Review Article

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Genital *Chlamydia trachomatis*: An update

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Chlamydia trachomatis **is the most common cause of curable bacterial sexually transmitted infection (STI) worldwide. It manifests primarily as urethritis in males and endocervicitis in females. Untreated chlamydial infection in man can cause epididymitis and proctitis. Though most women with *Chlamydia* infection are asymptomatic or have minimal symptoms, some develop salpingitis, endometritis, pelvic inflammatory disease (PID), ectopic pregnancy and tubal factor infertility. It is associated with an increased risk for the transmission or acquisition of HIV and is also attributed to be a risk factor for the development of cervical carcinoma. Early diagnosis and treatment of infected individuals is required to prevent the spread of the disease and severe sequelae. Traditionally, tissue culture was considered the gold standard for the diagnosis. However, with the availability of newer diagnostic techniques particularly molecular methods which are not only highly sensitive and specific but are cost-effective also, the diagnosis has became fast and easy. The purpose of this review is to study the various aspects of genital *C. trachomatis* infection. Also the advances related to the clinical picture, various diagnostic modalities, prevention, treatment, drug resistance and control measures will be dealt with.**

Key words *Chlamydia trachomatis* - epidemiology - genetics - immunology - treatment

Introduction

Chlamydia trachomatis is the most common bacterial sexually transmitted infections worldwide[1], and women carry the major burden of the disease. These women are also a potential source of infection to their partners. It causes urethritis in men and mucopurulent cervicitis, urethritis, and endometritis in women. Mucopurulent cervicitis can lead to at least three types of complications[2] - ascending intraluminal spread of organism from cervix producing pelvic inflammatory disease (PID); ascending infection during pregnancy resulting in premature rupture of the membrane, chorioamnionitis, premature delivery and puerperal and neonatal infections (conjunctivitis and possibly intestitial pneumonia); and also an increased risk of the development of cervical carcinoma[2]. A 3- to 4-fold increased risk of transmission of HIV is an added cause

of concern[3]. The incidence of chlamydial infections in women has increased dramatically from 79 to 467 per 100,000 between 1987 and 2003[4]. According to the World Health Organization (WHO)[1], 101 million chlamydial infections are detected annually worldwide. The clinical presentation, course, complications and late sequelae of *C. trachomatis* closely resemble *Neisseria gonorrhoeae* infection.

C. trachomatis is also considered to be a leading cause of PID and female infertility worldwide. More than 13.5 per cent of women less than 25 yr old infected with *C. trachomatis* have lower genital tract infection, reducing to 4.4 per cent in women 25 yr and above[5]. In USA, approximately 20-30 per cent of PID cases have been attributed to *C. trachomatis*[6]. Recent studies from India have revealed the prevalence of *C. trachomatis* infection to be 23 per cent in gynaecology outpatient department (OPD)[7] and 19.9 per cent in STD patients[8].

It has been recovered from 30-60 per cent cases of salpingitis and PID[9] patients in India, while sero-prevalance is shown to be higher in at least one recent study[10]. An estimated 15-40 per cent of women with cervical chlamydial infections develop PID[11]. Twenty per cent of women who develop PID become infertile, 18 per cent develop chronic pelvic pain, and nine per cent have a tubal pregnancy[12].

Neonates are also at risk while passing through the contaminated birth canal during parturition. Screening young women for *Chlamydia* has been proven to be a cost-effective method of preventing PID. The US Preventive Services Task Force (USPSTF)[13] recommends that all women aged ≤24 yr receive routine screening for *Chlamydia*. However, insufficient evidence was found to recommend for or against routine screening for chlamydial infection in asymptomatic males[13].

The challenge being faced in the control of chlamydial disease is that as many as 70-80 per cent of women and up to 50 per cent men have asymptomatic infection[14]. This results in a large reservoir of unrecognized, infected individuals who are capable of transmitting the infection to their sexual partners. Further, the sequelae of *C. trachomatis* infection in women, namely PID, infertility and ectopic pregnancy, is the most costly outcome of any STD except HIV or AIDS[15]. This review covers the various aspects of genital chlamydial infection as also the clinical picture, various diagnostic modalities, prevention, treatment and control measures.

Chlamydia trachomatis - the microorganism

Chlamydiae are spherical or ovoid obligate intracellular bacteria that are ubiquitous. Intracellular parasitism of *Chlamydia* differentiates it from other bacteria. Unlike viruses, Chlamydiae possess both DNA and RNA, multiply by binary fission rather than self-assembly, contain their own ribosome, have a peptidoglycan free cell wall and are susceptibile to various antimicrobial agents[16].

Immunopathogenesis

C. trachomatis is a strong immunogen, which stimulates both humoral and cell mediated immune responses. In addition to the immunogenic antigens, the outcome of chlamydial infection depends on interaction and balance of cytokines secreted by the activated lymphocytes. Interferon gamma (IFN-**γ)** has been described as a single most important factor in host defense against *Chlamydia*, while disease susceptibility has been linked with enhanced expression of Interleukin- 10 (IL-10)[17]. Immune system changes or disturbances induced by *C. trachomatis* may favour its own survival in the infected host, and induce persistent infections.

