Study of the prevalence and association of ocular chlamydial conjunctivitis in women with genital infection by *Chlamydia trachomatis, Mycoplasma genitalium* and *Candida albicans* attending outpatient clinic

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Abstract

- **AIM:** To determine the association between chlamydial conjunctivitis and genital infection by *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Candida albicans*, in addition to the possible relationship between cultured bacterial pathogens and ocular genital chlamydial infection.

- **METHODS:** This study was performed on 100 (50 symptomatic and 50 asymptomatic) women attending the Gynecological and Obstetric outpatient clinic of Alzahra hospital, Alazhar University. Simultaneously a conjunctival swab was taken from these patients. Polymerase chain reaction (PCR) was done on DNA extracted from both vaginal and conjunctival swab samples. Culture for both vaginal and conjunctival swabs was also done.

- **RESULTS:** *Candida albicans* was the predominant organism isolated by culture in 20% and 40% of conjunctival and vaginal swabs respectively. By the PCR method, ocular *Chlamydia trachomatis* was present in 60% of symptomatic women, while genital *Chlamydia trachomatis* infection was present in 30% of symptomatic women. The results of this method also indicated that 25/50 (50%) vaginal swabs were positive with PCR for *Candida albicans* versus 15/50 (30%) were PCR positive in conjunctival swab. *Mycoplasma genitalium* was present in only 10% of vaginal swabs. Concomitant oculogenital PCR positive results for *Chlamydia trachomatis* and *Candida albicans* were 30% and 28% respectively.

- **CONCLUSION:** Ocular *Chlamydia trachomatis* was associated with genital *Chlamydia trachomatis* in a high percentage of women followed by *Candida albicans*. Cultured bacterial organisms do not play a role in enhancement of *Chlamydia trachomatis* infection.

- **KEYWORDS:** *Chlamydia trachomatis*; *Mycoplasma genitalium*; *Candida albicans*; vaginal swabs; polymerase chain reaction

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INTRODUCTION

The composition of the normal ocular flora plays an essential role in the normal flora of the eye, maintenance of surface homeostasis and in preventing ocular infection. The interaction between normal flora and ocular surface is important for the study of ocular pathology. An effective treatment requires a proper identification of the colonizing organisms and the understanding of its relation with surface structures and host immune response[1]. The bacterial flora of the female genital tract is highly dynamic and the local microbial community consists of a large multitude of different species. The balance therein may be disturbed due to physiologic variability or the presence of disease-causing microorganisms. The latter may exert a specific effect on the local ecology, which may lead to major shifts in the nature of the microbial population. A different status or a variable mode of behavior of the host may cause similar effects as well. These changes in the natural stability of the microbial populations can have profound effects on host susceptibility to other pathogenic microorganisms [2]. *Chlamydia trachomatis* is a ubiquitous pathogen worldwide.
and causes ocular, urogenital and respiratory infections in humans. An estimated 92 million new cases of *Chlamydia trachomatis* infection occur each year[3].

*Chlamydia trachomatis* is classified into 15 distinct serovars based on antigenic variation of the ompA genes that encode the organism's major outer membrane protein (MOMP). The 15 different serovars display well-documented and unique tissue tropisms. Serovars A, B, Ba, and C are the causative agents of trachoma, the most common infectious cause of blindness worldwide. Serovars D-K are a major cause of urogenital tract infection worldwide but are not associated with blinding trachoma. However, serovars D-K can cause ocular infection when newborn infants acquire the organism during passage through the infected birth canal or when adults secondarily inoculate the eye with infected genital secretions. In humans, progression from uncomplicated mucosal infections to serious sequelae such as blindness or tubal factor infertility has been linked epidemiologically to either re-infection or persistent infection. Re-exposure or persistent infection is thought to drive an immunopathological inflammatory response resulting in tissue fibrosis and scarring [4]. About 0.3%-2% of genital infection with *Chlamydia trachomatis* are complicated by a chlamydial eye infection which is not sight threatening[5]. MOMP is regarded the dominant surface protein (60% of the total protein mass in the outer membrane), and consists of four variable domains interspersed between five constant domains. The four variable domains contain serovar specific epitopes. The five constant domains are highly conserved between the different serovars and contain several conserved CD4 and CD8 T cell epitopes[6].

*Chlamydia trachomatis* has currently emerged as the most common sexually transmitted (STD) pathogen. Chlamydial infection produces less severe symptoms than other STD diseases. These deceptively mild symptoms allow the infection to go unnoticed with minimal patient awareness until secondary or tertiary symptoms develop, also have a high risk to develop conjunctivitis and pneumonia. The sequelae of undetected and thus untreated infections like acute salpingitis and pelvic inflammatory disease lead not only to significant morbidity but far more importantly to infertility. Infertility due to *Chlamydia trachomatis* represents a preventable type of infertility, if detected early[7].

Early diagnosis is mandatory to avoid serious complications especially with the development of effective treatment. Confirmation of chlamydia infection usually depends on taking an appropriate specimen and a suitable laboratory-based diagnostic test[8].

