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Review

Microbiology Section

Genital *Chlamydia trachomatis:* A Review

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ABSTRACT

Globally, Sexually Transmitted Infections (STIs) are a serious public health concern. The most common bacterial STI pathogen is *Chlamydia trachomatis (C. trachomatis)*. Over 80% of individuals with this infection remain asymptomatic, which may lead to undiagnosed cases. A persistent infection with *C. trachomatis* can spread to the upper genital tract, resulting in Pelvic Inflammatory Disease (PID). If left untreated, this may lead to long-term sequelae, such as infertility. Early diagnosis and treatment of infected individuals are required to prevent the spread of the disease and its severe consequences. Tissue culture was once thought to be the most reliable method of diagnosis. However, diagnosis has become quick and simple with the development of improved diagnostic tools, especially molecular techniques, which are not only very sensitive and specific but also cost-effective. This review elucidates the effects of genital infections caused by *Chlamydia* on human health and recommends further research on the frequency, prevalence, and pathogenic mechanisms of *C. trachomatis* Infection (CTI) to gain a better understanding of the long-term consequences caused by this illness. Lastly present review confirms that the evidence presented can encourage changes in the country's healthcare system in the future. For instance, future infection control measures could be planned, prevention campaigns could be created to increase public awareness of the risk factors for this infection, and screening techniques for *Chlamydia* could be updated.

Keywords: Infertility, Pathogenesis, Pelvic inflammatory disease, Tubal factor infertility

INTRODUCTION

Chlamydiae are common, ovoid or spherical, obligatory intracellular microorganisms. *Chlamydia* is distinct from other bacteria due to its intracellular parasitism. Chlamydiae differ from viruses in that they possess both DNA and RNA, divide via binary fission instead of self-assembly, have their own ribosomes, and have a cell wall free of peptidoglycan. They are also sensitive to a number of antimicrobial substances. Previously, the generic names suggested for these agents were Miyagawanella and Bedsonia. However, due to their distinct developmental cycle, they are categorised under a different order [1].

The first human case of *C. trachomatis* was reported in 1907, based on intracytoplasmic inclusions found in conjunctival epithelial cells taken from trachoma patients' conjunctival scrapings. It was also known as Chlamydozoa. In Greek, "Chlamy" means "cloak" [2]. For thousands of years prior to this discovery, trachoma was widely recognised as a blinding ocular disease in humans. Halberstaedter and von Prowazek identified a trachoma-like agent in a case of newborn blennorrhoea. Both Heyman B and Fritsch H et al., discovered trachoma-like inclusions in the mother's cervix and the scrapings of neonatal conjunctivitis [3,4]. Lindner K described the same intracytoplasmic inclusions in newborns with ophthalmia neonatorum, which were also detected in their parents' urethral cells, and *Chlamydia* has since been identified as the cause of STIs [5]. In 1913, the pathology of lymphogranuloma venereum and its role in sexual transmission were described [1].

In 1957, Tang et al., identified the bacteria from trachoma patients using hen's eggs. Following this, the same technique was used to identify the bacteria from individuals with genital and ocular diseases in London [6]. *Chlamydia* was identified as a bacterium in the 1960s and was discovered to be one of the major bacterial infections affecting humans [7]. Gordon and Quan's study in 1965 on *C. trachomatis* isolation using irradiated McCoy cells marked a significant advancement and was a major step in understanding chlamydial infections [8, 9]. Their technique allowed for cultures from genital material, which was more feasible than isolation from eggs. The use of McCoy cells provided a greater understanding of both the clinical aspects of *Chlamydia* and its basic biology. The significance of *C. trachomatis* in neonatal pneumonia and its association with PID was postulated. *C. trachomatis* was identified as a major aetiological factor for acute salpingitis and PID in females, and subsequent research indicated that the consequences of this organism were more common in women than in men. Inanimate media cannot be used to cultivate *Chlamydia*. Trachoma Inclusion Conjunctivitis (TRIC) and Lymphogranuloma Venereum (LGV) are the two strains or biovars of *C. trachomatis* that were separated based on the diseases they produce [1].

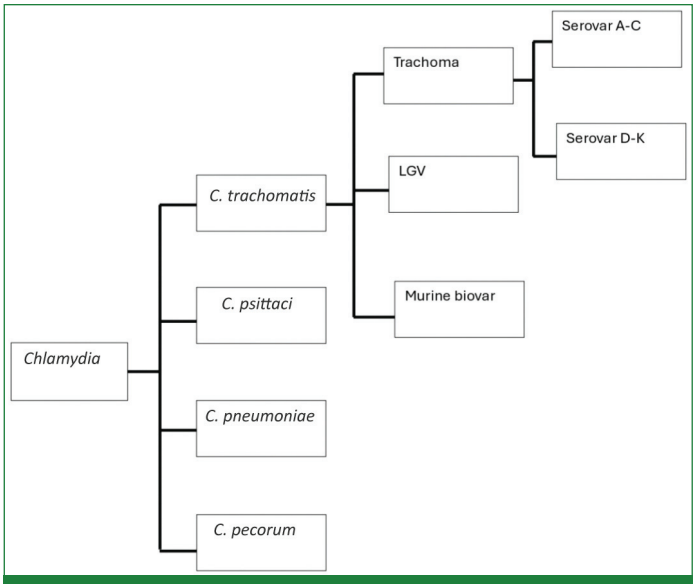
This study is beneficial for understanding the effects of genital infection caused by *Chlamydia* trachomatis on human health and recommends further research on the incidence, prevalence, and pathogenic mechanisms of CTI and female infertility in India for a better understanding of the long-term sequelae caused by CTI. Such an elaborate review on CTI and female infertility has not been published elsewhere in South India.

Taxonomy and Classification

Chlamydia belongs to the distinct order Chlamydiales, family Chlamydiaceae, phylum Chlamydiae. The family Chlamydiaceae comprises two genera, *Chlamydia* and Chlamydophila, which have since been combined into a single genus, *Chlamydia*. *C. trachomatis*, *C. pneumoniae, C. psittaci*, and *C. pecorum* are the four species that fall under the genus *Chlamydia* [1,10,11].

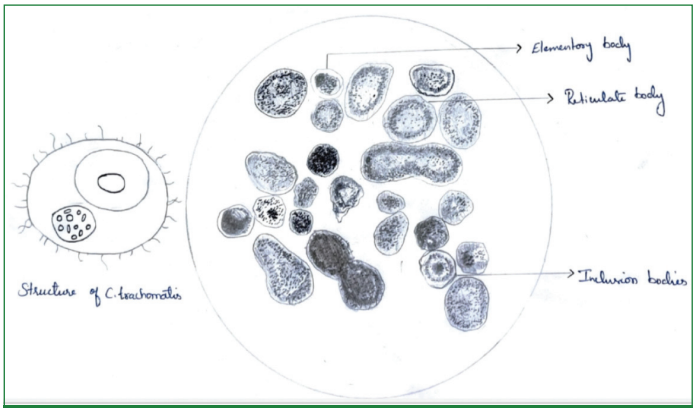
***Chlamydia trachomatis*:** *C. trachomatis* is a human pathogen that primarily causes infections of the eyes, urogenital tract, and in neonates. Depending on the diseases it produces, *C. trachomatis* was previously divided into two strains or biovars, called LGV and TRIC. *C. trachomatis* has 15 serovars that can be identified by micro-immunofluorescence, and the classification of genital CT is mentioned in [Table/Fig-1]. In regions where trachoma is endemic, Types A-C are mainly isolated from people suffering from ocular conditions. Types D-K are associated with infections of the

genital tract or newborn infections acquired from the mother during childbirth, although they can also cause ocular infections in adults in developed nations. Only patients with LGV are isolated from types L1-L3. There are two subspecies serogroups known as B and C complex serogroups that comprise the 15 immunotypes [1,12].