C. trachomatis infection may be primary or a chronic recurrence/ re-infection.

(i) Primary infection: A serial infection of the mucosal cells is seen during the primary infection. The damaging and infected epithelial cells secrete numerous pro-inflammatory chemokines and cytokines, including IL-1, IL-6, IL-8, granulocyte - macrophage colony stimulating factor (GM-CSF), growth regulated oncogene, and tumour necrosis factor alpha (TNF-α)[18,19]. The released cytokines cause vasodilatation, increased endothelial permeability, activation and influx of neutrophils, monocytes and T-lymphocytes, and elevated expression of adhesion molecules. In addition, it stimulates other cells to secrete cytokines. Neutrophils appear to play a role in reducing the initial amplification of *C. trachomatis* and possibly in limiting the spread within the female genital tract. IL-1 is secreted initially by the undamaged cells and stimulates the secretion of other cytokines from other non-infected cells, like TNF-α[20]. During the same period, *Chlamydia* passes via lymphatic vessels to local lymph nodes. The decaying epithelial cells release a few elementary bodies which are phagocytosed by neutrophills through phagolysosomes.

T lymphocytes mainly T helper cells (Th1) play an important role during early phase of infection,

which, due to *Chlamydia* antigen-induced activation, secrete IFN-γ, necessary for infection regression. It increases the potential of various phagocytes to destroy *Chlamydia* and stimulates the secretion of other cytokines, including IL-1. IL-1, in turn, by stimulating the secretion of IL-2 by Th1 cells causes increased replication of cytotoxic lymphocytes and natural killer cells[21]. The role of secretory IgA has also been established in the neutralization of primary infection[22].

An intimate relationship between chlamydia and the host immune system has been described by Paavoven[23]. It has been observed that a single acute episode of chlamydial infection cannot lead to serious sequelae associated with this infection, persistent infection may be responsible for the grave consequences.

(ii) Chronic infection - recurrence/reinfection: Chronic infection, associated with persistence of *Chlamydia* in the host cells, recurrent infection or reinfection are more dangerous. A delayed hypersensitivity reaction or rarely type 3 hypersensitivity reactions (Arthus reaction) is observed in long term or recurrent stimulatory action of chlamydial antigens[23]. Antibodies are not involved in the delayed type of reaction developing within 24-48 h due to antigen interaction with specifically sensitized Th1 lymphocytes. Processes which occur during these reactions lead to tissue damage, fibrosis and cicatrization within the affected organs. Irreversible consequences like PID leading to mechanical infertility, ectopic pregnancy, chronic pelvic pains and chronic urethritis may occur. After a single episode of salpingitis about one in 10 patients become infertile because of tubal occlusion. After 2-3 episodes, infertility ensues in about 35-70 per cent cases. In several studies, repeated chlamydial infection was associated with PID and other reproductive sequelae, although it was difficult to determine whether the risk per infection increased with recurrent episode[25]. Lack of treatment or improper therapeutic management may result in chronic infection. A significant role of dietary factors like insufficient supply of tryptophan, L-isoleucine, and cysteine in diet, as well as certain cytokines like INF-γ, TNF-α, transforming growth factor has been observed[26].

Formation of atypical chlamydial forms *in vitro* has been demonstrated in INFγ treated cells. The atypical forms[26] are large, non-infectious, have reduced metabolic activity, and do not replicate, yet remain alive. Such atypical forms display decreased levels of chlamydial major outer membrane protein (MOMP) and lipopolysaccharide (LPS) antigens but continue with high production of chlamydial heat shock protein 60 (hsp60), which is capable of inducing chronic inflammation and scarring. Chronic and occult infections pose several diagnostic and therapeutic problems. Due to the variable antigenic structure of atypical forms, the routine diagnostic methods do not always identify them. Moreover, these forms have reduced MOMPs which lead to decreased transport of antibiotic across the cell. Therefore, in case of chronic infections, therapy frequently results in failure.

Reinfection is due to the repeated infection, while recurrence is caused by the presence of a *Chlamydia* reservoir in the lymph node and spleen[26]. Macrophages have been found to play an important role in the recurrence of infection as *C. trachomatis* circulates within the macrophages, finding a temporary shelter in the lymph nodes, spleen and serous cavities. It has been observed that recurrences were more frequent in young patients with prolongation of the active period in comparison with patients in older age group[27]. The less common spread of infection in the older age group has been attributed to low exposure to *C. trachomatis* and by physiological changes which reduce sensitivity to the acquisition[26].

Risk factors and demographic factors for *Chlamydia trachomatis* infection

The most common demographic correlate[16] of infection with chlamydial infection in women is young age (<20 yr). This could be explained by the anatomic differences in the cervix of the younger women, wherein the squamo-columnar junction, a primary host target for *C. trachomatis*, is everted and thus more exposed. Other factors associated with chlamydial infection include unmarried status, nulliparity, black race and poor socio-economic condition[28]. A large number of sexual partners, a new sexual partner, lack of use of barrier contraceptive devices and concurrent gonococcal infection are also known to be associated with chlamydial infection[29]. Cervical chlamydial infections are also found to be associated with the use of oral contraceptives[29].

Epidemiology

C. trachomatis was detected in 23.0 per cent of patients attending gynaecology OPD[7] and among 19.9 per cent patients attending STD clinic in a study from New Delhi[8].

In Mumbai, in a study conducted in female sex workers (FSW) and married contacts, attending a STD clinic 23.2 per cent chlamydial positivity was found[30]. In a study from Aligarh[31], *C. trachomatis* was detected in 28.1 per cent of infertile women. The prevalence of *C. trachomatis* in asymptomatic and symptomatic women attending a gynaecology clinic at Delhi was 4 and 30.4 per cent, respectively[32].

Anti-chlamydial IgG antibodies were present in 68 per cent of women with infertility, 50 per cent with bad obstretic history (BOH) and 10 per cent of healthy pregnant women, in a study conducted in Amritsar district in Punjab, India[33]. Joyee *et al*[34] found the prevalence of *Chlamydia* in STD patients to be 30.8 per cent, while in another study, prevalence of *C. trachomatis* infection in male patients with urethritis was found to be 17.5 per cent[35].