The increasing prevalence of chlamydial disease has generated much interest in the development of a sensitive, specific and rapid techniques for the diagnosis of chlamydial infections. Diagnosis is frequently based on bacterial isolation in tissue culture media which is tedious and slow. polymerase chain reaction (PCR) has been used to detect *Chlamydia trachomatis* infections[9]. Mycoplasmas are the smallest free living bacteria, lacking a cell wall, colonies may take up to 3wk to develop and are usually very small[10].

One of its 4 human species, *Mycoplasma genitalium* is so fastidious and immensely difficult to isolate from clinical specimens. It lives on the ciliated epithelial cells of the urinary and genital tracts in humans. Its existence was reported in 1981 [11], and was eventually identified as a new species of *Mycoplasma* in 1983. It is a STD pathogen and is a cofactor in HIV transmission [12]. Specifically it causes cervicitis, pelvic inflammatory infection in women and urethritis in both men and women[11-14].

It is the third smallest genome sized organism [15]. The synthetic genome of *Mycoplasma genitalium* named *Mycoplasma genitalium* JCVI-1.0 was produced in 2008, becoming the first organism with synthetic genome. In 2014, researchers discovered a new protein called Protein M from *Mycoplasma genitalium*[16].

The development of PCR methods made it possible to detect *Mycoplasma genitalium* more readily. Fungal infections of the eye are important amongst the clinical conditions responsible for ocular morbidity and blindness. *Candida albicans* is implicated in a variety of ocular infections including keratomycosis, endophthalmitis and dacryocystitis[17].

Endogenous fungal endophthalmitis represents an intraocular dissemination of a systemic fungal infection. Among the different fungal species, *Candida albicans* is the most common cause of infection followed by *Aspergillus* species[18]. *Candida albicans* are commensal organisms that reside in the human body and are found normally in the female genital tract. These fungi are kept in check by the host normal immune response[19].

Genital candidiasis occurs mainly in women referred as vulvovaginal candidiasis (VVC). VVC is one of the most common causes of infectious vaginitis. Approximately three-quarters of women will experience an episode of VVC at least once in their life and 5%-8% of them will have more than four attacks within a year; this condition has been designated as recurrent vulvovaginal candidiasis (RVVC)[20]. VVC is classified by the World Health Organization (WHO) as a pathological condition that is frequently STD. VVC has been considered to be an important worldwide public health problem[21].

**SUBJECTS AND METHODS**

**Subjects** The study adhered to the tenets of the Declaration of Helsinki and was approved by Ethics Committee of Gynecological and Obstetric Department of Alzahra Hospital, Alazhar University, Cairo, Egypt. All patients were...
informed regarding the procedure with written consent. This study was carried out after obtaining the approval from the Ethical Committee of Gynecological and Obstetric Outpatient Department of Alzahra Hospital, Alazhar University, Cairo, Egypt.

This study was performed on 100 (50 symptomatic and 50 asymptomatic) women attending the Gynecological and Obstetric Outpatient Clinic of Alzahra Hospital, Alazhar University. Women attending outpatient clinic with symptoms: vaginal discharge, itching and lower abdominal pain ($n=50$) formed the study group while women in outpatient clinic for contraceptive advice, follow up after abortion and delivery ($n=50$) formed the control group. We exclude those women who are not sexually active; below 15 years old and above 45 years old, pregnant, menstruating and receiving antibiotics. A full history was taken from them including concomitant eye symptoms e.g., redness, discharge, itching, pain, photophobia, e.t.c. Those with concomitant eye symptoms were chosen in our study. We exclude cases with allergic conjunctivitis.

**Samples** Vaginal swabs were collected from both groups. Simultaneously a conjunctival swab was taken from these patients. The samples were divided in two parts: one part stored at -70°C for PCR evaluation, another part was used immediately for culture diagnosis. Vaginal and conjunctival swabs were transported in Amies transport medium for culture.

**Culture Diagnosis** The samples were inoculated directly onto sheep's blood agar, chocolate agar, Sabouraud dextrose agar (SDA) and Wilkins Chalgenre anaerobic agar. Deep inoculation in brain heart infusion (BHI) broth was also done. The inoculated media were incubated at 37°C, examined daily, and discarded after 5d if no growth was observed. The inoculated SDA was incubated at 27°C, examined daily and discarded after 14d if no growth was detected while Wilkins Chalgren anaerobic agar was incubated anaerobically in anaerobic jar at 37°C and examined after 5d. Following adequate growth of the fungal isolates on SDA, identification was done based on its macroscopic and microscopic features using lactophenol cotton blue stain solution. If positive bacterial growth was obtained on the different culture media, the standard biochemical tests were performed and further identification was done up to the species levels using the API STAPH and API 20 NE systems.