[Table/Fig-1]: Classification of genital *C. trachomatis*.

Morphological characteristics [Table/Fig-2]: Chlamydiae occur in two forms: the infective, non dividing Elementary Body (EB), which lives extracellularly, and the non infective Reticulate Body (RB), which divides intracellularly via binary fission. The EB is a 300 nm diameter spherical, electron-dense coccus with numerous ribosomes and a rigid trilaminar wall. Its surface features patches of regularly arranged hemispheric projections. Haemagglutinin is present on its surface, and it contains equal amounts of DNA and RNA. The EB is also relatively impermeable and resistant to trypsin and sonication. In comparison, the RB is larger (up to 1000 nm), has non rigid walls, and does not contain haemagglutinin. It contains three to four times more RNA than DNA, is more permeable, and is susceptible to trypsin and sonication [1].

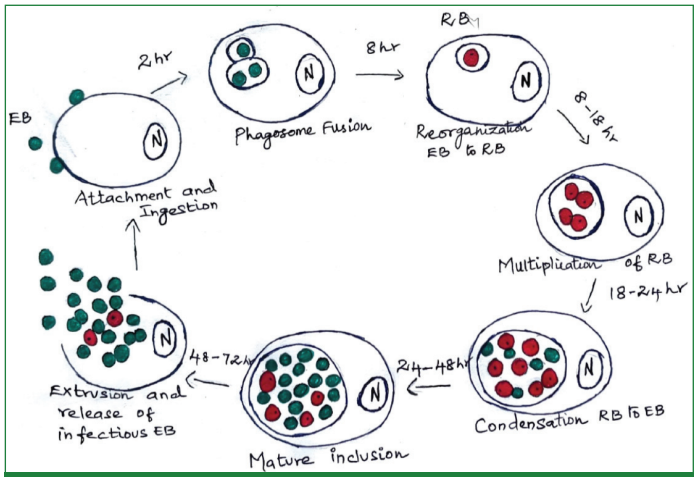


[Table/Fig-2]: Microscopic image of *C. trachomatis*.

After attaching and entering the host cell, the EB evolves into an RB, characterised by structural alterations in the organisation of the outer membrane and the relaxation of the condensed nucleoid. The replicative, intracellular form of Chlamydiae is known as the RB. *Chlamydia*, sometimes referred to as energy parasites, depend on their host for ATP, as they are unable to produce a net gain of ATP. Consequently, cell-free growth of *Chlamydia* has not been achieved, and cell culture techniques or yolk sacs are required for in vitro development. Another distinctive feature of *Chlamydia* is its envelope structure, which is Gram-negative and consists of an inner membrane and an outer membrane containing lipopolysaccharides. Despite possessing penicillin-binding proteins and being susceptible to medications that inhibit the production of peptidoglycan, such as penicillin G and D-cycloserine, *Chlamydia* appear to lack peptidoglycan in their envelope [13].

Additionally, each EB of Chlamydiae has 18-22 distinct dome-shaped surface projections that are evenly spaced and extend about 20 nm above the outer membrane. In the RB, the projections are arranged in a less ordered array. According to studies, these projections may penetrate the domes and could even pass through the membrane separating the RB. However, the purpose of the projections remains uncertain; they may function as ion channels, actively participate in nutrient acquisition, or act as anchors within the inclusion to bring *Chlamydia* close to the host cytoplasm [14].

Developmental cycle [Table/Fig-3]: Following attachment, the host cell endocytoses Chlamydiae, likely due to prostaglandin-mediated modifications to the fluidity of the host cell membrane. The EB loses its ability to spread infection after being endocytosed and begins to transform morphologically and molecularly into the RB. A cytoplasmic vacuole enclosed by the membrane of the host cell undergoes further development, increasing in size despite the host cell's inhibition of protein synthesis. Phagolysosome fusion is not triggered by endocytosis, allowing proliferation inside the vacuole. The RB divides through binary fission. After 8-12 hours, there are 4-16 RB within the vacuole. The RB continues to divide, and individual bodies become smaller. After 20 hours, some of the particles exhibit central condensation of cytoplasmic contents, which is linked to the formation of complexes between proteins and carbohydrates. These particles are identified as normal EB. At this point, the inclusion begins to push out the nucleus, and a substance that contains iodine-stainable glycogen forms inside it. After 48-60 hours, the cytoplasmic vacuole ruptures extracellularly to release the particles, at which point the remaining RB have differentiated into 100 or more infectious EBs. A proteinase specific to Chlamydiae may initiate the release process.



[Table/Fig-3]: Developmental cycle of *C. trachomatis*.

Energy and metabolic interactions: Energy and other intermediates are produced by the host cell, which are necessary for the developmental cycle of *Chlamydia*. The host cell must compete with Chlamydiae for these resources. The nutritional condition of the host cell may regulate chlamydial replication. Interferon and lymphokine-altered macrophages can prevent replication. One molecule of their own ADP is exchanged for one molecule of host ATP by Chlamydiae; however, they do not produce ATP on their own. Although Chlamydiae possess a DNA-dependent RNA polymerase, nucleoside triphosphates from the host cells are required for RNA synthesis. Similarly, while certain proteins specific to Chlamydiae are synthesised during the embryonic cycle, some amino acids necessary for protein synthesis are not produced by *C. trachomatis*. The site of infection may be related to the varying amino acid requirements of different strains [1,15].

Pathogenesis of *C. trachomatis*: Humoral and cell-mediated immunity are triggered by the potent immunogen *C. trachomatis*. The outcome of chlamydial infection is determined by the balance and interaction of immunogenic antigens and cytokines generated by activated lymphocytes. One of the most crucial elements in the host's defense against *Chlamydia* is Interferon Gamma (IFN-γ), whereas increased expression of interleukin-10 has been associated with disease susceptibility. By disrupting or altering the immune system, *C. trachomatis* may enhance its own survival in the infected host, potentially leading to chronic infections. Chlamydial infections can present either as acute or chronic recurrent infections [16,17].

Clinical Manifestation

In female: Asymptomatic chlamydial infections can account for up to 80% of cases. Cervicitis, urethritis, and endometritis are among the clinical signs of *C. trachomatis* infections in women. Cervicitis can lead to three different types of complications: PID, preterm birth, premature membrane rupture, and infection of the newborn can occur due to the organism's ascending spread from the cervix. Additionally, there is an increased risk of developing cervical cancer. PID, tubal scarring, Ectopic Pregnancy (EP), and infertility can result from ascending infections [12,15,18-20].

In male: Men who exhibit symptoms may complain of dysuria and may have a mucoid or watery urethral discharge, which can be clear, sparse, or only evident after the urethra has been milked. A chlamydial infection may cause inflammation of the testes and epididymides. *C. trachomatis* is one of the most common pathogens associated with epididymitis in sexually active men under 35 years of age. Acute epididymitis can result in hydrocele, epididymal enlargement, and testicular pain. One-third of patients with Non Gonococcal Urethritis (NGU) exhibit the complete reactive arthritis triad, formerly known as Reiter syndrome, which includes urethritis, uveitis, and arthritis. These infections are often symptomatic, unlike those caused by serovars linked to genital tract infections. Symptoms such as fever, anorectal discomfort, discharge, tenesmus, rectal bleeding, and constipation are frequently present [21-25].

Lower genital tract infections: *C. trachomatis* is one of the primary causes of urethritis and dysuria in women, and its presence can lead to bacteriuria without significant bacteriuria. Mucopurulent cervicitis is suggestive of urethral syndrome, despite the absence of urethral inflammation and *C. trachomatis* infection. Cervicitis caused by *C. trachomatis* is frequently asymptomatic, but if left untreated, it can spread to the upper genital tract, resulting in PID. Reports indicate that subclinical PID is present in 64% of women with chlamydial mucopurulent cervicitis.