A study from UK[36] has shown that health care settings had higher prevalence estimates than population based studies**.** Among less than 20 years, prevalence estimates were 17.3 per cent in genitourinary clinics, 12.6 per cent in antenatal clinics, 12.3 per cent in termination of pregnancy clinics, 10.7 per cent in youth clinics, 10.0 per cent in family planning clinics compared to 5.0 per cent in population based studies. Vuylsteke *et al*[37] reported 7.3 per cent prevalence of *C. trachomatis* in females attending STD/genitourinary clinic in Belgium. In Europe[38], *C. trachomatis* infection prevalence was estimated to be 5 to 12 per cent for women undergoing termination of pregnancy. Studies in Latin America show *C. trachomatis* prevalence rates of 1.9 to 4.5 per cent in Chile, Peru, Brazil, and Mexico[39,40] and 12.2 per cent[41] in women attending family planning clinics in Jamaica.

Diagnosis

***Clinical diagnosis*:** Clinical picture of the patients suffering from chlamydial infection could be misleading as up to 70-80 per cent of the infected women and 50 per cent of the infected men are asymptomatic. Typically, a female with uncomplicated chlamydial infection will present with odourless, mucoid vaginal discharge without pruritis. Dysuria without frequency or urgency will be complained of if urethra is involved. Further, in PID, history of severe abdominal pain with high fever, dyspareunia, prolonged menstrual cycles and intermenstural bleeding can be elicited. On examination, cervicitis with a yellow, cloudy, mucoid discharge can be seen from the os. The cervix tends to bleed easily when scraped with spatula or brush. Urinalysis will reveal the presence of >5 WBC/HPF (high power field), which is suggestive of urethritis[13]. Chlamydial infections cannot be distinguished from other urethral infections clinically. Amine test (*i.e.*, significant odour release on addition of KOH to the vaginal secretion) can help differentiate chlamydial infections from other lower genital tract infections but has a low specificity.

Chlamydial infection in males manifests as urethritis in 15-55 per cent of the affected less than or equal to 35 yr, occasionally epididymitis may be seen[2]. Mild to moderate clear to white urethral discharge is seen in the morning before the patient voids. In epididymitis, history of unilateral testicular pain with scrotal erythema, tenderness or swelling over the epididymis may be elicited. The diagnosis can be established by the presence of mucopurulent discharge from penis which on Gram staining shows >5 WBC/HPF and absence of intracellular Gram negative diplococci. Reiter's syndrome may be a rare complication of untreated chlamydial infection. A reactive arthritis that includes triad of urethritis/cervicitis in females, conjuntivitis and painless mucopurulent eruption on palms and soles of feet is seen in Reiter's syndrome[29]. Female are more commonly affected than males. There is asymmetrical multiple joint involvements with predilection for lower extremities.

***Laboratory diagnosis*:** Asymptomatic nature of the disease and the increasing spectrum of infections caused by *C. trachomatis* emphasize the need for the sensitive and reliable laboratory methods.

Proficiency in specimen collection and transport is paramount to accuracy in diagnostic testing. Both the sensitivity and specificity of diagnostic tests for *C. trachomatis* have been shown to be directly related to the adequacy of the specimen. The host cells that harbour the organism should be included in the specimen collection as the chlamydiae are obligate intracellular pathogens, especially in techniques involving direct visualization of the organism.

The choice of sampling sites can influence the likelihood of recovering the pathogen. A 10-20 per cent increase in the recovery of *C. trachomatis* from genital tract has been observed if both cervical and urethral specimens are taken in comparison to cervical sampling only[42]. Endocervical swab, vaginal/introital swab, vulval swab as well as urethral and rectal swab and first catch urine are the common samples taken from the female patients. Urethral and rectal swab

and first catch urine sample can also be collected from male patients in addition to other specific samples like prostatic fluid.

Quality assurance of collection and transport of the specimen: Specimen adequacy can be determined by visualization of squamo-columnar cells during microscopy. A specimen is considered adequate if it contains one columnar/ metaplastic cell per slide. The likelihood of isolation is optimized if the specimen is refrigerated immediately after collection at 2-8˚C. The time between sample collection and processing should ideally be less than 48 h, if that is not possible these may be frozen at -70 ˚C until processed[16]. Foetal bovine serum (2-5%) helps to preserve the viability of chlamydiae in specimen, which is to be frozen. Two-molar sucrose phosphate (2-MSP) or sucrose glutamate phosphate are the most commonly used transport medium. Synthetic transport media for culture and some non- culture tests have been developed and approved for diagnostic use, *i.e.* M4 transport medium, Flex Trans medium and new M4 synthetic/universal medium.

The laboratory diagnosis of *Chlamydia* consists of the following methods:

(i) Specific tests

Cell culture: Isolation of the organism is the definitive method for the diagnosis of chlamydial infection. *Chlamydia* is an obligate intracellular pathogen and, therefore, requires embryonated hen's egg or animal cell lines for culture. Such culture methods are technically difficult, labour intensive, cumbersome and expensive, and have not been widely adopted as a routine test performed in general clinical laboratories. However, three *in vitro* systems have been used for culture of chlamydiae *viz.* mouse inoculation (intraperitoneal, intracranial and intravenous), yolk sac-inoculation (7-8 day old chick embryo yolk sac inoculation) and cell-culture lines[16]. The most commonly used cell lines include- HeLa 229 cells, McCoy cells, BHK21 and BGMK cells[24].