**Polymerase Chain Reaction**

**Sample collection and DNA extraction** DNA from all vaginal and conjunctival swabs was extracted within one month of receipt. Briefly, DNA was extracted from each vaginal and conjunctival swab using QIA amp DNA Blood Mini kit and QIA amp DNA Micro extraction kit from Qiagen respectively according to manufacturer's instructions. QIA shredder was also used to harvest the lysate.

**DNA Amplification**

**Conditions for Candida species** PCR reaction mix (25 μL for Candida genotyping: the mix contained DNA 1 μL, taq (5 U/μL) 0.2 μL, primers (0.125 μL Forward + 0.125 μL Reverse) (50 pmole), dNTPs 4 μL (2 mmol/L), 5x buffer (Go Taq reaction buffer) 5 μL complete with dist.H2O. PCR program: initial denaturation 95°C for 5min, denaturation 95°C for 30s, annealing 61°C for 30s, extension 72°C for 20s, 40 repeating replication cycles, final extension 72°C for 7min. By using CAFOR 2-9 and CAREV3-9 primers, product size 402 bp (add 210 μL dist.H2O to CaFor2-9 and 260 to CaRev3-9).

**Conditions for Chlamydia trachomatis** The primers used were derived from highly conserved regions of the published DNA sequences for the MOMP of C. trachomatis serovars[22-24]. All serovars produced the same intensity 144 bp fragment[23]. In brief, 2 μL of DNA extracts was processed in a 30 μL reaction volume containing PCR buffer [10 mmol/L Tris (pH 9.0), 50 mmol/L KCl, 0.01% gelatin], 200 μmol/L deoxynucleoside triphosphates, 2.5 mmol/L MgCl2, 0.5 μmol/L each primer, and 1 U of Taq polymerase. Amplifications were carried out in a mastercycler. The first cycle, consisting of a 5min denaturation at 94°C, was followed by 35 cycles each of 30s at 94°C, 45s at 56°C, and 1min at 72°C, with a final extension for 10min at 72°C. The PCR products were visualized in 2% agarose gels containing 0.5 μg of ethidium bromide/mL.

**Conditions for Mycoplasma genitalium** Reaction mixture was prepared in a PCR tube by combining the reagents as follows: PCR master mix 12.5 μL, DNA template 2.5 μL, Primer1 MgpaW1 2 μL, Primer2 MgpaR 2 μL, H2O 6 μL, total volume 25 μL. PCR tubes were then placed in a thermal cycler and PCR amplification was done according to the following program.

Temperature cycling program, step 1: initial denaturation at 95°C for 10min. Step 2: 35 cycles of denaturation at 94°C for 40s; annealing at 56°C for 40s; elongation at 72°C for 40s. Step 3: final extension at 72°C for 15min. PCR products were visualized and a band of 495 bp in size indicates a positive result for Mycoplasma genitalium.

The primers used in this study, their sequence, product size and references are: 1) *Chlamydia trachomatis*, 144 bp, Fallah et al [9]: CT1: CCT/CGT/GGG/AAT/CCT/GCT/GCT/GAA CT4: GTC/GAA/AAA/GAA/GTCATCCAGTA/GTA. 2) *Candida albicans*, 402 bp, Jaeger et al [23]: CAFOR2 -9: GGG AGG TAG TGA CAA TAA ATA AC; CAREV3 -9: CGT CCC TAT TAA TCA TTA CTA CGA T. 3) *Mycoplasma genitalium*, 495 bp, Nassar et al[26]: MgPaW1: -5-AAG TGG AGC GAT CAT TAC TAA C-3; MgPaR1: -5-CCG TTG TTA TCA TAC CTT CTG A-3.

**Statistical Analysis** Data was analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL,
USA). Numerical data were expressed as mean and standard deviation and range. Qualitative data were expressed as frequency and percentage. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric *T*-test). Kappa test was used to evaluate agreement between two diagnostic methods. The tests were two-tailed. A *P* <0.05 was considered significant.

**RESULTS**

The study included 100 women, 50 symptomatic and 50 asymptomatic serving as control group. Symptomatic women with concomitant eye symptoms were chosen for our study. Their mean age was 28.6y, with a minimum and maximum range of 22 to 45y respectively, standard deviation 4.6y.

In the 50 conjunctival swabs, *Candida albicans* was isolated from 20% of cases, while each of *Staphylococcus aureus* and *Staphylococcus epidermidis* was isolated from 8% of cases and finally *Streptococcus pneumoniae* was isolated from only 4% of cases.

In the 50 vaginal swabs, *Candida albicans* was isolated from 40% of cases, followed by *Staphylococcus aureus* (16%), each of *Pseudomonas aeruginosa* and *Streptococcus agalactiae* was isolated from 12% of cases and *Klebsiella spp.* was isolated from only 5% of cases.