Pelvic Inflammatory Disease (PID)

PID, also known as salpingitis, is an infection of the fallopian tubes, uterus, and surrounding structures that is unrelated to pregnancy or surgery. The infectious bacteria that cause PID typically arise from the cervix, vagina, and upper genital canal. PID is one of the most serious complications of sexually transmitted infections (STIs), with over half of reported cases attributed to *C. trachomatis* and *N. gonorrhoeae*, or both. Each recurrence of PID doubles the risk of tubal disease, which can lead to EP or infertility and potential chronic tubal damage. Women (18%) with laparoscopically diagnosed PID will experience chronic pelvic pain, while 9% will have ectopic pregnancies, and 20% will become infertile due to tubal scarring [17,26]. Acute PID symptoms may include ovarian abscesses, pelvic peritonitis, endometritis, and salpingitis. Additionally, the appendix and liver capsule may become infected due to the ascending infection.

To diagnose PID, a patient must present with lower abdominal pain, bilateral adnexal tenderness, cervical motion tenderness, and no evidence of another plausible diagnosis. According to Cates W and Wasserheit JN, PID is often silent or asymptomatic. They found a strong association between serum anti-chlamydial antibodies and tubal factor infertility (TFI) or EP in patients both with and without a history of PID [27].

At the time of diagnosis, women with PID often have damaged fallopian tubes. Studies in monkey and mouse models have shown that antibiotic treatment, with or without anti-inflammatory medications, has little effect on tubal inflammation. Chlamydial PID is believed to cause tubal damage through an immunopathological mechanism. Numerous studies have identified a link between PID, TFI, or EP and serum antibodies to chlamydial heat shock protein 60 (cHSP60). HSP antibodies have been associated with several conditions in women, including chlamydial PID, a history of PID, cervicitis, laparoscopically confirmed tubal blockage, the site of tubal inflammation, and the presence of adhesions [28].

Acute salpingitis: Acute salpingitis is caused by an ascending infection from the lower genital tract, leading to tubal constriction, postinflammatory fibrosis, and peritubal adhesions. It is characterised by erythema and oedema of the fallopian tubes and is diagnosed through laparoscopy. The oedema associated with salpingitis increases intraluminal agglutination, causing the fallopian tubes to adhere together and become dysfunctional, partially or completely obstructed. Pelvic pain may result when this agglutination develops into a thick, filmy pelvic adhesive disease [29,30].

Non gonococcal urethritis: When *Neisseria gonorrhoeae* is not the causative agent of urethritis, the condition is referred to as NGU. Several studies have reported that up to 13-50% of NGU cases show evidence of *Chlamydia trachomatis* in urethral specimens [31].

Postgonococcal urethritis: The persistence of NGU following treatment for gonococcal urethritis is termed postgonococcal urethritis. It is caused by dual infection, with *C. trachomatis* responsible for up to 80% of postgonococcal urethritis cases [32].

Lymphogranuloma venereum: LGV is a systemic STI caused by *C. trachomatis* serovars L1–L3. The disease is endemic in South America, Southeast Asia, India, and East and West Africa. The primary LGV lesion typically appears 3-12 days after infection. Additional manifestations during the initial stage may include salpingitis, endometritis, or urethritis. The secondary stage usually develops up to six months after infection and is characterised by regional lymphadenopathy, oedema, and inflammation. This stage is also associated with systemic dissemination of LGV. The tertiary stage is marked by rectal stricture or stenosis, fistula formation, perirectal abscesses, and proctocolitis [1].

Infertility: Infertility has a significant social and psychological impact. Globally, it is estimated that 8-12% of couples are affected. In India, approximately 25% of couples experiencing infertility require medical assistance each year. Among Indian women of reproductive age, about 11.8% are estimated to have primary infertility. A World Health Organisation (WHO) multicentre study found that 37% of women and 50% of men were infertile [33]. Tubal diseases include peritubal adhesions, salpingitis, hydrosalpinx, and distal or proximal tubal obstruction. TFI can result from PID, a prior history of EP, previous surgery or sterilisation, and prior peritonitis. Hydrosalpinx may develop as a consequence of severe tubal disease, particularly distal tubal blockage, and has been associated with infertility [34].

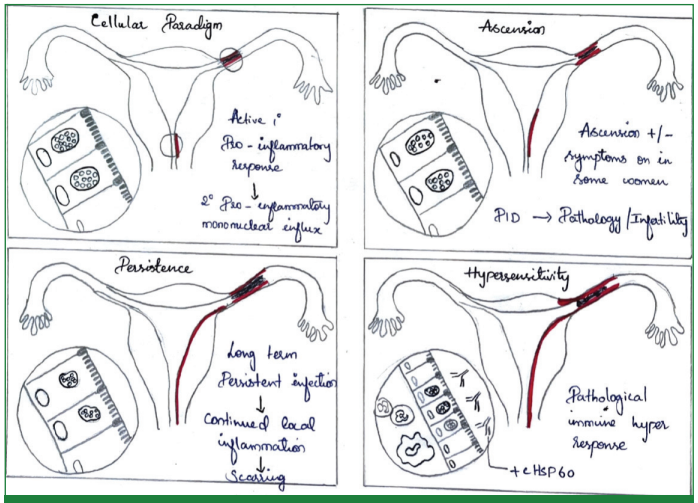
Infertility occurs in approximately 3% of women with genital tract CTI [35]. Recurrent CTI in female patients are known to increase the likelihood of long-term sequelae such as TFI [36]. Although most patients are asymptomatic, reinfection or persistent infection with *C. trachomatis* causes more severe tubal damage than infections caused by other pathogens [37].

Tubal factor infertility: Peritubal adhesion formation, tubal scarring, and tubal blockage due to hydrosalpinx are the main pathological alterations linked to TFI following acute salpingitis. Immune-mediated mechanisms are believed to contribute to adhesion and scarring caused by *C. trachomatis*. Animal model studies have shown

that recurrent chlamydial infection of the fallopian tubes leads to pathological changes associated with hydrosalpinx formation and a delayed-type hypersensitivity response. In human fallopian tube tissue, chlamydial DNA has been detected in TFI patients, and its persistence may trigger immune-mediated tissue damage [38]. Numerous serological studies have demonstrated a link between the development of tubal pathology and CTI. Additionally, they have shown an association between TFI and the presence of chlamydial HSP60 antibodies [39,40].

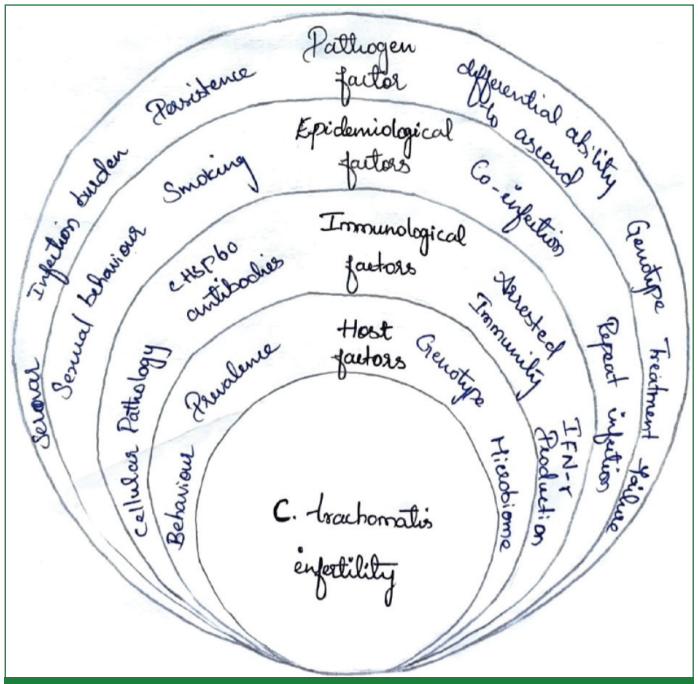
Proposed model for the development of infertility following CTI [Table/Fig-4]: The mechanisms underlying the onset of infertility following *C. trachomatis* infection are most commonly described by four models:

1. The Ascension Model
2. The Persistence Model
3. The Cellular Paradigm
4. The cHSP60-Induced Delayed Hypersensitivity Model.



