Incidence of genital *Chlamydia trachomatis* infection in sexually active women attending the Mymensingh Medical College Hospital

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Introduction

*C. trachomatis* belongs to the genus chlamydia, family *Chlamydiaceae*. It is an obligate intracellular bacteria and the causative agent of genital chlamydial infection. The genital chlamydial infection is the most common Sexually transmitted diseases (STD) and major cause of infertility for millions of women in the world particularly in developing countries. It is a major public health concern in both industrialized and developing countries (WHO 2001).

According to WHO global estimates in 1999, four major STD's among 15-49 years old people were syphilis, gonorrhoea, chlamydia and trichomoniasis and the total number of new cases of these curable STDs was about 340 million. Among these, chlamydial incidence was around 92 million (27%) affecting more women (50 million) than men (42 million). The largest number of new cases (42.89 million) occurred in the region of South and Southeast Asia which was about 46.6% of the total (WHO 2001). In 2008, reported cases of three common STDs in United States (US) were Chlamydia 1,210,523 (0.4%), Gonorrhoea 336,742 (0.1%) and Syphilis 46,277 (0.01%) respectively. Chlamydia was four time’s more common than gonorrhoea, comprised the largest proportion of all STDs. It is estimated that the reported (diagnosed) cases are less than 10% of total cases (CDC 2008).

In 1990, WHO study amongst pregnant women have shown a prevalence rate ranges from 5.7 % to 17% in Western Pacific region, 1.9% to 12.2% in Latin America and Caribbean, 2.7% to 8% in Europe and 6% to 13% in Africa (Paul et al. 1999; Passay et al. 1998). Few reports from developing countries show the prevalence in pregnant
women ranging from 7% to 45%. Affluent society is affected at a high rate but complications are more in the poor, so the greatest burden falls on the developing countries (Garbase et al. 1998; Yasodhara 2001).

In Bangladesh, there are a few sporadic prevalence reports. Chowdhury, Rahman & Zaman (1989) reported 20% prevalence of chlamydial infection among all sexually transmitted diseases in Bangladesh. In another study among commercial sex workers (CSWs), Chlamydia antigen was tested by enzyme linked immunosorbent assay (ELISA) and 26.6% cases was found to be positive (Islam et al. 2000). Chlamydia antigen was also detected by Immunochromatographic (ICT) test in symptomatic women attending at Mymensingh Medical College Hospital and 58.3% cases was found to be positive (Shamsuzzaman, Parveen and Hossain 2003).

Women are at higher risk than men and young women (aged 15 to 24) are more susceptible than older (Cates et al. 1990). Women infected with chlamydia are up to five times more likely to become infected with HIV if exposed (CDC 2008).

*C. trachomatis* has group specific lipopolysaccharide (LPS) antigen and species specific and immunospecific antigen which can be best detected by immunofluorescence. It exists in more than 19 immunotypes, types D-K causes genital tract infections. It is found only in humans and transmitted by close personal contact. It can not synthesize its own ATP independently, so it is called ‘energy parasite’. It has a unique cell wall and unique developmental (replicative) cycle. It lacks muramic acid in its cell wall. The biphasic developmental cycle, consists of elementary body (EB) and reticulate body (RB), is different from all other bacteria (Suchland & Stamm 1991; Black 1997; Levinson 2009).
C. trachomatis is a major cause of genital infection such as cervicitis, urethritis and pelvic inflammatory diseases (PIDs). Chlamydial infections during pregnancy cause a variety of perinatal complications including inclusion conjunctivitis, pneumonia and delivery of low-birth-weight infants, premature rupture of membranes, ectopic pregnancy, spontaneous abortions, postpartum endometritis and perinatal death. Ectopic pregnancies are the leading cause of pregnancy-related deaths in the first trimester and account for 9 percent of perinatal complications.

Symptoms usually manifest non-specifically as excessive vaginal discharge, per vaginal bleeding, lower abdominal pain or burning sensation in micturation. The greatest challenge to control chlamydial diseases is that as many as 70%-80% of women who are infected, do not experience symptoms and remain undiagnosed and untreated. So asymptomatic individuals are important reservoir of infection for others than symptomatic genital tract infections (Stoner & Moncada 1983; Stamm & Cole 1986; Black 1997; Schachter, Chernesky 2005).

