between CT infections and women's infertility. Due to the effects of *chlamydia*l infection on the genital tract of Mosul infertile women.
2. Negative bacterial cultures enhance the likelihood of chlamydia infection in instances of infertility, and the immune-chromatographic card test for chlamydia antigens identification is a quick, accurate, and affordable tool for the detection of chlamydia infection.
3. The direct gram stain of the vaginal swabs could predict the type of bacteria that grows on cultures in more than half of the cases.
4. infertile and fertile chlamydia-positive cases are associated with increased PMN counts (> 10/hpf) in both direct gram stain and wet preparation of vaginal swabs.
5. The presence of clue cells and *Trichomonal* infection increases the possibility of chlamydia infection.

6. The direct gram stain of the vaginal swabs could predict the type of bacteria that grows on cultures in more of the cases.
7. The high prevalence of extragenital bacterial STIs in MSM, and co-infection with HIV and syphilis, suggest that screening is valuable in identifying cryptic pregnancy and contributing to better care.

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