[Table/Fig-4]: Four models of development of pathology associated infertility due to CTI.

Host and pathogen factor associated with development of infertility due to CTI [Table/Fig-5]: Pathogen-related factors such as serovar type, infectious load, antibiotic susceptibility, and persistence contribute to chlamydial infertility on a broader scale. Population-level factors include age, sexual behaviour, smoking status, co-infections, and previous infection history. Although immunological variables are common to all individuals, cellular pathology, cHSP60 antibodies, and IFN-γ–mediated immune responses can all influence the development of sequelae. Hormonal status, genetics, and microbiota composition also vary among individuals [41].



[Table/Fig-5]: Host and pathogen factor associated with development of infertility due to CTI.

Risk factors and demographic factors: The most prevalent risk factor is young age (under 20 years). Other risk factors associated with CTI include Black race, nulliparity, unmarried status, and low socio-economic background. Additional risk factors include having multiple sexual partners, a new sexual partner, failure to use barrier contraceptives, and concurrent gonococcal infection. The use of oral contraceptives has also been associated with cervical chlamydial infections [2,12,17].

Co-infection: The two most common bacteria causing lower genital tract infections are *C. trachomatis* and *Neisseria gonorrhoeae*. Since the clinical symptoms and signs of both infections are similar, laboratory confirmation is essential. In resource-limited settings, the syndromic management approach involves treating both urethral discharge and related conditions simultaneously.

Women with gonorrhea are more likely to have *C. trachomatis* co-infection than women without gonorrhea. The likelihood of acquiring *Chlamydia* is increased by 15-40% among individuals with gonorrhea. Moreover, patients co-infected with *N. gonorrhoeae* and *C. trachomatis* shed higher amounts of *C. trachomatis* than those infected with *C. trachomatis* alone. These findings suggest that gonococcal infection may increase susceptibility to *Chlamydia* or reactivate latent chlamydial infection.

PGU is frequently caused by *C. trachomatis*, which cannot be eradicated with conventional gonorrhea therapy. Reports indicate that the prevalence of *C. trachomatis* and *N. gonorrhoeae* co-infection ranges from 1.1% to 67% [42-46].

C. trachomatis has also been found to co-occur with bacterial vaginosis (12.7%), candidiasis (10.9%), syphilis (3.6%), and chancroid (1.8%). Co-infection with *N. gonorrhoeae* was not observed in one study. Additionally, two patients had multiple infections, including bacterial vaginosis, *Candida albicans*, Human Immunodeficiency Virus (HIV), and syphilis; another had syphilis, HIV, and *Candida albicans* co-infection. In another study, *C. trachomatis* was detected in 30.8% of patients with sexually transmitted diseases (STDs). Among these, 50% of HIV-positive cases were also *C. trachomatis*-positive, while 30% of *Chlamydia*-infected cases were co-infected with HIV [47].

Epidemiology: India has a high prevalence of genital chlamydial infection. In Bombay, chlamydial antigen was detected in 38.46% of patients with PID [48]. In rural areas, the prevalence of CTI among women was 5.9%, compared to 1.8% in urban areas [49]. Using fluorescent staining, *C. trachomatis* was detected in 15% of asymptomatic women in Maharashtra [50]. Enzyme immunoassay (EIA) detected *C. trachomatis* in cervical specimens of 33% of PID patients [51].

In New Delhi, the Chlamydiazyme test kit revealed that the prevalence of *C. trachomatis* was 18.6% during labour and 17% during mid-pregnancy [52]. A Polymerase Chain Reaction (PCR) assay based on the *Chlamydia* plasmid detected 50% positivity in STD patients from endocervical swabs [53]. In Bombay, the prevalence of *Chlamydia* was 23.2% among female sex workers attending an STD clinic [44]. In urban slum areas, women presenting with vaginal discharge and symptomatic women had chlamydial antigen positivity rates of 12.2% and 28.7%, respectively [54,55].

Using ELISA and cell culture methods on endocervical and blood samples, seropositivity rates of 55% and 5.5% were found among women with secondary infertility and in the control group, respectively, in Aligarh [56]. In Orissa, PCR testing of cervical swab

specimens revealed a *C. trachomatis* prevalence of 7.04% among patients visiting gynaecology outpatient departments [57]. The prevalence of *C. trachomatis* was reported as 10.5% in men and very low among women in Karnataka [58].

In Karnataka, the seropositivity rate for *C. trachomatis* was 25.4% [59], and the overall prevalence was reported as 23% [60]. In Chidambaram, a higher seroprevalence of 52% was observed among patients attending STD clinics [61]. In New Delhi, 3, 11, and 9 positive cases were detected by ELISA, DFA, and PCR, respectively, from endocervical and urethral swabs [62].

In both Europe and the United States, genital infections caused by *C. trachomatis* are the most prevalent STIs, with the highest incidence among young people. Although the CDC recorded 1,758,668 cases of *Chlamydia* in the U.S., an estimated 2.86 million infections occurred annually in 2018 [12]. Young individuals aged 15-24 years accounted for two-thirds of new *Chlamydia* infections [63]. According to a systematic review, the prevalence of *C. trachomatis* among women of reproductive age in Africa was 8.9% in Eastern Africa, 7.2% in Middle Africa, 5.9% in Southern Africa, and 7.4% in Western Africa [64].

Population-based studies show that the prevalence among individuals under 30 years old ranges from 2% to 6% in the Netherlands, Denmark, and the United Kingdom. However, rates among young women attending STD clinics are significantly higher, exceeding 10% [65]. A Dutch study found that 2.9% of women aged 15-29 who initially tested negative during population-based screening became positive after one year [66]. Surveys from Latin America report *C. trachomatis* prevalence rates ranging from 1.9% to 4.5% in Chile, Peru, Brazil, and Mexico, and 12.2% among women attending family planning clinics in Jamaica [67-69].

Laboratory diagnosis: The asymptomatic nature of *C. trachomatis* infection underscores the need for sensitive and accurate diagnostic techniques. The accuracy of diagnostic testing depends heavily on specimen collection and transportation. The adequacy of the specimen directly affects the specificity and sensitivity of tests for *C. trachomatis*. Because *Chlamydia* is an obligate intracellular pathogen, specimens must contain host cells that harbour the organism—this is particularly critical for methods requiring microscopic examination of the pathogen. The recovery of the pathogen is influenced by the sampling site. Collecting both cervical and urethral specimens, rather than cervical samples alone, has been shown to increase the recovery rate of *C. trachomatis* from the genital tract by 10-20%. In female patients, the most common sample types include endocervical, vaginal/introital, vulval, urethral, and rectal swabs, as well as first-catch urine. In male patients, urethral and rectal swabs, prostatic fluid, and first-void urine samples are typically collected [70].

Quality assurance and transport of specimen: Microscopic examination for squamocolumnar cells can be used to assess specimen adequacy. A specimen containing at least one columnar or metaplastic cell per slide is considered sufficient. Immediately after collection, specimens should be refrigerated at 2–8 °C to maximise the likelihood of pathogen recovery. Samples should ideally be processed within 48 hours; if this is not feasible, they may be stored at –70 °C until processing.

Adding 2-5% foetal bovine serum helps maintain the viability of *Chlamydia* in frozen specimens. Commonly used transport media include sucrose-glutamate-phosphate or 2M sucrose phosphate (2-MSP). Synthetic transport media developed and approved for culture and non-culture diagnostic assays include M4 transport medium, FlexTrans medium, and the newer M4 synthetic/universal medium [71].