Tests to diagnose C. trachomatis is now encouraged because a positive test can facilitate referral and treatment of patient as well as sexual partners and can provide guidance for management of patients who do not respond to therapy as expected. It may not be diagnosed unless the right samples are taken and appropriately handled. The most common failure is in detecting and notifying the problems, so that re-infection occurs ((Black 1997; FitzGerald et al. 1998).

Definitive diagnosis of chlamydia can be made by isolating the organism in cell culture. Cell culture was considered the ‘gold standard’ for diagnosis of genital C. trachomatis
infection because of its high specificity (close to 100%) (Lees, Newnan and Garland 1988). Problem associated with cell culture isolation of chlamydia includes technical complexity, long turnaround time and stringent requirements related to collection, transport and storage of specimens. These have driven the development of commercially available non-cultural methods with higher sensitivities. Now, culture for detection of chlamydiae in clinical specimens is generally performed only in specialized laboratories. It has specificity of 100% but its sensitivity ranges from 35%-86%. So, the increasing demand for Chlamydia diagnosis favors a method which is less time consuming and less labor intensive and large number of specimen can be processed more quickly and economically (Thames et al.1984;Smith et al.1987;Krech, Bleckmann & Paatz 1989;Altaie et al. 1992;Black 1997).

There are several Nucleic Acid Amplification (NAA) methods such as polymerase chain reaction (PCR), ligase chain reaction (LCR), transcription mediated amplification (TMA) tests used for the detection of C. trachomatis. Although all the NAA assays appear to be highly sensitive but the actual sensitivity with the clinical specimen is lower (90%-96%) because of sampling variability and inhibition of amplification reactions by factors in the specimen. Because of these maneuvers are costly, time consuming and require validation, these maneuvers are not recommended at this time on a routine basis (Chernesky 2005; Mahony, Chong and Jang 1998).

Nucleic Acid Hybridization (NAH) tests for detection of C. trachomatis infection have been used in some parts of the world. The overall sensitivity and specificity of this method are about 85% and 98%-99% respectively but the equipment needed to process samples and detect infectious agent is costly and not suitable for developing countries(eds Brooks et al. 2007).
Among the various laboratory methods for diagnosis of *C. trachomatis* infection, microscopical examination for demonstration of intracytoplasmic inclusion bodies (IB) by iodine staining is rapid, simple, less-time consuming technique where specimen can be preserved indefinitely at -70°C. Inclusion bodies are highly sensitive in conjunctival infection (exceeds 90%) whereas in genital infections it is relatively insensitive (Gordon et al. 1969; eds Joklik et al. 1992 ). Direct cytological examination by Giemsa staining of fixed smear detects typical intracytoplasmic inclusions of infected epithelial cells obtained from involved sites. It is simple to perform, inexpensive and provides a permanent preparation. It also provides a rapid diagnosis but sensitivity is very low (15%-40%) (eds Joklik et al. 1992; Chun and Yin 1998; Chernesky 2005).

Direct Fluorescence Antibody (DFA) test involving monoclonal antibodies has been presented as a rapid alternative method for detecting *Chlamydia trachomatis* infections (Lipkin et al.1986; Tilton 1988). Several DFA assays are commercially available and use monoclonal antibodies directed against major outer membrane protein (MOMP) or Lipopolysaccharide (LPS). The anti-MOMP monoclonal antibodies are prepared against *C. trachomatis*; therefore, they are species-specific. The immunofluorescence technique is now widespread use in clinical medicine. The test is reactive with all known human serovars of *Chlamydia trachomatis* to detect extracellular elementary bodies (EB) or occasionally larger reticulate bodies (RB) (Lipkin et al.1986). Immunofluorescence staining is highly specific, combines only the antigen for which the antibody has been prepared. The sensitivity and specificity is approximately 75% to 85% and 98%-99% respectively compared with culture. The procedure is rapid, taking about 30 minutes to perform (Newhall 1994; Chernesky 2005).
Enzyme Linked Immunosorbent Assay (ELISA) detects serum antibodies much more commonly and are higher than trachoma because of relatively greater antigenic mass of chlamydial genital tract infection. In genital secretions, antibody can be detected during active infections and directed against the immunotypes. The sensitivity and specificity for IgG ELISA is 29.4% and 100% respectively (Jenab et al. 2009).