The positivity of PCR results for the 50 conjunctival swabs revealed that *Chlamydia trachomatis* was positive in 30 (60%) and *Candida albicans* positive in 15 (30%) of cases, while *Mycoplasma genitalium* was PCR negative in all cases. As for the 50 vaginal swabs obtained, the PCR results indicated that *Candida albicans* exhibited a high positivity PCR in half of the cases, 25 (50%), followed by *Chlamydia trachomatis* which was positive in 15 (30%) of cases. Regarding *Mycoplasma genitalium*, only 5 (10%) were PCR positive out of the 50 vaginal swabs.

The culture results (vaginal and conjunctival) of the 30 positive *Chlamydia trachomatis* conjunctival swabs by PCR showed that the bacterial growth pattern for the conjunctival culture indicated that gram positive cocci were the only organisms isolated with *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated in 3 (10%) cases each, followed by *Streptococcus pneumoniae* in 2 (6.7%) cases, while vaginal culture growth pattern showed that both fungal and bacterial organisms were isolated, with *Staphylococcus aureus* and *Streptococcus agalactiae* isolated in 7 (23.3%) and 4 (13.3%) cases respectively, Gram negative *Klebsiella spp.* and *Pseudomonas aeruginosa* were isolated in 5 (16.7%) and 3 (10%) cases respectively, while the fungus *Candida albicans* was cultured in 6 (20%) cases.

The conjunctival and vaginal culture in the 15 positive vaginal *Chlamydia trachomatis* by PCR showed that the map of organisms isolated from the conjunctival culture depicted the isolation of *Staphylococcus epidermidis* and

| Table 1 Relation between *Chlamydia trachomatis* conjunctival and *Chlamydia trachomatis* vaginal PCR |
|---------------------------------|---------------------------------|---|
| **CT VAG PCR** | **CT CONJ PCR** | **Total** |
| **Negative** | **Positive** | **Total** |
| Count | 20 | 15 | 35 |
| % within CT VAG PCR | 57.1 | 42.9 | 100.0 |
| % within CT CONJ PCR | 100.0 | 50.0 | 70.0 |
| **Positive** | Count | 0 | 15 | 15 |
| % within CT VAG PCR | 0.0 | 100.0 | 100.0 |
| % within CT CONJ PCR | 0.0 | 50.0 | 30.0 |
| **Total** | Count | 20 | 30 | 50 |
| % within CT VAG PCR | 40.0 | 60.0 | 100.0 |
| % within CT CONJ PCR | 100.0 | 100.0 | 100.0 |

CT: *Chlamydia trachomatis*; VAG: Vaginal; CONJ: Conjunctival; PCR: Polymerase chain reaction. Significant moderate association between the *Chlamydia trachomatis* conjunctival and *Chlamydia trachomatis* vaginal (Kappa=0.444, *P*<0.001).

*Staphylococcus aureus* in 2 (13.3%) and 1 (6.7%) cases respectively; while 80% of the cases gave a negative culture, while vaginal culture organisms isolated were a mixture of gram positive and gram negative, with *Staphylococcus aureus* and *Klebsiella spp.* were isolated in 4 (26.7%) cases each, followed by *Pseudomonas aeruginosa* isolated in 2 (13.3%) cases.

The relation between conjunctival and vaginal *Chlamydia trachomatis* by PCR as seen in Table 1, shows the agreement between the two results, in which the percentage of positive agreement and negative agreement for them was 50% and 100% respectively. By statistical analysis using Kappa measurement of agreement, there was a significant moderate agreement between conjunctival *Chlamydia trachomatis* and vaginal *Chlamydia trachomatis* (Kappa=0.444).

In addition, none of the patients were conjunctival *Chlamydia trachomatis* negative and vaginal *Chlamydia trachomatis* positive by PCR, while 50% of the patients were conjunctival *Chlamydia trachomatis* positive and vaginal *Chlamydia trachomatis* negative by PCR. The difference between the results were significant (*P*<0.001).

In Table 2, which shows the agreement between conjunctival and vaginal *Candida albicans* by PCR, it is seen that there is a significant fair association between those two results. The percentage of positive and negative agreement was 80% and 62.9% respectively (Kappa=0.360). Also 52% of patients were conjunctival negative and vaginal positive for *Candida albicans* by PCR, while 12% were conjunctival positive and vaginal negative *Candida albicans* by PCR. A fair significant difference between the results is seen (*P*=0.005).

A statistical agreement between the two tests was not significant due to the small number of *Mycoplasma genitalium* PCR positive data and in addition all *Mycoplasma genitalium* tests for conjunctival swabs by PCR
were negative. Similarly a statistical difference between the results of the two tests was not reached for the same reason. From the results obtained in the relation between *Chlamydia trachomatis* conjunctival PCR and *Candida albicans* vaginal PCR, it is seen that among the 30 patients who were positive conjunctival *Chlamydia trachomatis*, 8 were only positive vaginal *Candida albicans* by PCR, giving a 26.7% positive agreement; while among the 20 patients who were negative for *Chlamydia trachomatis* by conjunctival swabs, only 3 were negative vaginal *Candida albicans*, with a 15.0% negative agreement. Thus by statistical analysis there is a significant inverse moderate association between *Chlamydia trachomatis* conjunctival PCR and *Candida albicans* vaginal PCR (Kappa=-0.560, P<0.001).