Chlamydia can be diagnosed in the laboratory using the following techniques

Specific tests

Cell culture: The most reliable method for diagnosing chlamydial infection is isolation of the bacterium. Because *Chlamydia* is an obligate intracellular pathogen, it must be cultivated using animal cell lines or embryonated hen eggs. However, these culture methods are not routinely used due to their high cost, labor-intensive nature, and technical complexity. Three in-vitro systems have been used for *Chlamydia* cultivation: cell lines, mouse inoculation (intracranial, intraperitoneal, intravenous), and yolk sac inoculation (in 7–8-day-old chick embryos). The most commonly used cell lines are McCoy, HeLa 229, BHK21, and BGMK cells. To improve culture sensitivity, cells may be pretreated with polycations or DEAE-dextran, the inoculum can be centrifuged onto the cell monolayer, and anti-metabolites such as cycloheximide or cytochalasin B can be added to the culture medium.

C. trachomatis cell monolayers are typically grown in drum or shell vials on glass coverslips, or in multiwell culture plates. The shell vial technique is more sensitive for clinical specimens because it reduces the risk of cross-contamination. Before inoculation, specimens should be sonicated to release chlamydial inclusions and disrupt host cells. Prior to adding the inoculum, the culture medium covering the cell monolayer should be removed and replaced with sufficient transport medium containing the specimen to fully cover the monolayer and prevent desiccation.

Eagle's Minimum Essential Medium (EMEM), the most widely used growth medium, is enriched with vitamins, amino acids, 5–10% foetal calf serum, additional glucose, and 2-glutamine. After inoculation, the cultures are incubated for two to three days at 37°C. Subsequently, immunofluorescent staining is used to visualise chlamydial inclusions. Depending on the laboratory and culture system, the sensitivity of isolation varies from 70% to 85%. Because of its 100% specificity, this method has historically been considered the "gold standard" for diagnosing *C. trachomatis*. However, due to its high cost, labour-intensive nature, and technical demands, it is beyond the capacity of most private and public laboratories [17,70].

Direct Fluorescent Assay (DFA): The DFA remains one of the most effective diagnostic methods and provides the significant advantage of using *Chlamydia*-specific antibody staining for direct specimen evaluation. This assay employs fluorescein isothiocyanate-conjugated monoclonal antibodies (FITC-MAb) against MOMP or LPS to rapidly identify elementary bodies (EBs) in smears. EBs appear as distinct, sharply defined, apple-green, disk-shaped (300 nm) particles, while reticulate bodies (RBs) appear about three times larger with a fluorescent halo [71,72].

Preparing a bedside smear and transporting it for processing eliminates the need for strict specimen transportation conditions. DFA has been reported to have a sensitivity of 80-90% and a specificity of 98-99% compared to culture. Its high specificity is due to the staining properties and the unique morphology of chlamydial inclusions. DFA is the only diagnostic method that allows visual assessment of epithelial cells while also evaluating specimen adequacy. Although it is rapid and simple, specialised expertise is required for microscopic analysis and interpretation of results. This method is recommended for low-volume laboratories. For identifying *Chlamydia* in endometrial or tubal specimens, DFA is reported to be more sensitive than culture [71,72].

Enzyme Linked Immunosorbant Assay: Both the antigen and antibody for *C. trachomatis* can be detected using ELISA. Several commercially available ELISA kits are designed for this purpose, most of which detect chlamydial LPS, which is more soluble than MOMP. Compared with cell culture, Enzyme Immunoassay (EIA) tests have demonstrated a sensitivity of 62-96% and specificity of 86-99%. This assay is suitable for laboratories without access to

cell culture. However, several studies worldwide, including in India, have shown that the sensitivity of ELISA is lower than that of DFA and PCR [62,73,74].

Cytology: Cytology is an easy-to-use, inexpensive, and widely available diagnostic test. Compared with culture, the technical procedures involved are faster and simpler. The most commonly used staining methods include iodine, Giemsa, and immunofluorescence. Additional stains such as immunoperoxidase, immunoferritin, May-Grünwald, Giemenez, Macchiavello, and acridine orange can also be used to identify chlamydial inclusions in exfoliated cells. Among these, immunofluorescence is the most sensitive technique, followed by Giemsa and iodine staining [70].

Molecular methods: Traditional diagnostic methods have limitations such as low sensitivity, longer test times, and high costs. Consequently, assays that directly detect DNA or RNA sequences have been developed. The PACE 2 and PACE 2C (Probe Assay Chemiluminescence Enhanced) tests are commercially available DNA probe assays capable of detecting both *C. trachomatis* and *N. gonorrhoeae* from a single sample [75]. Since in-vitro cell culture techniques replaced the yolk sac method for isolating the organism from clinical specimens, the most significant advancement in chlamydial diagnosis has been the introduction of assays based on Nucleic Acid Amplification Techniques (NAATs). NAATs are at-least 20-30% more sensitive and 100% specific compared to other methods. They also allow screening for infections in asymptomatic individuals using non invasive samples such as urine—a major advantage, as most chlamydial infections in both men and women are asymptomatic.

PCR is the most well-known DNA amplification technique. Depending on the primer design, PCR can be genus-, species-, group-, or strain-specific. Genes targeted for *C. trachomatis* diagnosis include MOMP, the endogenous plasmid, the phospholipase gene, and the 16S and 23S rRNA genes. A positive result does not depend on the viability or intactness of the target organism because nucleic acid amplification technologies detect nucleic acid sequences; therefore, sample transport is not a critical issue [76].

Roche Diagnostics developed the first FDA-approved PCR test for *C. trachomatis* detection (Amplicor PCR) in the United States. Since 1993, Amplicor PCR has been extensively evaluated for both urogenital and urine specimens, showing overall sensitivity and specificity of 90% and 99-100%, respectively. It is approved for use with urine, urethral, and cervical specimens. Quantitative real-time PCR can determine the bacterial load of *C. trachomatis* in the vaginal tract, ranging from 10 to over one million organisms per milliliter of secretion.

Newer molecular assays such as the Abbott m2000 system, Strand-Displacement Amplification (SDA), and Transcription-Mediated Amplification (TMA) have been developed with advances in molecular biology. NAATs remain the most sensitive tests for detecting and diagnosing genital tract chlamydial infections [12,17,77,78].

Non specific tests: A rapid dipstick test for urine samples, the Leukocyte Esterase (LE) test, detects enzymes produced by polymorphonuclear cells to identify urinary tract infections. *C. trachomatis* and *N. gonorrhoeae* are among the pathogens that can produce positive LE test results. The sensitivity and specificity of the LE test for detecting chlamydial infections range from 31% to 100% and 83% to 100%, respectively. It is considered the most effective screening tool for male adolescents [17,79].

Rapid point of care (POC) tests: Rapid tests for *C. trachomatis* use the EIA method, primarily based on latex immunodiffusion or membrane capture. Although several commercial kits are available, none have been thoroughly evaluated. Compared with laboratory-based EIAs, rapid tests are generally less sensitive and specific. Therefore, a positive result from a rapid test should always be considered presumptive and confirmed by a laboratory test [17,72].

Serology: Serological testing is generally not useful for diagnosing genital tract infections caused by *C. trachomatis*. Since these infections produce long-lasting antibodies, a positive result cannot distinguish between current and past infections.

Prevention of *C. Trachomatis* Infection (CTI)

According to CDC guidelines for the prevention and management of STIs, prevention strategies should include: Educating and counseling at-risk individuals on safer sexual behaviours. Effective detection and treatment of infected individuals. Identification of asymptomatic and symptomatic persons unlikely to seek diagnostic or treatment services. Assessment, care, and counseling of sexual partners of infected individuals. Pre-exposure vaccination for infections preventable by vaccines [12].