In Bangladesh, no study in relevant to this topics had been found. Having the described background, the present study was carried out on sexually active symptomatic and asymptomatic women to diagnose genital *Chlamydia trachomatis* infection by non culture techniques.

**Objectives:**

**General objective:**

To find out the incidence of genital *Chlamydia trachomatis* infection in sexually active women attending at Mymensing Medical College Hospital.
Specific objectives:

1. To diagnose genital chlamydia by detecting antigen from endocervical swabs by Direct Fluorescent Antibody Test (DFAT).

2. To identify genital chlamydia by detecting inclusion bodies from endocervical swabs by Giemsa and Iodine staining techniques.

3. To detect the seroprevalence of genital *Chlamydia trachomatis* infection by detecting IgG from serum.

4. To find out the genital chlamydial infection rate in pregnancy.

5. To find out the measures of validity of IgG ELISA (Enzyme-Linked Immuno sorbent Assay), Giemsa staining and Iodine staining methods in comparison with DFAT.
Review of Literature

2.1 Historical background of *Chlamydia trchomatis*

The description of Chlamydial disease is ancient, trachoma being one of the earliest human diseases have been recognized since antiquity. It was described in the Ebers papyrus (1500BC). Lymphogranuloma Venerum(LGV) was described in the 8th century. However, the genital tract infections were not recognized until identification of the organism in the 20th century. *Chlamydia trchomatis* was first isolated from patients with LGV in 1930s and from genital tract (other than LGV) in 1959 (Schachter and Alexander 1998).

The name trachoma was first used by Dioscorides in 60 AD. From the Middle Eastern reservoir, it spreaded through the Europe from the time of Crusades of Napoleon. Chlamydia was first detected in 1907 by light microscopy in conjuctival scrapings.

In 1903, Neisser undertook a journey to the island of Java together with two other famous scientistists — Ludwig Halberstaedter and Stanislaus von Prowazek. As it often happens, the latter, against the will of Neisser, inoculated the eyes of orangutans with trachoma material obtained from eye scrapings from patients with trachoma and found that the monkeys developed conjunctival inclusions. Similar inclusions were found in the conjunctiva of infants, the cervix of their mothers, and the urethral scrapings from male patients with non-gonococcal urethritis. But it was not until 1930 that Philip Thygeson and his colleagues were the first ones to prove the causal baby-mother-NGU connection (Dimitrakov 2002).
In 1950, Bedson and Thygeson described the unique developmental cycle of Chlamydia. Bedson referred to this agent as an obligate intracellular parasite with the bacterial affinities, a concept not generally accepted for another 30 years. In the period of 1930-1950, the clinical and epidemiological features of chlamydial diseases were described and there were search for in vitro isolation for Chlamydia. Attempts to develop a wide variety of tissue culture system and embryonated hen’s egg isolation failed. Machiavello in 1944 and Stewart in 1950, claimed the organisms growth in yolk sac of embryonated eggs, but their findings could not be confirmed. This was done by Tang et al. in 1957, in China, to clearly isolate the agent of trachoma in yolk sac and to demonstrate that this material caused inclusion positive conjunctivitis in the monkey model (Schachter and Alexander 1998). Collier and Sowa confirmed these findings in African studies in the Gambia and an explosion of trachoma research followed and it became clear from the studies that it was not a virus. In 1959 Jones et al. isolated the Chlamydia using yolk sac technique. In 1960, Chlamydia was confirmed to be bacteria, with the discovery that it contains both DNA and RNA, ribosomes, and cell wall structures typical of bacteria (Mayer 2005).