Table 3 Relation between *Chlamydia trachomatis* conjunctival PCR and *Candida albicans* vaginal PCR

<table>
<thead>
<tr>
<th>MG VAG PCR</th>
<th>CT CONJ PCR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within MG VAG PCR</td>
<td>42.2</td>
<td>57.8</td>
</tr>
<tr>
<td>% within CT CONJ PCR</td>
<td>95.0</td>
<td>86.7</td>
</tr>
</tbody>
</table>

CA: *Candida albicans*; VAG: Vaginal; CT: *Chlamydia trachomatis*; CONJ: Conjunctival; PCR: Polymerase chain reaction. No association between the *Chlamydia trachomatis* conjunctival PCR and *Mycoplasma genitalium* vaginal PCR (Kappa=0.069, P=0.336).

Table 4 Relation between *Chlamydia trachomatis* conjunctival PCR and *Mycoplasma genitalium* vaginal PCR

<table>
<thead>
<tr>
<th>MG VAG PCR</th>
<th>CT CONJ PCR</th>
<th>Total</th>
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<tr>
<td>Negative</td>
<td>Positive</td>
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<tr>
<td>Count</td>
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</tr>
<tr>
<td>% within MG VAG PCR</td>
<td>40.0</td>
<td>60.0</td>
</tr>
<tr>
<td>% within CT CONJ PCR</td>
<td>100.0</td>
<td>100.0</td>
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</tbody>
</table>

CA: *Candida albicans*; VAG: Vaginal; CT: *Chlamydia trachomatis*; CONJ: Conjunctival; PCR: Polymerase chain reaction. Significant inverse moderate association between the *Chlamydia trachomatis* conjunctival PCR and *Candida albicans* vaginal PCR (Kappa=-0.560, P=0.001).

**DISCUSSION**

Chlamydia genital infections are the most prevalent STD bacterial infections in the world. WHO estimated that 90 million new cases of chlamydial infections occurred globally, and prevalence is highest in persons aged ≤25y [27] which in accordance with our results. Data show that the annual incidence of adult chlamydial conjunctivitis is increasing and correlates with the annual incidence of genital chlamydial infection in the region[28].

The aim of the research was to determine the prevalence of *Chlamydia trachomatis* conjunctivitis and urogenital infections coexistence in adult females as there is general consensus that ocular infection in adults results from autoinoculation of infected genital secretions [29-30] from the patient or from his/her partner. Fifty women with genital symptoms were chosen for our study. Their mean age was 28.6y, with a minimum and maximum range of 22 to 45 respectively, standard deviation 4.6y.
In our study, the positivity of PCR results for the 50 conjunctival swabs revealed that *Chlamydia trachomatis* was positive in 30 (60%). As for the 50 vaginal swabs obtained, the results indicated that *Chlamydia trachomatis* was positive in 15 (30%) of cases. By analysis of our results, we found a positive and negative agreement of 50% and 100% respectively for concomitant oculogenital *Chlamydia trachomatis* by PCR (Kappa=0.444) (Table 1). In addition, none of the patients were conjunctival *Chlamydia trachomatis* negative and vaginal *Chlamydia trachomatis* positive by PCR. This substantiate that autoinoculation was a method of transmission of the organism from genital secretions to the eye [28-30]. This contradicts the study made by Tullo et al. [31]. Tullo et al. [31] suggested that chlamydial infection of the eye complicates no more than 1 in 300 chlamydial infections of the genital tract in adults. Stenberg and Mardh [30] showed that up to 77% of patients with symptomatic ocular infection have positive concomitant genital infection.

In addition, Postema et al. [9] concluded that 74% of women with chlamydial conjunctivitis had concomitant genital chlamydial infection. We find our results (50% positive agreement) are in close agreement with the latter 2 authors, but was not consistent with that reported by Insler et al. [28] who concluded that thirty patients attending a STD disease clinic were evaluated for genital and ocular infection with chlamydia. Eight patients had positive conjunctival immunofluorescent staining. Yet, 50% of the patients had *Chlamydia trachomatis* in the conjunctiva by PCR while the concomitant vaginal swabs were negative for *Chlamydia trachomatis*. This was in close accordance with study made by Postema et al. [9]. They reported that a genital infection with *Chlamydia trachomatis* could not be proven in 38% (24/64) of the patients with chlamydial conjunctivitis. This would give rise to speculation as to the source of ocular infection for these patients, when their vaginal samples were negative. In a study done by Stenberg and Mardh [30] they suggested that the genital infection may heal before the eye infection begin. They observed a mean delay of over 2wk between the first visit to the eye hospital and the 1st visit to the STD disease department.