Treatment: Treatment for *Chlamydia trachomatis* infection depends on the patient's age, the site of infection, and whether the infection is complicated. Treatment regimens also vary during pregnancy. For uncomplicated infections, the CDC recommends either a single oral dose of azithromycin 1 g or doxycycline 100 mg taken orally twice daily for seven days. Other treatment options include ofloxacin 300 mg orally for seven days or erythromycin 500 mg orally four times a day [1,12,17].

Antibiotic resistance: Mourad A et al., were the first to report decreased sensitivity to erythromycin in 1980 [80]. The first documented case of reduced tetracycline sensitivity was reported by Jones RB et al., in 1997 [81]. Compared with control MICs of 0.125-0.25 mg/L, they identified five isolates from tubal infertility cases with Minimum Inhibitory Concentrations (MICs) for tetracycline ranging from 4 to >8 mg/L. These isolates were sensitive to ciprofloxacin and ofloxacin but resistant to erythromycin, clindamycin, and sulfonamides. Tetracycline resistance was also reported in France in 1997. In 2000, Somani J et al., described multidrug-resistant isolates of *C. trachomatis* associated with azithromycin treatment failure [82].

Antibiotic resistance in *C. trachomatis* differs considerably from that in most other bacteria. As an intracellular pathogen, *Chlamydia* must be tested for antibiotic susceptibility based on its ability to replicate within host cells under different antibiotic concentrations. Moreover, unlike most bacteria, *C. trachomatis* strains showing resistance to common antibiotics such as tetracycline rarely exhibit complete resistance. Instead, *C. trachomatis* demonstrates "heterotypic resistance" in-vitro- where both susceptible and resistant bacteria co-exist within the same population. Thus, although every organism in a population may have the potential to exhibit resistance, only a small fraction express it at any given time.

When the Minimum Chlamydicidal Concentration (MCC) is tested, the small proportion of resistant organisms may proliferate and form inclusions. Therefore, if both MIC and MCC tests are not conducted, heterotypic resistance in some *C. trachomatis* strains may go undetected. Strains exhibiting heterotypic resistance often display numerous abnormal inclusions, with the ratio of atypical to typical inclusions increasing as the total number of inclusions decreases-until all inclusions are either abnormal or absent. This supports the hypothesis that resistance within a chlamydial population is heterogeneous [82].

The mechanism underlying heterotypic resistance in *C. trachomatis* remains unclear. Multidrug resistance is believed to be phenotypic rather than genotypic [82]. Additionally, a prolonged or intermediate phase of the organism's life cycle that is more resistant to antimicrobial agents may develop due to heterotypic resistance. This may result from an unidentified alteration in the growth rate or life cycle, or it may be mediated by mechanisms such as efflux pumps that prevent antibiotics from entering the cell wall or chlamydial inclusion. Further research is required to validate these hypotheses [82].

Currently, there is limited information regarding the treatment of clinically resistant CTI. In-vitro studies suggest that resistance to ofloxacin may confer cross-resistance to other fluoroquinolones, including ciprofloxacin. Although several newer quinolones such as trovafloxacin, sparfloxacin, grepafloxacin, and tosufloxacin have similar or higher MIC values against *C. trachomatis*, they should be evaluated against ofloxacin-resistant strains [81]. Since conventional treatments such as doxycycline and azithromycin have been effective in treating *C. pneumoniae* infections in recurrent cases, a prolonged course of these agents may be beneficial for resistant *C. trachomatis* infections [83].

Studies have shown that azithromycin (1 g single dose) and doxycycline (100 mg twice daily for seven days) have high antibacterial efficacy against *C. trachomatis*, with microbiological cure rates exceeding 95% within 2-5 weeks and minimal evidence of antimicrobial resistance. However, women with high bacterial loads have been observed to exhibit multidrug-resistant *C. trachomatis* strains, whereas men who abstained from sexual activity after treatment did not show resistance [84].

CONCLUSION(S)

C. trachomatis is one of the most prevalent STIs. It infects epithelial cells of both male and female reproductive tracts. Although the infection is largely asymptomatic, chronic or recurrent infections are often associated with PID. In women, chronic chlamydial infections can result in serious complications such as infertility, EP, and persistent pelvic pain. Therefore, monitoring and controlling this infection, as well as understanding the true prevalence and consequences, are of utmost importance. This review highlights the impact of genital *Chlamydia* infections on human health and emphasises the need for further research on the prevalence, pathogenic mechanisms, and correlation between CTI and female infertility in India to gain a deeper understanding of its long-term effects.

The study also compares the efficacy of current *C. trachomatis* detection techniques and discusses preventive medical measures that could help interrupt the cycle of transmission. Finally, the evidence presented here may guide improvements in the national healthcare system. Future infection control initiatives could include targeted prevention campaigns to increase public awareness, enhanced screening programs, and updated diagnostic protocols for *Chlamydia*.

REFERENCES

[1] Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's principles and practice of infectious diseases E-Book: 2-volume set. Elsevier health sciences; 2019 Aug 8.

[2] Taylor-Robinson D. The discovery of *Chlamydia trachomatis*. Sex Transm Infect. 2017;93(1):10.

[3] Heyman B.Über die Fundarte der Prowazekschen Korperchen [On the locations of Prowazek's corpuscles]. Berl Klin Wochenschr 1910;47:663-66.

[4] Fritsch H, Hofstätter A, Lindner K. Experimentelle Studien zur Trachomfrage [Experimental studies on the trachoma question]. Arch f Ophth. 1910;76:547.

[5] Lindner K. Gonoblennorrhoe, einschlussblennorrhoe und trachoma [Gonoblenorrhea, inclusion blenorrhea and trachoma]. Albrecht von Graefes. Arch Ophthalmol. 1911;78:380.

[6] Jones BR, Al-Hussaini MK, Dunlop EM. Infection of the eye and genital tract by TRIC Agent. III. Ocular Syndromes associated with Infection of the Genital Tract by TRIC Agent. Br J Vener Dis. 1964;40:19–24.

[7] Moulder JW. Interaction of chlamydiae and host cells in vitro. Microbiological Reviews. 1991;55(1):143-90.

[8] Mahony JB, Chernesky MA. Effect of swab type and storage temperature on the isolation of Chlamydia trachomatis from clinical specimens. J Clin Microbiol. 1985;22(5):865-67.

[9] Mahony JB, Luinstra KE, Sellors JW, Chernesky MA. Comparison of plasmid-and chromosome-based polymerase chain reaction assays for detecting Chlamydia trachomatis nucleic acids. J Clin Microbiol. 1993;31(7):1753-58.

[10] Sachse K, Bavoil PM, Kaltenboeck B, Stephens RS, Kuo CC, Rosselló-Móra R, et al. Emendation of the family Chlamydiaceae: Proposal of a single genus, *Chlamydia*, to include all currently recognized species. Sys App Microbiol. 2015;38(2):99-103.

[11] Mackie TJ. Mackie & McCartney practical medical microbiology. Harcourt Health Sciences; 1996.

[12] Centers for Disease Control and Prevention. Chlamydia-CDC Fact Sheet. Centers for Disease Control and Prevention, Atlanta, GA 2011 [Internet].

[13] Barbour AG, Amano KI, Hackstadt T, Perry LI, Caldwell HD. *Chlamydia trachomatis* has penicillin-binding proteins but not detectable muramic acid. J Bacteriology. 1982;151(1):420-28.

[14] Matsumoto A. Structural characteristics of chlamydial bodies. Microbiology of Chlamydia. 1988:21-45.

[15] Stephens R. "Cell biology of chlamydial infection." In Proceedings of the 8th International Symposium on Human Chlammydial Infections. Societa Editrice Esculapio, Bologna, Italy. 1994a, pp. 377-386.

[16] Rank RG, Ramsey KH, Pack EA, Williams DM. Effect of gamma interferon on resolution of murine chlamydial genital infection. Infection and immunity. 1992;60(10):4427-29.