In 1964, Chlamydia was first recovered from the urethra of men. In 1965, Gordon and Quann first described the cell culture method for isolation of C.trachomatis from clinical specimen on irradiated McCoy cells. In the period of 1965-1985, the development of cell cultures for isolation, microimmunofluorescence (MIF) methods for antigen detection, immunotyping and measurement of host response permitted the clinical and epidemiological characteristics of C.trachomatis disease. 1985 -1995 nonculture tests became available for chlamydia (Xuan, Baudouin & Garcher 2001).
2.2 Epidemiology of *C. trachomatis*

2.2.1 Prevalence of *C. trachomatis*

Chlamydial genital tract infections have a world wide distribution. Among the Sexually Transmitted Diseases (STDs), *C. trachomatis* infection is the most prevalent disease today. World Health Organisation (WHO) report published in 2001 provides estimates of the extent of the world's STD epidemics in 1999. WHO reported that, around 92 million (27%) among 340 million new curable STDs (syphilis, gonorrhea, chlamydia and trichomoniasis) were due to chlamydial infections. Estimated new cases of chlamydia infections (in millions) among adults in 1999 were 3.93 (4.2%) in North America, 5.22 (5.6%) in Western Europe, 3.15 (3.4%) in North Africa and middle Europe, 5.97 (6.5%) in Eastern Europe & central Asia, 15.89 (17.2%) in Sub-Saharan Africa, 42.89 (46.6%) in South and South East Asia, 5.3 (5.7%) in East Asia and Pacific, 0.3 (0.3%) in Australia and New Zealand, 9.31 (10.1%) in Latin America and Caribbean. The largest number of new cases occurred in the region of South and Southeast Asia (WHO 2001).

In 1990, WHO study amongst pregnant women have shown a prevalence rate ranges from 5.7% to 17% in Western Pacific region, 1.9% to 12.2% in Latin America and Caribbean, 2.7% to 8% in Europe and 6% to 13% in Africa (WHO 2001). Few reports from developing countries show the prevalence in pregnant women ranging from 7% to 45% (Yasodhara 2001).
Infection rates can vary enormously between countries in the same region and between urban and rural populations. In general, the prevalence of STDs tends to be higher in urban residents and in young adults. Individuals from lower-income households, minorities that live in inner-city areas, unmarried, adolescents, young and sexually active adults are the most likely to become infected with Chlamydia (WHO 2001). Chlamydial infection rates have been increasing since the late 80s. In the United States (US) estimated annual incidence is 3 -5 million cases with a peak incidence in late teens and early twenties (Mayer 2005).

*Chlamydia trachomatis* infections are the most commonly reported notifiable disease in US. They are the most prevalent of all STDs and since 1994, have comprised the largest proportion of all STDs reported to CDC. From 1999 to 2002, the overall prevalence of chlamydial infection was 2.2% (males 2.0% and females 2.5%). In 2008, reported cases of STDs by US state health department was Syphilis 46,277 (0.01%), Chlamydia 1,210,523 (0.4%), Gonorrhoea 336,742 (0.1%) and Chancroid 00 (0.0%) respectively. In 2007, reported cases of chlamydia was 1,108,374 (0.3%) which was significantly lower than that of 2008. Recent studies also demonstrate the high prevalence of chlamydial infections among the general US population. Under-reporting is substantial since most cases are asymptomatic because 75% of women and 50% of men are not aware of their infection (CDC 2008).

Chlamydia causes more than 250,000 cases of epididymitis and 250,000 to 500,000 cases of PID every year in the United States (US). Women infected with chlamydia are up to five times more likely to become infected with HIV, if exposed (Wikimedia 2010). Age is the most important risk factor for chlamydial infection. The burden of chlamydia appears higher among women especially those of younger age. Among women, highest
age-specific rates of reported chlamydia in 2008 were 3,275.8 cases per 100,000 females (3.3%) among 15 to 19 years of age and 3,179.9 cases per 100,000 females (3.2%) among 20 to 24 years of age. Among men, age-specific rates was highest in the 20 to 24 year old age group, 1,056.1 cases per 100,000 males (1%), which was substantially lower than the rates among women. *C. trachomatis* infections both of the vision organ and urinogenital tract affect mainly young people (CDC 2008).