Considering the strong association between chlamydial eye and genital infections and that chlamydial infections are often asymptomatic (70%-80% of women and up to 50% of men) [34-38], any physician treating a patient with a chlamydial genital infection should consider the probability of an ocular infection in this patient. Especially the ophthalmologists are important in this respect. Candida is a genus of yeasts and is the most common cause of fungal infections worldwide. Many species are harmless commensalism or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease. *Candida albicans* is representative of the important pathogens causing exogenous fungal endophthalmitis, endogenous endophthalmitis conjunctivitis and keratitis [30]. *Candida albicans* are found normally in the female genital tract. These fungi are kept in check by the host normal immune response. When a break down in the host's immune system occurs, fungi may spread throughout the body including the eye [29].

VVC is a mucosal infection caused by Candida species which is one of the most common clinical disorder in women of reproductive age [30-35]. The colonization and adherence of Candida species in epithelial cells are the first step in initiating of infection and creating VVC. Candida species has some adhesion molecules such as agglutinin like sequence and HWP1 in attachment to host cell surface [39,40]. Moreover *Candida albicans* is the most frequent colonizer and responsible for vaginal candidiasis. It is estimated that 75% of women will experience at least one episode during their life and 50% of them experience multiple episodes as well. The incidence of VVC is the highest rate in 20-40 year old women [41-42].

Despite availability of reliable and rapid methods for yeasts identification, precise diagnosis of yeasts continues to remain controversial based on the phenotypic criteria such as chlamydospores abundance, germ tube formation, and colony colors on the CHROMagar Candida medium. Identification of Candida isolates at the molecular level is crucial to assist in early diagnosis and for timely prescription of appropriate antifungal drugs [43].

The conventional identification of pathogenic fungi in the clinical microbiology laboratory involves the examination of colony and microscopic morphologies and the assessment of various biochemical reactions [43,44]. It often requires three or more days, and may be inaccurate [45].

This study used both culture and PCR to identify *Candida albicans* in a concomitant vaginal and conjunctival swabs obtained from symptomatic women attending the gynaecology outpatient clinic. By comparing both culture and PCR methods, our results showed that *Candida albicans* was isolated and detected in 20% and 30% of the 50 total conjunctival swabs by culture and PCR, respectively. As for the 50 vaginal samples, culture and PCR methods were able to detect *Candida albicans* in 40% and 50% of the cases, respectively. The culture and PCR results were almost similar in detection of the organism from oculogenital samples. In a study done by Liguori et al. [46] comparing biological methods to PCR for *Candida albicans* identification and stated the disadvantage of the former method in its lower...
sensitivity and specificity and suggested using it for screening and preliminary assays, while pointing out the precise and simple to implement PCR method. In their study, they reported the prevalence of *Candida albicans* among vaginal samples to be 65.1%, which was moderately higher than our results (50%). But these results differed from those obtained by Caldwell *et al.* where they reported the prevalence of vaginal candidiasis in the studied women population was 21% by PCR method, and *Candida albicans* was isolated by culture in only 6% of cases. The latter result is much lower when compared to our result (40% isolation rate), and also inconsistent from those obtained in other studies describing *Candida albicans* as the principal organism in infection statistics. 

Roudbary *et al.* stated that PCR method showed *Candida albicans* as the most frequently detected species (87.2%) in genital samples, and this result differed from our results, where the PCR method detected *Candida albicans* in half of the total 50 vaginal samples (50%). A logical explanation for this detection rate in our study could be due to the increased anti fungal resistance and recurrent candidiasis leading to the emergence of non albicans species and this is supported by, who isolated Candida glabrata as the second element after *Candida albicans*. Likewise, this was in agreement with, and with Rad *et al.* who reported that *Candida albicans* and Candida glabrata were the 2 most common causes of vulvo vaginal candidiasis in their studied population. 

Mintz and Martens conducted a study on prevalence of Candida spp. by culture and PCR in women with recurrent vulvovaginal symptoms. They reported that 30 out of 103 women (29.1%) tested positive for Candida spp., and that of these 30 positive women, 15 (50%) were positive for *Candida albicans* in vaginal swabs by culture/or PCR in each women. These results were consistent with our findings. Martens *et al.* reported an agreement rate of 43% between culture and PCR, where Candida was positive by culture in 21% of vaginal samples and 18% positive only by PCR. These findings were not consistent with our obtained results. These discrepancies in agreement between our results and those of others may be partially attributed to detection thresholds set by different labs. 

The overall incidence of ocular candidiasis in our patient study by culture and PCR (20% and 30% respectively) was observed in similar studies, with a comparable number of patients. Meanwhile, Khalid *et al.* reported a higher incidence of *Candida albicans* isolation (54%) by culture comparable to our results (20%). In a study done by Gaudio *et al.* comparing cultured PCR results, they reported that the sensitivity of PCR approximated that of culture in the detection of fungi in ocular surface scrapings, a finding that was similar to ours (30% and 20% respectively), although they studied a lower number of cases (30 clinical specimens) than ours. 