[17] Malhotra M, Sood S, Mukherjee A, Muralidhar S, Bala M. Genital *Chlamydia trachomatis*: An update. Indian J Med Res. 2013;138(3):303-16.

[18] Bleker OP, Smalbraak DJ, Schutte MF. Bartholin's abscess: The role of *Chlamydia trachomatis*. Sexually Transmitted Infections. 1990;66(1):24-25.

[19] Paavonen J, Eggert-Kruse W. *Chlamydia trachomatis*: Impact on human reproduction. Human Reproduction Update. 1999;5(5):433-47.

[20] Özdemir-Karabağ AÖ. Chlamydia: The female reproductive system and infertility. InChlamydia-Secret Enemy From Past to Present 2023 May 31. IntechOpen.

[21] Stamm WE, Koutsky LA, Benedetti JK, Jourden JL, Brunham RC, Holmes KK. *Chlamydia trachomatis* urethral infections in men: Prevalence, risk factors, and clinical manifestations. Ann Int Med. 1984;100(1):47-51.

[22] Hedger MP. Immunophysiology and pathology of inflammation in the testis and epididymis. J Andrology. 2011;32(6):625-40.

[23] Keat A, Thomas BJ, Taylor-Robinson DA. Chlamydial infection in the aetiology of arthritis. British Medical Bulletin. 1983;39(2):168-74.

[24] Hannu T. Reactive arthritis. Best Pract Res Clin Rheumatol. 2011;25(3):347-57.

[25] de Vries HJ, Zingoni A, White JA, Ross JD, Kreuter A. 2013 European Guideline on the management of proctitis, proctocolitis and enteritis caused by sexually transmissible pathogens. International journal of STD & AIDS. 2014;25(7):465-74.

[26] Malik A, Jain S, Hakim S, Shukla I, Rizvi M. *Chlamydia trachomatis* infection & female infertility. Indian J Med Res. 2006;123(6):770.

[27] Cates Jr W, Wasserheit JN. Genital chlamydial infections: epidemiology and reproductive sequelae. Am J Obstet Gynecol. 1991;164(6):1771-81.

[28] Stamm WE, Peeling RW, Money D, Brunham RC, Hawes SE, Stevens CE. Prevalence and correlates of antibody to chlamydial hsp-60 in *C.trachomatis* infected women. InEighth International Symposium on Human Chlamydial Infection 1994 Jun.

[29] Osser S, Persson K, Liedholm P. Tubal infertility and silent chlamydial salpingitis. Human Reproduction. 1989;4(3):280-84.

[30] Jaiyeoba O, Soper DE. Cervicitis and pelvic inflammatory disease. Sexually Transmitted Diseases. 2012:14-25.

[31] Janier M, Lassau F, Casin I, Grillot P, Scieux C, Zavaro A, et al. Male urethritis with and without discharge: A clinical and microbiological study. Sexually Transmitted Diseases. 1995;22(4):244-52.

[32] Mardh P, Paavonen J, Puolakkainen M. (Eds.). "Genital and associated infections in the male. In Chlamydia. New York and London, Plenum. 1989, pp. 137-150.

[33] Walker MH, Tobler KJ. Female Infertility. 2022 Dec 19. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan. PMID: 32310493. infertility.

[34] Nackley AC, Muasher SJ. The significance of hydrosalpinx in in vitro fertilization. Fertility and Sterility. 1998;69(3):373-84.

[35] Ray K. *Chlamydia trachomatis* & infertility. Indian J Med Res. 2006;123(6):730.

[36] Den Hartog JE, Morré SA, Land JA. *Chlamydia trachomatis*-associated tubal factor subfertility: Immunogenetic aspects and serological screening. Human Reproduction Update. 2006;12(6):719-30.

[37] Cunningham KA, Beagley KW. Male genital tract chlamydial infection: implications for pathology and infertility. Biology of Reproduction. 2008;79(2):180-89.

[38] Patton DL, Askienazy-Elbhar M, Henry-Suchet J, Campbell LA, Cappuccio A, Tannous W, et al. Detection of Chlamydia trachomatis in fallopian tube tissue in women with postinfectious tubal infertility. Am J Obstet Gynecol. 1994;171(1):95-101.

[39] Dutta R, Jha R, Gupta S, Gupta R, Salhan S, Mittal A. Seroprevalence of antibodies to conserved regions of Chlamydia trachomatis heat shockproteins60 and 10 in women in India. Br J Biomedsci. 2007;64(2):78-83.

[40] Dutta R, Jha R, Salhan S, Mittal A. *Chlamydia trachomatis*-specific heat shock proteins 60 antibodies can serve as prognostic marker in secondary infertile women. Infection. 2008;36(4):374-78.

[41] Menon S, Timms P, Allan JA, Alexander K, Rombauts L, Horner P, et al. Human and pathogen factors associated with *Chlamydia trachomatis*-related infertility in women. Clinical Microbiology Reviews. 2015;28(4):969-85.

[42] Bala M, Mullick JB, Muralidhar S, Kumar J, Ramesh V. Gonorrhoea & its co-infection with other ulcerative, non-ulcerative sexually transmitted & HIV infection in a Regional STD Centre. Indian J Med Res. 2011;133(3):346-49.

[43] Tapsall JW, Kinchington M. The frequency of co-infection with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in men and women in eastern Sydney. Pathology. 1996;28(1):84-87.

[44] Divekar AA, Gogate AS, Shivkar LK, Gogate S, Badhwar VR. Disease prevalence in women attending the STD clinic in Mumbai (formerly Bombay), India. International Journal of STD & AIDS. 2000;11(1):45-48.

[45] Donati M, Di Francesco A, D'Antuono A, Pignanelli S, Shurdhi A, Moroni A, et al. *Chlamydia trachomatis* serovar distribution and other concurrent sexually transmitted infections in heterosexual men with urethritis in Italy. Eur J Clin Microbiol Infect Dis. 2009;28(5):523-26.

[46] Lyss SB, Kamb ML, Peterman TA, Moran JS, Newman DR, Bolan G, et al. *Chlamydia trachomatis* among patients infected with and treated for *Neisseria gonorrhoeae* in sexually transmitted disease clinics in the United States. Ann Int Med. 2003;139(3):178-85.

[47] Joyee AG, Thyagarajan SP, Sowmya B, Venkatesan C, Ganapathy M. Need for specific & routine strategy for the diagnosis of genital chlamydial infection among patients with sexually transmitted diseases in India. Indian J Med Res. 2003;118:152-57.

[48] Jalgaonkar SV, Pathak AA, Thakar YS, Kher MM. Enzyme immunoassay for rapid detection of *Chlamydia trachomatis* in urogenital infections. Indian J Sex Transm Dis. 1990;11(1):23-26.

[49] Alexander R, Mathai E, Nayyar V, Mathew M, Jasper P. Low prevalence of chlamydial endocervical infection in antenatal South Indian women. Genitourinary Medicine. 1993;69(3):240.

[50] Joshi JV, Palayekar S, Hazari KT, Shah RS, Chitlange SM. The prevalence of *Chlamydia trachomatis* in young women. Nat Med J India. 1994;7:57.

[51] Shrikhande SN, Joshi SG, Zodpey SP, Saoji AM. *Chlamydia trachomatis* in pelvic inflammatory disease. Indian J Pathol Microbiol. 1995;38(2):181-84.

[52] Paul VK, Singh M, Gupta U, Buckshee K, Bhargava VL, Takkar D, et al. *Chlamydia trachomatis* infection among pregnant women: prevalence and prenatal importance. National Mat Med J India. 1999;12:11-13.

[53] Gopalkrishna V, Aggarwal N, Malhotra VL, Koranne RV, Mohan VP, Mittal AD, et al. *Chlamydia trachomatis* and human papillomavirus infection in Indian women with sexually transmitted diseases and cervical precancerous and cancerous lesions. Clin Micrbiol Infect. 2000;6(2):88-93.