Females are more affected than male. The incidence of chlamydial infection in women increased dramatically between 1987 and 2003, from 79 to 467 per 100,000 (from 0.08% to 0.46%) (Miller 2006). From 2004 through 2008, the chlamydial infection rate in men increased by 45.0% (from 145.6 to 211.1 cases per 100,000 males) compared with 21.5% increase in women during the same period (from 480.6 to 583.8 cases per 100,000 females). In 2008, the overall rate of reported chlamydial infection among women in US was 583.8 cases per 100,000 females (around 0.6%) and among men 211.1 cases per 100,000 males (0.2%). The rate in female was almost three times higher than the rate among men (CDC 2008).

In addition to gender disparities, there are also racial and ethnic disparities. Non white had a greater incidence than whites. In 2008, chlamydia rates increased for all racial and ethnic groups. The rate of chlamydia among blacks was 1,519.3 cases per 100,000 (1.5%) and among whites was 173.6 cases per 100,000 (0.17%). The rate was over eight times higher in blacks than that of whites (CDC 2008).

*C. trachomatis* is currently classified into 19 serovars. Serovars D to K are chiefly responsible for urogenital infections; serovars E, F, and D account for up to 60% to 70% of these infections. Serovers D, E, G are predominant in pregnant women. Mixed
infections with two or more strains are evident in 3%-8 % of highly active populations. Approximately 35%-50% of non-gonococcal urethritis (NGU) is due to *C. trachomatis*: biovar- trachoma (Mayer 2005).

### 2.2.2 Habitat, Transmission and Reservoir of infection

*C. trachomatis* infect only human.

**Habitat** - Human genital tract and eye.

**Transmission**: Transmitted by close personal contact with infected person, e.g., sexually or by passing through the birth canal of infected mother. Transmission from men to women and women to men are equally efficient.

**Reservoir**: Individuals with genital tract infections are reservoir of infection for others.

**Recurrence** is caused by presence of Chlamydia in the lymph nodes and spleen of reservoir. Incidence of recurrent infection was calculated to 29% (12% to 46%) during the 24 weeks of follow up. Previous or present sexually transmitted diseases other than *C. trachomatis* were significantly associated with recurrence (Kjaer 2000). Chlamydia can persist in macrophages even for 10 days. They circulate with macrophages round the body, finding a temporary shelter in the lymph nodes, spleen and serous cavities (Stefanow, Puchalska & Pucilo 2003).

**Incubation period**: 1 to 3 weeks.

### 2.3 Microbiology of *C. trachomatis*

#### 2.3.1 Taxonomy of *C. trachomatis*

**Phylum**: chlamydiace (a bacteria, all of which are intracellular parasites of eukaryotic cells.)
**Kingdom:** bacteria (microscopic and unicellular)

**Class:** Chlamydiae

**Order:** Chlamydiales

**Family:** Chlamydiaceae

**Genus:** Chlamydia

**Species:** *C. trachomatis*

**Domain:** Prokaryote, any unicellular microorganism that lack a nucleus.

**Division:** Gram negative

(Mellissa’s Microbe 2005).

**Serovars**

Different major outer membrane protein

A through L

**Biovars** of *C. trachomatis* – 3(three):

- Trachoma biovar- serovars A, B, Ba or C
- Urethritis biovar are - serovars D-K.
- Lymphogranuloma venereum (LGV) biovar are - serovars L1, L2, L2a, L2b and L3.

(Fredlund et al. 2004; Mayer 2009).

The word *chlamys* is Greek for "cloak draped around the shoulder." This describes how the intracytoplasmic inclusions caused by the bacterium are "draped" around the infected cell's nucleus (Clark n.d.).

Genital infections are caused by B and D through K including the sreovars Da and Ia and the genovarint Ja (Wang & Grayston 1991).

**2.3.2 Physiology and Structure**
Morphology

The *C. trachomatis* are Gram negative, strict intracellular bacterial parasites, does not have a regular morphology, can not be grown in artificial media. The *Chlamydia trachomatis* are small, typically coccoid or rod shaped, non-motile, lacking flagella and non pilliated. They possess unusual cylindrical surface projections, averaging 18 in number and arranged in a hexagonal array. They have a rigid cell wall but do not have typical peptidoglycan layer, do not stain well with Gram stain, cluster of bacteria can be stained with Giemsa stain rather than the cell. Their cell walls resemble those of gram negative bacteria but lack muramic acid (Levinson 2009).