The sensitivity of cell culture for isolation of chlamydiae is enhanced by the pre-treatment of cell by polycations, DEAE-dextrans, centrifugation of the inoculum on to the cell monolayer and incorporation of anti-metabolites such as cycloheximide or cytochalasin B into the cell culture medium[42]. Cell monolayer for culture of *C. trachomatis* is grown in drum or shell vials on glass coverslips or in the wells of multiwell cell culture dishes. The shell vial method is more sensitive for clinical specimen than multiwell cell culture due to less chances of cross-contamination[42]. Prior to inoculation, the specimen should be sonicated to disrupt the host cells and to separate chlamydial inclusions. To inoculate the cell cultures, the overlying culture medium should be removed and replaced with enough of specimen in the culture transport medium to cover the monolayer and prevent drying.

The most commonly used growth medium is Eagles Minimal Essential Medium (EMEM) supplemented with amino acids and vitamins, foetal calf serum (5-10), extra glucose (0.056 m) and 2-glutamine[42]. After inoculation, the cultures are incubated at 37^{o}C for 2-3 days. The chlamydial inclusions are then observed by immunofluorescent staining. The sensitivity of the isolation has been shown to range from 70-85 per cent depending on the laboratory and the culture system used. Traditionally, this is the "gold standard" for the diagnosis of *C. trachomatis* as it is 100 per cent specific[42]. Unfortunately, it is beyond the capabilities of most private and public laboratories due to its technical demand, labour intensity and high cost.

Direct fluorescent test (DFA): The DFA test adds the considerable advantage of *Chlamydia* specific antibody staining to direct examination of specimen and remains one of the most useful diagnostic techniques. In this test, rapid identification of elementary bodies in smears with flourescein isothiocynate- conjugated monoclonal antibodies (FITC-Mab) against MOMP or genus specific LPS are used. Elementary bodies appear as distinct, sharply outlined, apple green, disk shaped (300 nm) particles and reticulate bodies appear about three times larger than elementary bodies having a fluorescing halo.

This procedure does not require stringent conditions for specimen transportation as a bed side smear is prepared and subsequently transported for processing. With the use of MOMP of *C. trachomatis* the sensitivity and specificity of DFA is found to be 80-90 per cent and 98-99 per cent, respectively in relation to culture[43]. The high specificity of DFA is attributed to its dependence on the visualization of distinctive morphology and staining characteristics of chlamydial inclusions. The DFA is the only diagnostic test available that permits simultaneous assessment of specimen adequacy by visualization of epithelial cells present in the smear. It is rapid and simple (turnaround time about 30 min) but microscopic examination and interpretation of results requires expertise. This method is, therefore, recommended for low volume laboratories. This test can also be applied to extragenital sites. It is reported

to be more sensitive than culture for the detection of *Chlamydia* in endometrial or tubal specimen[42].

ELISA (enzyme linked immunosorbant assay): ELISA is available for the detection of *C. trachomatis* antigen. Several commercially available ELISA kits are available for the purpose. Most of these detect chlamydial LPS which is more soluble than MOMP. The enzyme immunoassay (EIA) tests have been reported to have a sensitivity of 62-96 per cent and a specificity of 86-99 per cent in comparison to cell culture[44]. This test is suitable for laboratories without access to cell culture. However, different large and small studies across the world including India have reported poor sensitivity of ELISA in comparison to DFA and PCR[45-47].

Cytology: Cytology is an easily available, simple to use and cost-effective diagnostic test. It does not require precautions for specimen storage and transport, and non-viable/non-infectious particles can also be detected. The quality of the clinical specimen can be assessed by the microscopic technique and the technical procedures used in these tests are usually quicker and simpler to perform than culture. Giemsa, immunoflourescence and iodine staining methods are most commonly used. Other stains like immunoperoxidase, immunoferritin, May Grunwald, Giemenez, Macchiavello and acridine orange can also be used for detecting chlamydial inclusion in exfoliated cells. The presence of intracytoplasmic inclusions is pathogonomic for chlamydial ocular infections in neonates, however, this method is not recommended for diagnosing conjunctivitis or genital infection in adults due to the lack of sensitivity. Of the three methods, immunofluorescence offers the highest sensitivity followed by Giemsa and then iodine staining[42].

Molecular methods: The traditional methods of diagnosis have several limitations which include low sensitivity, long testing time and high cost. Therefore, tests based on the direct recognition of DNA and RNA sequences are devised. The commercially available DNA probe for the detection of *Chlamydia* is PACE 2 test (Probe Assay Chemiluminescence Enhanced)[48], capable of detecting *Neisseria gonorrhoeae* also, which is a non-isotopic DNA probe for the detection of specific parts of r-RNA of *C. trachomatis* in the endocervical and urethral specimen. Another DNA probe, PACE 2C test has also been developed which simultaneously detects both *C. trachomatis and N. gonorrhoeae* from a single specimen; however, further evaluation of PACE 2C is required before its use in diagnostics. These tests employ a chemiluminescent DNA probe that hybridizes to a species- specific sequence of chlamydial 16S rRNA. Once the DNA-rRNA hybrid is formed, it is adsorbed onto a magnetic bead and the chemiluminescent response is detected quantitatively with a luminometer. Since actively dividing chlamydiae contain up to 10^4 copies of 16S rRNA, the PACE 2 test should theoretically be more sensitive than antigen detection systems. The sensitivity of PACE 2 relative to a DNA amplification standard has not yet been well evaluated but has been reported to be 77 to 93 per cent[48] in one study.