Imran and Al-Asadi were able to isolate Candida spp. from 35 (21%) conjunctival swabs out of 165 patients by culture; and reported that *Candida albicans* was detected with the highest frequency by PCR (33%). We find that our results are in close agreement with their study regarding culture and PCR in conjunctival samples (20% and 30% respectively). Saha and Das pointed out that *Candida albicans* was a common pathogen in 45.8% of keratitis cases. Oude Lashof *et al.* reported an epidemic of ocular candidiasis in 370 patients of which 13% gave consistent finding with a positive diagnosis of ocular fungal infection. Their results showed high prevalence of ocular candidiasis (56.4%) with *Candida albicans* (21.2%) as the most frequent cause of ocular candidiasis. 

We find that these results are more or less coincident with our results regarding PCR and culture methods. On the other hand, Durand mentioned high prevalence of ocular *Candida albicans*, accounting for 92% of cases in Iraq who explained this high rate due to lesser care and imprecise diagnosis. This result was contrary to ours, which identified *Candida albicans* by culture and PCR at a much lower percentage and we accredit this low rate to inadequacy of diagnosis. 

In our study in an attempt to show the possible association of *Candida albicans* (by culture) in chlamydial conjunctivitis patients. Our results revealed that *Candida albicans* was genitally isolated in 20% of the total chlamydial conjunctivitis patients (30 cases); while it was not isolated in any of the ocular samples. This could be attributed to that culture method was not adequate in the isolation of *Candida albicans* from ocular samples or due to the presence of *Chlamydia trachomatis* in the eye and preventing the habitation of other pathogens by competence; in addition the vaginal environment favours the growth of *Candida albicans* more than the ocular environment, thus *Candida albicans* being one of the vaginal flora organisms. 

These results highlight the demerits of the routine conventional assay, which is also in agreement with the reports of Ahmed *et al.* which from their view confirm that phenotypic tests does not always give much credit for complete *Candida albicans* identification. We agree with their statement and that of Ferrer and Alio that molecular methods are increasingly used for the rapid detection of *fungal spp.* as a whole. 

In the present study, we tried to evaluate the agreement between the results of concomitant oculogenital *Candida albicans* by PCR in the studied patients, using the Kappa measurement of agreement. In addition, the difference between the results of the ocular and the genital *Candida albicans* by PCR performed on the same group of patients was evaluated using the McNemar's test (Table 2). From the
obtained results, there was a high detection rate for *Candida albicans* in concomitant vaginal and conjunctival swabs (80%), elucidating a significant fair positive agreement for the two results. Likewise, the percentage of negative agreement (62.9%) was fairly significant (Kappa=0.360). This agreement of concomitant oculo-genital *Candida albicans* infection substantiates the autoinoculation transfer of infection in individual patients, as reported by Melvin[63] who stated that it was usually considered, albeit with little or no direct evidence, that infected genital tract secretion is transferred to the eye by the hands.

Hassan *et al* [64] presented a case of *Candida albicans* endogenous endophthalmitis by PCR in a patient with vaginal *Candida albicans* infection and claimed the transfer of the fungus through the blood stream, where yeasts can originate from a primary fungal infection leading to candidaemia. Their results are different from ours and this is attributed to the nature of location of the fungus, where in our study, *Candida albicans* was detected in the conjunctiva swabs, a superficial layer of the eye, thus easily infected by autoinoculation through contaminated hands; while in their study[64], the candidal infection affected the deep tissues of the eye (endophthalmitis), thus necessitating the transfer of pathogen through the blood stream.

Another study done by Postema *et al* [5] was partially consistent with our results, where they found no relationship between the eye infected and whether the individual was left or right handed, although they assumed that eye infection was the result of autoinfection with infected genital secretion and reported a 74% rate of concomitant urogenital *chlamydial* infection, they could not account for a concomitant genital infection with the pathogen in 38% of patients. Their explanation was based on the suggestions of Stenberg and Mardh [38] who claimed that genital infection may heal before the eye infection begins. In addition to the possible use of antibiotics prior to genital samples taken.

We attempted to evaluate the possible relation between chlamydial conjunctivitis and genital infection with *Candida albicans* by PCR in the studied patients group. From the results obtained in this work and by statistical analysis, it was found that there was a significant inverse agreement regarding this association. This was not expected, based on the fact that chlamydial eye infection is a chronic disease, necessitating its treatment with long term antimicrobials, thus encouraging overgrowth of commercial *Candida albicans* in the mucous membranes especially the vagina[65].

Several studies were undertaken addressing this possible relation between *Chlamydia trachomatis* eye infection and fungal infection. Barchiesi *et al* [66] stated that individuals with chronic eye infections and exposed to broad spectrum antibiotics for a long period are more disposed to fungal infection.

Calderone and Clancy [66] reported that vaginal candidiasis is commonly observed in immune competent people with predisposing factors such as antibiotics. On the other hand, the results obtained by the works of Katharine Sturm-Ramirez *et al* [67] were in accordance with those of our study. They studied the prevalence of *Chlamydia trachomatis* and distribution of risk factors and reported that yeast infections were identified among 39 cases in 722 patients tested (5.9%).