[54] Vishwanath S, Talwar V, Prasad R, Coyaji K, Elias CJ, de Zoysa I. Syndromic management of vaginal discharge among women in a reproductive health clinic in India. Sex Transm Infect. 2000;76(4):303-06.

[55] Garg S, Sharma N, Bhalla P, Sahay R, Saha R, Raina U, et al. Reproductive morbidity in an Indian urban slum: need for health action. Sex Transm Infect. 2002;78(1):68-69.

[56] Malik A, Jain S, Rizvi M, Shukla I, Hakim S. *Chlamydia trachomatis* infection in women with secondary infertility. Fertility and Sterility. 2009;91(1):91-95.

[57] Dwibedi B, Pramanik JM, Sahu P, Kar SK, Moharana T. Prevalence of genital Chlamydia infection in females attending an Obstetrics and Gynecology out patient department in Orissa. Indian J Dermatol Venereol Leprol. 2009;75:614.

[58] Becker M, Stephen J, Moses S, Washington R, Maclean I, Cheang M, et al. Etiology and determinants of sexually transmitted infections in Karnataka state, south India. Sex Transm Dis. 2010;37(3):159-64.

[59] Salmani MP, Mindolli PB, Vishwanath G. Seroprevalence of *Chlamydia trachomatis* infection in women with bad obstetric history and infertility. The Journal of Communicable Diseases. 2011;43(4):243-47.

[60] Patel AL, Sachdev D, Nagpal P, Chaudhry U, Sonkar SC, Mendiratta SL, et al. Prevalence of Chlamydia infection among women visiting a gynaecology outpatient department: evaluation of an in-house PCR assay for detection of *Chlamydia trachomatis*. Ann Clin Microbiol Antimicrobiol. 2010;9(1):24.

[61] Prathiba G, Innocent DJ. Incidence of *Chlamydia trachomatis* infection in infertile urban population. J Ecobiotechnology. 2010;2(3):07-11.

[62] Mukherjee A, Sood S, Bala M, Satpathy G, Mahajan N, Kapil A, et al. The role of a commercial enzyme immuno assay antigen detection system for diagnosis of *C. trachomatis* in genital swab samples. Indian J Med Microbiol. 2011;29(4):411-13.

[63] Satterwhite CL, Torrone E, Meites E, Dunne EF, Mahajan R, Ocfemia MC, et al. Sexually transmitted infections among US women and men: Prevalence and incidence estimates, 2008. Sex Transm Dis. 2013;40(3):187-93.

[64] Hussen S, Wachamo D, Yohannes Z, Tadesse E. Prevalence of *chlamydia trachomatis* infection among reproductive age women in sub Saharan Africa: A systematic review and meta-analysis. BMC Infectious Diseases. 2018;18(1):596.

[65] Land JA, Van Bergen JE, Morre SA, Postma MJ. Epidemiology of *Chlamydia trachomatis* infection in women and the cost-effectiveness of screening. Human Reproduction Update. 2010;16(2):189-204.

[66] Veldhuijzen IK, Van Bergen JE, Götz HM, Hoebe CJ, Morré SA, Richardus JH, et al Study Group. Reinfections, persistent infections, and new infections after general population screening for *Chlamydia trachomatis* infection in the Netherlands. Sex Transm Dis. 2005;32(10):599-604.

[67] Oliveira FA, Pfleger V, Lang K, Heukelbach J, Miralles I, Fraga F, et al. Sexually transmitted infections, bacterial vaginosis, and candidiasis in women of reproductive age in rural Northeast Brazil: A population-based study. Memorias do Instituto Oswaldo Cruz. 2007;102:751-56.

[68] Behets FM, Ward E, Fox L, Reed R, Spruyt A, Bennett L, et al. Sexually transmitted diseases are common in women attending Jamaican family planning clinics and appropriate detection tools are lacking. Sex Transm Infect. 1998;74:S123-S127.

[69] Gunn RA, Hillis SD, Shirey P, Waterman SH, Greenspan JR. *Chlamydia trachomatis* infection among Hispanic women in the California-Mexico border area, 1993: establishing screening criteria in a primary care setting. Sex Transm Dis. 1995:329-34.

[70] Ward ME, Chlamydia RG. In: Collier L, Balows A, Sussman A, editors. Topley and Wilsons microbiology and microbial infection. 9th ed. New York: Oxford University Press Inc. 1999. p. 1331-36.

[71] Thejls H, Gnarpe J, Gnarpe H, Larsson PG, Platz-Christensen JJ, Ostergaard L, et al. Expanded gold standard in the diagnosis of *Chlamydia trachomatis* in a low prevalence population: Diagnostic efficacy of tissue culture, direct immunofluorescence, enzyme immunoassay, PCR and serology. Sex Transm Infect. 1994;70(5):300-03.

[72] Dhivya G, Sridharan KS, Reddy NS, Ramesh A. Seroprevalence of *Chlamydia trachomatis* among infertile female patients: A cross-sectional study. Nat J Lab Med. 2024;13(2):MO10-MO13.

[73] Dhivya G, Sridharan KS, Nellepalli SR, Kumar PK, Ramesh A, Katta D. Detection of chlamydial heat shock protein 60 and 10 antibody among female infertility. Journal of Global Infectious Diseases. 2024;16(2):68-72.

[74] Altwegg M, Burger D, Lauper U, Schar G. Comparison of Gen-Probe Pace-2, Amplicor Roche, and a conventional PCR for the detection of *chlamydia-trachomatis* in genital specimens. Med Microbiol Lett.1994;3(4):181-87.

[75] Schachter J, Hook EW, Martin DH, Willis D, Fine P, Fuller D, et al. Confirming positive results of nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis*: all NAATs are not created equal. J Clin Microbiol. 2005;43(3):1372-73.

[76] Michel CE, Sonnex C, Carne CA, White JA, Magbanua JP, Nadala Jr EC, et al. *Chlamydia trachomatis* load at matched anatomic sites:implications for screening strategies. J Clin Microbiol. 2007;45(5):1395-402.

[77] Dhivya G, Sridharan KS, Reddy NS, Kumar PK, Ramesh A, Katta D. Molecular identification of *chlamydia trachomatis* among infertile females. Journal of Pure & Applied Microbiology. 2024;18(1):430-37.

[78] Ingram DL, Runyan DK, Collins AD, White ST, Durfee MF, Pearson AW, et al. Vaginal *chlamydia trachomatis* infection in children with sexual contact. The Pediatric Infectious Disease Journal. 1984;3(2):97-99.

[79] Hammerschlag MR. The intracellular life of chlamydiae. InSeminars in pediatric infectious diseases. 2002 Oct 1 (Vol. 13, No. 4, pp. 239-248). WB Saunders.

[80] Mourad A, Sweet RL, Sugg N, Schachter J. Relative resistance to erythromycin in *Chlamydia trachomatis*. Antimicrobial Agents and Chemotherapy. 1980;18(5):696-98.

[81] Jones RB, Van der Pol B, Johnson RB. Susceptibility of *Chlamydia trachomatis* to trovafloxacin. J Antimicrob Chemother. 1997;39(suppl_2):63-65.

[82] Somani J, Bhullar VB, Workowski KA, Farshy CE, Black CM. Multiple drug-resistant *Chlamydia trachomatis* associated with clinical treatment failure. J Infect Dis. 2000;181(4):1421-27.

[83] Miyashita N, Niki Y, Kishimoto T, Nakajima M, Matsushima T. In vitro and in vivo activities of AM-1155, a new fluoroquinolone, against *Chlamydia* spp. Antimicrob Chemother. 1997;41(6):1331-34.

[84] Horner P. The case for further treatment studies of uncomplicated genital *Chlamydia trachomatis* infection. Sex Transm Infect. 2006;82(4):340-43.

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