The development of tests based on nucleic acid amplification technology (NAAT) has been the most important advancement in the field of chlamydial diagnosis since *in vitro* cell culture techniques replaced the yolk sac for culture and isolation of the organism from clinical specimens. NAAT is at least 20-30 per cent more sensitive (capable of detecting as little as a single gene copy) and 100 per cent specific[49,50]. It offers the opportunity to use non-invasive samples like urine to screen for infections in asymptomatic individuals who would not ordinarily seek clinical care. This is a critical advantage, since the majority of chlamydial infections in women and a significant proportion of infections in men are asymptomatic. The most widely known of the DNA amplification technologies is PCR. PCR can be genus, species, group, or strain specific depending on the primer design. Genes targeted for diagnosis of *C. trachomatis* are the MOMP gene, the endogeneous plasmid, the phospholipase gene and the 16S and 23S rRNA gene. Since all nucleic acid amplification technologies detect nucleic acid targets, these do not depend on either viability or an intact state of the target organism for a positive result. Hence, transportation of sample is not a critical issue[49]. Although it has not been well studied, the "window" for the culture-negative, PCR positive state following therapy with doxycycline appears to last up to 3 wk[29]. After this time, patient specimens become both culture and PCR negative.

The PCR test for detection of *C. trachomatis* developed by Roche Diagnostics, Basel Switzerland (Roche-Amplicor) was the first PCR test to be approved by the FDA in the United States[51]. Since 1993, Amplicor PCR has been relatively well evaluated for both urogenital and urine specimens, with an overall sensitivity and specificity of 90 and 99 to 100 per cent, respectively[51]. Amplicor PCR is approved for cervical, male urethral and male urine specimens.

With the explosion of molecular biology techniques newer assays like the m2000 system (Abbott) as well as strand-displacement amplification (SDA) (BD ProbeTec strand displacement amplification developed by Becton Dickinson and Company, Diagnostic Systems, Franklin Lakes, N.J.) and transcription-mediated amplification (TMA) (APTIMA system by Gen-Probe, Inc., San Diego) became available for *C. trachomatis*. Although popular in the developed countries, their high initial and maintenance cost prevent their use in resource-poor settings.

The burden of *C. trachomatis* organisms in the genital tract (chlamydial load) can be detected by quantitative real-time PCR and can vary from 10 to over a million organisms/ml of genital tract secretions[52]. This is likely to influence the performance of different nucleic acid amplification tests, which do not routinely distinguish between people with high and low chlamydial loads. Differences in chlamydial load have been reported to be associated with the presence of clinical symptoms, the transmissibility and persistence of infection, and the risk of developing chronic sequelae[53]. Hence, there is a critical role of quantification in the diagnosis and treatment of chlamydial infections.

The NAATs are the most sensitive tests for the screening and diagnosis of chlamydial and gonococcal infections of the genital tract[54-56]. However, doubts regarding their performance in low prevalence areas are reported[57,58]. In 2002, the CDC recommended to confirm all positive NAATs for *C. trachomatis* when the positive predictive value of the test is <90 per cent[59]. However, the true specificities of NAAT methods are found to be >99 per cent[54,55].

The CDC has also suggested several possible strategies for confirmation[59] which include *(i)* testing a second specimen with a different NAAT having equal or higher sensitivity to the first test, *(ii)* performing a different NAAT having equal or higher sensitivity to the first test targeting a different nucleic acid sequence on the original specimen, *(iii)* repeating the original test on the original specimen, and *(iv)* bringing the patient back for a retest.

However, limitations described are that most clinicians will not collect two samples for the same evaluation, nor is it feasible to bring back the patient to collect another sample, and most laboratories do not have the facilities / capability to perform two different NAATs.

The concept of confirmatory testing is not new[57]. However; it complicates the handling of a NAAT positive sample and adds cost to an already expensive screening test. Also, there is still room for improving the sensitivity of NAATs, perhaps by better specimen preparation, automation, or target concentration.

(ii) Non-specific tests

Leukocyte esterase (LE) test is a rapid dipstick test for use with urine specimens. This test is designed to detect urinary tract infections by detecting the enzyme produced by the polymorphonuclear (PMN) cells. Positive LE test results occur with infections caused by a number of different agents including *C. trachomatis* and *N. gonorrhoeae.*

The sensitivity of the LE test for detection of *C. trachomatis* infection varies widely from 31 to 100 per cent, and specificities range from 83 to 100 per cent[60]. The LE test has been considered the best screening test for adolescent males and, according to most reports, should not be used for testing specimens from women or older men due to unsatisfactory performance.

(iii) Rapid point of care (POC) tests

Rapid tests, also called "point-of-care" tests for *C. trachomatis* employ EIA technology in formats based primarily on membrane capture or latex immunodiffusion. Rapid tests are performed in physician's offices, do not require sophisticated equipment, and can be completed in about 30 min. Results are read visually and are thus qualitative. Though several kits are commercially available, but none has been well evaluated. In general, the rapid tests are significantly less sensitive and specific than laboratory-performed EIAs. Compared with PCR, the sensitivity and specificity of the Clearview test (Unipath Ltd., UK) were 53.8 and 99.1 per cent, respectively, with endocervical swab specimens, and 31.1 and 95.2 per cent with vaginal swab specimens from Filipino women[61]. The rapid tests offer an advantage over conventional laboratory tests only when results are required immediately for patient management. Rapid tests should not be used in a low-prevalence population or for asymptomatic individuals due to the potential for false-positive results. The results of a rapid test should always be considered presumptive and, if positive, should be confirmed by a laboratory-performed test.