In support of our findings were the results obtained by Schachter [68]. They stated that the presence of yeast in genital specimens was associated with lower risk of *Chlamydia trachomatis* infection, explaining that yeast infection indicates an overgrowth of the organism in the genital tract, thus inhibiting further infection with *Chlamydia trachomatis* or indirectly hindering the growth of *Chlamydia trachomatis* by rendering the genital environment inhospitable. Consequently a low auto transmission of *Chlamydia trachomatis* infection from the hands to the eyes and a decreased detection rate of ocular *Chlamydia trachomatis* infection. This was consistent with our results elucidating the inverse significant difference between the results, where 85% of the patients were conjunctiva *Chlamydia trachomatis* negative and vagina *Candida albicans* positive by PCR, and 73.3% were positive conjunctiva *Chlamydia trachomatis* and negative vagina *Candida albicans* by PCR (*P*<0.001).

The aforementioned authors further stated that the use of antibiotics could affect the balance of vaginal flora there by limiting or even cleaning chlamydial infections. The association of chlamydial ocular infection with other pathogens was studied by several workers, Kaul *et al* [69] who showed the association of *Chlamydia trachomatis* with bacterial vaginosis. Concurrent gonorrhea which could reactivate latent chlamydial infection was reported by Batteiger *et al* [70]. In addition Avery and Sullivan [71] stated that chlamydial ocular infections were also associated with concomitant syphilis.

Bagshaw and Edwards [72], McCormack *et al* [73] respectively, elucidated the assumption that *Chlamydia trachomatis* can occur in patients with other studies, but not to a statistical significance.

*Mycoplasma genitalium* was first discovered in 1981, but more extensive studies of *Mycoplasma genitalium* could not be conducted until the development of sensitive and specific PCR techniques, because the bacterium is extremely difficult to grow. In addition to urogenital and urine samples, *Mycoplasma genitalium* has been detected by PCR in rectal, synovial fluid, and respiratory tract specimens[74].

In our study all *Mycoplasma genitalium* tests for conjunctival swabs by PCR were negative and this was not in agreement with studies by Bjornellius *et al* [75] who described the first case of detection of *Mycoplasma genitalium* in a
conjunctival swab specimen from a patient with chronic conjunctivitis. Both the conjunctival swab and the urine specimens were positive for *Mycoplasma genitalium* DNA. At a follow-up visit 6 wk later, the clinical signs and symptoms had diminished. They attributed this case of *Mycoplasma genitalium*-associated conjunctivitis to have been caused by contamination of the eye with genital secretions, substantiated by the fact that the patient was found to have urethritis and prolonged intermittent dysuria and that urine samples were found to be positive for a strain of *Mycoplasma genitalium* with a sequence identical to the sequence obtained from the conjunctival specimen.

Mycoplasmas are often present in the genital tract of women who have no symptoms or abnormalities; they are usually found together with other more virulent organisms, and they have been isolated from sterile sites without producing disease.

Regarding the prevalence of *Mycoplasma genitalium* in the genital swabs of the female patients in our study, only 5 (10%) were PCR positive out of the 50 vaginal swabs. Our results showed a moderately higher percentage than a study made by Walker et al. where they reported a prevalence of *Mycoplasma genitalium* to be 2.4% among young Australian women. They explained this relatively low percentage in their study by speculating that *Mycoplasma genitalium* is a rather conflicting organism in its transmission either concomitantly or sexually. In addition, they compared the prevalence of *Chlamydia trachomatis* with *Mycoplasma genitalium* in genital specimens of women and found that *Chlamydia trachomatis* prevalence was higher (4.9%) than that of *Mycoplasma genitalium* (2.4%) and this was in agreement with our study. In our study, the prevalence of genital *Chlamydia trachomatis* compared to *Mycoplasma genitalium* was 30% and 5% respectively. And this was attributed to: it is possible that *Mycoplasma genitalium* is less infectious than *Chlamydia trachomatis*: it requires a greater "exposure" or direct genital or cervical contact to acquire *Mycoplasma genitalium*. This is supported by the 100 fold lower organism load among samples from women with *Mycoplasma genitalium* compared with *Chlamydia trachomatis* and the finding that *Mycoplasma genitalium* was more strongly associated with unprotected sex than *Chlamydia trachomatis*.

Clearly, further partner studies are needed to investigate the transmission dynamics for *Mycoplasma genitalium* and *Chlamydia trachomatis* to determine if and how transmission dynamics differ. This explanation was consistent with that given by Walker et al. We can conclude that ocular *Chlamydia trachomatis* was associated with genital *Chlamydia trachomatis* in a high percentage of women followed by *Candida albicans*.

Cultured bacterial organisms do not play a role in enhancement of *Chlamydia trachomatis* infection.

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Oculogenital chlamydial infection