In conclusion, although culture is 100 per cent specific, its estimated sensitivity may be as low as 50 per cent. Majority of laboratories have moved away

from culture due to the expense involved, time and technical difficulties. Thus, instead of culture as a diagnostic gold standard, the expanded gold standard/ defined reference standard, *i.e.*, commonly consistent result with two non-culture techniques is considered to be useful as research tool[43].

(iv) Serology

The serological tests are generally not useful in the diagnosis of genital tract infections caused by *C. trachomatis*. Antibodies elicited by *C. trachomatis* infection are long lived and a positive antibody test will not distinguish a previous from a current infection.

New variant of *Chlamydia trachomatis*

A new variant *Chlamydia trachomatis* (nvCT) strain has been recently isolated in Sweden (2006)[62], which has a 377 bps deletion in a portion of the plasmid that is the target area for some of the NAATs. Consequently these tests often give false negative results when presented with this strain. Therefore, it is important to select primers for NAAT carefully particularly those targeting the endogenous plasmids. The symptoms and treatment of this strain do not differ from those for normal chlamydiae. So far, this strain has been found in Sweden and Norway. The clinicians and microbiologists should remain vigilant for suspicious negative results as well as unexplained fall in positive results. However, other commercially available NAAT systems that use a different sequence (Gene Probe Aptima Combo AC 2, Probe Tech, BD, *etc.*) accurately detect this agent.

***Chlamydia trachomatis* and pelvic inflammatory disease**

Twenty per cent of the women with chlamydial lower genital tract infection will develop PID[63] and 4 per cent will develop chronic pelvic pain[2]. The clinical spectrum of chlamydial PID ranges from subclinical endometritis to frank salpingitis, tubo-ovarian masses, pelvic peritonitis, periappendicitis and perihepatitis. However, symptomatic chlamydial infections represent only the tip of the iceberg of all chlamydial infections as majority of genital chlamydial infections are asymptomatic.

***Chlamydia trachomatis* and pregnancy**

The prevalence of *C. trachomatis* infection in pregnant women ranges from 2-35 per cent[42]. Pregnant women with chlamydial infection are at increased risk for adverse outcomes of pregnancy and post-partum PID. Sequelae like still birth, low birth weight, neonatal death, decrease gestational periods, preterm delivery and premature rupture of membranes (PROM) have been reported[16]. Nine per cent of the women with chlamydial infection who develop PID have tubal pregnancy[59]. Early pregnancy loss or recurrent pregnancy loss may be induced by asymptomatic chlamydial infection through the operation of immune mechanism.

***Chlamydia trachomatis* and infertility**

Chlamydial PID is the single most important preventable cause of infertility. Approximately, 3 per cent women with chlamydial genital tract infection develop infertility. After a single episode of PID, the risk of tubal factor infertility is approximately 10 per cent, each repeat episode doubles the risk[64]. Although the majority of patients are asymptomatic but re-infection/persistent infection with C. *trachomatis* leads to more severe tubal damage than other agents.

The role of *C. trachomatis* in the development of urethritis, epididymitis and orchitis in men is widely accepted. Though the role of this organism in prostatitis is controversial, but up to 35- 50 per cent incidence has been reported in patients with prostatitis[65]. Infection of the testes and the prostrate is implicated in the deterioration of sperm (decrease sperm motility, increase proportion of sperm abnormalities, significant reduction in sperm density, sperm morphology and viability and increased likelihood of leucocytospermia) affecting fertility. Chlamydial infection may also affect the male fertility by directly damaging the sperm as sperm parameters, proportion of DNA fragmentation and acrosome reaction capacity are impaired. However, the role of *C. trachomatis* in male infertility is not yet proven.

***Chlamydia trachomatis* and HIV**

Chlamydial infection of the genital tract facilitates the transmission of HIV. This is confirmed by various studies[12,15,23]. The combined epidemiology of these infections may partly be due to the fact that STDs including *C. trachomatis* and HIV have common sexual/ behavioural risk factors. But, *C. trachomatis* and HIV have inter-relationship independent of the sexually transmissible risk factors[34]. The possible inter-relationship between HIV infection and *C. trachomatis* includes *(i)* the invasive intracellular pathogenesis of *C. trachomatis* can cause substantial damage to the genital epithelial layer that may facilitate HIV infection, and

(ii) the immunological changes due to HIV infection may favour chlamydial infection.

On the other hand, immunosuppression due to HIV may lead to more aggressive chlamydial disease conditions like PID in those who are infected. Thus, early diagnosis and treatment of chlamydial infections are important to prevent HIV risk and devastating clinical consequence.

Chlamydia trachomatis and co-infection with other STI/ RTI/ infections

C. trachomatis and *N. gonorrhoeae* are the two most common bacterial causes of lower genital tract infection. Clinical findings need to be corroborated with the laboratory investigations as the signs and symptoms of both are indistinguishable. Therefore, in the syndromic approach used in resource-limited settings, urethral discharge (UD) is simultaneously treated for both. *C. trachomatis* is recovered more often from women who acquire gonorrhoea than from similarly exposed women who do not acquire gonorrhoea. In individuals with gonorrhoea, there exists a 15-40 per cent higher risk of acquiring *Chlamydia*. Further, individuals infected with both *C. trachomatis* and *N. gonorrhoeae* shed larger number of *C. trachomatis* than those infected with *C. trachomatis* alone. These data suggest that acquisition of a gonococcal infection either reactivates a persistent chlamydial infection or increases the susceptibility of the host to *Chlamydia*. Post-gonococcal urethritis is often due to *C. trachomatis* infection which is not cured by conventional therapy against gonorrhoea. Co-infection of *C. trachomatis* with *N. gonorrheae* has been reported to range between 1.1 to 67 per cent[66-70].

In a study in STD patients in New Delhi, 19.9 per cent prevalence of *C. trachomatis* was observed[9]. The co-infection of *C. trachomatis* with bacterial vaginosis was found to be 12.7 per cent, candidiasis in 10.9 per cent cases, syphillis in 3.6 per cent cases and chancroid in 1.8 per cent cases. However, co-infection with *N. gonorrheae* was not found. Two cases with multiple infections were also reported (*i.e.* one with *C. trachomatis*, *Candida albicans*, HIV and syphilis and the other with *C. trachomatis*, *C. albicans*, HIV and bacterial vaginosis). In another study, the prevalence of *C. trachomatis* in STD patients was found to be 30.8 per cent[34]. Thirty per cent of the *Chlamydia* infected cases had HIV infection, while the analysis revealed that 50 per cent of the HIV positive cases happened to be proven *C. trachomatis* positive cases.

Prevention of *Chlamydia trachomatis* infection

The control of STD is a public health priority and the importance of these infections has increased in salience over the past decade, with the growing evidence of co- transmission of HIV. The CDC guidelines[59] for the prevention and control of STDs are based on five major concepts: *(i)* Education and counselling on safer sexual behaviour in persons at risk. *(ii)* Identification of asymptomatic infected persons and of symptomatic persons unlikely to seek diagnostic and treatment services. *(iii)* Effective diagnosis and treatment of infected persons. *(iv)* Evaluation, treatment and counselling of sex partners of persons infected with a STD. *(v)* Pre-exposure immunization for vaccine preventable diseases.

The CDC strongly recommends that all sexually active women (≤25 yr) and women at increased risk of infection should be routinely screened for *Chlamydia*. However, screening for chlamydial infection is not recommended for men, including those who have sex with other men[71]. Prevention of *C. trachomatis* infection can be done at primary, secondary and tertiary levels. Primary prevention involves preventing both exposure to and acquisition of chlamydial infection through lifestyle counselling and health education. Clinicians play an important role by enquiring about the risk taking sexual behaviour, by encouraging screening tests for those at risk, by ensuring that partners are evaluated and treated and by counselling about safe sex practices. Effective school based health programmes should be implemented among adolescents. Unfortunately, primary prevention has not gained popularity especially in the developing world[72]. Secondary prevention means early detection of asymptomatic disease by screening in order to prevent the drastic sequelae of chlamydial infection. Chlamydial infection fills the general pre-requisite for disease prevention by screening, since these are highly prevalent, are associated with significant morbidity, can be diagnosed, and are treatable. Recent advances like testing non-invasive specimen, utilization of nucleic acid amplification tests and single dose therapy using azithromycin further enhance the efforts to prevent chlamydial infection. Tertiary prevention of acute and chronic chlamydial infection of the upper genital tract has largely failed because by the time patient becomes symptomatic substantial tubal damage already occurs.

Treatment of urogenital *Chlamydia trachomatis* infection

The treatment of chlamydial infection depends on the site of infection, the age of the patient, and whether the infection is complicated or not. Treatment also differs during pregnancy.

Uncomplicated infection: The CDC recommends 1 g azithromycin orally in a single dose, or 100 mg doxycycline orally twice a day (bd) for seven days for uncomplicated genito-urinary infection. Alternate regimens include erythromycin 500 mg orally four times a day (qid) or ofloxacin 300 mg orally (bd) for seven days.

Compared with the conventional therapy, azithromycin has advantage of having better compliance being administered in the physicians' chamber. All the other regimens have similar cure rates and adverse effect profiles. Patients should be instructed to abstain from sexual intercourse for seven days after the treatment initiation. Both the partners should be treated simultaneously in order to prevent re-infection of the index patient. Patient need not be re-tested after completing the treatment, unless the symptoms persist or re-infection is suspected.

Chlamydial infection with PID: Recurrent chlamydial infection increases the risk for developing ectopic pregnancy and PID. PID can be treated on an outpatient basis unless indicated (accompanied by severe illness, nausea, vomiting, high-grade fever, tubo-ovarian abcess or intolerance or unresponsiveness to oral therapy). The CDC has recommended ofloxacin 400 mg orally (bd) or levofloxacin 500 mg orally once a day (od) with or without metronidazole 500 mg orally (bd) for two weeks. In case of intolerance to the above mentioned regimen, ceftriaxone 250 mg intramuscular (im) or cefoxitin 2 g (im) as a single dose with concurrent probenicid 1 g orally in single dose plus doxycycline 100 mg orally (bd) with or without metronidazole 500 mg orally (bd) for two weeks[13].

Treatment during pregnancy: Levofloxacin, ofloxacin and doxycycline are contraindicated during pregnancy. Therefore, azithromycin 1 g orally in a single dose or amoxycillin 500 mg orally thrice a day (tds) is recommended. Amoxycillin is reported to be more effective and with fewer side effects than erythromycin in treating antenatal chlamydial infection. Alternatively, erythromycin base 500 mg orally (qid) is a safe and effective alternative[13]. Testing for cure is indicated in patients who are pregnant and should be performed three weeks after completion of treatment. If the risk of re-exposure is high, screening should be repeated throughout pregnancy.

Multidrug resistant and heterotypic resistant *Chlamydia trachomatis*

In 1980, Mourad *et al*[73] were the first to report the reduced sensitivity to erythromycin. Decreased sensitivity to tetracycline was first reported by Jones *et al*[74] in 1997. They identified five isolates from cases of tubal infertility which had minimum inhibitory concentration (MICs) to tetracycline of 4 to >8 mg/l, compared with control MICs of 0.125 to 0.25 mg/l. The isolates were also resistant to erythromycin, clindamycin and sulphonamide, but sensitive to ciprofloxacin and ofloxacin. Tetracycline resistance was also reported from France in 1997[75]. In 2000, Somani *et al*[76] reported multidrug resistant isolates of *C. trachomatis* associated with treatment failure with azithromycin.

The characteristics of antibiotic resistance of *C. trachomatis* differ significantly from those of other bacteria in several ways. First, because chlamydiae are intracellular pathogens, antimicrobial susceptibility must be determined by their ability to proliferate within a host cell in the presence of varying concentrations of antibiotic. Second, unlike the case for most bacteria, when *C. trachomatis* organisms are found to be resistant to typically effective antibiotics such as tetracycline, the resistance is not absolute. In fact, *C. trachomatis* displays what is known as "heterotypic resistance" *in vitro*; that is, the chlamydial population contains both susceptible and resistant organisms. Thus, although it is possible that all organisms within a population may be capable of expressing resistance, only a small proportion does so at any one time. Testing for the MCC (defined as lowest concentration of drug that permitted no inclusions to be formed on passage on an antibiotic free medium) may allow the small percentage of organisms that were resistant to the first exposure to antibiotic (MIC) to then multiply and form inclusions[76]. Heterotypic resistance exhibited by some *C. trachomatis* strains, therefore, may be missed unless both MIC and MCC testing is done. In strains that exhibit heterotypic resistance, many aberrant inclusions are seen, and the proportion of atypical to typical inclusions gradually increases along with a decrease in the overall number of inclusions until all inclusions become aberrant or absent, which is reinforcing the fact that the resistance exhibited by individual organisms within the chlamydial population

is heterogenous (defined as heterotypic resistance). The mechanisms underlying heterotypic resistance in *C. trachomatis* is not known. It is hypothesized that multidrug resistance in *C. trachomatis* is phenotypic in nature rather than genotypic[76]. Also, heterotypic resistance may be a byproduct of some undefined alteration of the growth rate or life cycle, resulting in a longer phase or intermediate stage that is more refractory to the antimicrobial agents. Alternatively, it may be mediated by some kind of mechanisms that exclude the drug from cell wall or chlamydial inclusion (*e.g.* efflux pump)[76]. Further studies are required to prove these hypotheses.

There are no data regarding management of clinically resistant *C. trachomatis* infection. *In vitro* data suggest that resistance to ofloxacin imparts resistance to other fluoroquinolones, such as ciprofloxacin. Although many of the newer quinolones, including trovafloxacin, sparfloxacin, grepafloxacin and tosufloxacin have equal or greater MICs for *C. trachomatis*, these need to be tested against an ofloxacin-resistant strain[74,77]. Perhaps a prolonged course of therapy with a standard agent such as doxycycline or azithromycin would be effective against resistant *C. trachomatis* disease, because such therapy has been efficacious against *C. pneumoniae* infection in cases of relapse[78].

Azithromycin 1 g immediately and doxycycline 100 mg twice daily have shown good antimicrobial activity against *C. trachomatis* and studies have demonstrated >95 per cent microbiological cure at 2-5 wk, with antimicrobial resistance being hardly reported[72]. However, there are evidences of multidrug resistance to *C. trachomatis* in women with high bacterial load but not in men who had been sexually inactive after treatment[72].

Vaccines

Vaccination could be substantially more effective than other biomedical interventions in controlling epidemics of *Chlamydia* infection. Currently, the best public health intervention available is increasing the rate of screening and treating infected individuals. Administrating a protective vaccine to adolescents before their first sexual experience could induce a significant reduction in prevalence which could not be obtained by screening teenagers, even with a coverage of 100 per cent[79]. Unfortunately, no protective vaccines, either fully or partially, are available although there have been many attempts to develop one. The immunological characteristics of the genital tract and the tropism of *Chlamydia* for mucosal epithelial cells emphasize that a *C. trachomatis* vaccine must induce both mucosal and systemic protective responses[80]. The research goal for an efficacious human chlamydial vaccine has faced key challenges to define the elements of protective immunity to facilitate vaccine evaluation, the judicious selection of appropriate vaccine candidates that possess stable antigenic and immunologic properties and the development of effective delivery vehicles and adjuvants to boost immune effectors to achieve long term protective immunity. Progress in the functional immunobiology of *Chlamydia* has established the essential immunologic paradigms for vaccine selection and evaluation, including the obligatory requirement for a vaccine to induce T- helper type 1 immune response that controls *Chlamydia*. Major inroads are however, required in the construction and development of novel and effective delivery systems, such as vectors and adjuvants.

Conclusion

Role of *C. trachomatis* in serious genitourinary complications in women and men is widely accepted. Further, it has been found to facilitate the acquisition and transmission of HIV infection. Information is now available on cell biology, bacterium- host cell interactions, disease producing mechanisms, host defense evading factors, transmission sources and antimicrobials used for treatment. Despite these advances, there are many lacunae which need to be addressed. Asymptomatic infection in the majority coupled with re-infection, recurrent and latent infections are the major challenges to the control of this bacterial STI. The best available intervention today is the early detection by screening and treatment of infected cases and their sexual partners. However, the ultimate intervention - the development of an effective vaccine is still far away and further research is required.

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