There are two morphologically distinct forms of chlamydiae: elementary body (EB) and reticulate body (RB) that distinguish from all other groups of bacteria (‘Wikimedia’ 2010).

A. Elementary body(EB)

EB are small cell, dense spherical body with an electron dense nucleoid, 0.2 to 0.4 µm in diameter, which rivals mycoplasma for the designation “smallest of the prokaryotrs.” It is the extracellular, metabolically inert, non-replicating, ‘spore like’ infectious form of the organism, responsible for attaching to the target host cell promoting its entry. They possess an outer membrane that is extensively cross-linked by disulfide bonds. Because of the rigid outer membrane the EBs are resistant to harsh environmental conditions encountered when the chlamydia are outside their eukaryotic host cell. The EB bind to receptors on host cells and initiate infection. The EB have a high affinity for host epithelial cells and rapidly enter them. Most Chlamydia infects columnar epithelial cells but some can also infect macrophages (Mayer 2005).
B. Reticulate body (RB)

RBs are ovoid intracellular, non-infectious, metabolically active replicating form of the Chlamydia that divides by binary fission. It is larger than the EB, approximately 0.5 to 1.0 μm in diameter and devoid of an electron dense nucleoid. They possess a fragile membrane lacking extensive disulfide bonds characteristics of the EB.

Developmental cycle (Growth cycle)

A unique highly specialized biphasic developmental cycle characterizes the growth of these intracellular bacteria in their host cells. The cycle consists essentially of five major phases: (1) attachment and penetration of the EB, (2) transition of the metabolically inert EB into metabolically active form RB, (3) growth and division of the RB, producing many progeny, (4) maturation of the non infectious RB into infectious EB and (5) release of EB from the host cell. The infectious and non infectious stages exhibit unique morphological, biochemical and biological properties.

In the infectious stage, the bacteria are in a state similar to that of an endospore, where the outer membrane is resistant to the environment and allows it to exist without a host cell (Mayer 2005). The initial event in the infectious process begins with attachment of the EB to microvilli of susceptible columnar epithelial cell. The EB travels down the microvilli and localizes indentations of the host cell plasma membrane resembling coated pits. There are multiple adhesions, receptors, and mechanism of entry. Heparin sulfate-like proteoglycans on the surface of C. trachomatis is likely possibilities for mediating at least the initial interaction between EBs and host cells. Other potential adhesions include
the major outer membrane protein (MOMP). Glycosylated MOMP and other surface proteins (eds Brooks et al. 2007).

The EB internalized by receptor mediated endocytosis in clathrin coated pits, by pinocytosis via non-coated pits and or by phagocytosis. Shortly after entry into the host cell, the disulfide bonds of the EB membrane proteins are no longer cross linked and the EBs reorganize and undergo changes in their cell wall that result in a spheroplast transition to the larger RB form within the host cell endosome within 6-8 hours. In this state, the bacteria use the host cell’s ATP to reproduce through binary fission. The vacuole that encloses the bacteria protects the reticulate body from cellular lysosomes and prevents them from being eliminated by these defense mechanisms (Dimitrakov 2002). DNA, RNA and protein synthesis are initiated, permitting the growth of the RB and replication of RB by repeated binary fission at approximately 2-3 hours per generation.

The cell body has an incubation period of 7 to 21 days in the host. It contains no cell wall and is detected as an inclusion in the cell. The developing microcolony containing 100 to 500 progeny is termed as inclusions. The RBs mature into EBs when nutrients are depleted. Eventually, the entire vacuoles become filled with daughter EBs to form cytoplasmic inclusions and after 2 to 3 days, hundreds of EBs are released from the host cell by reverse endocytosis. The elementary bodies then go on to infect other host cells, where the process continues and the infection spreads. The reticulate body is dependent on a host cell for survival whereas the elementary body does not require a host cell to survive. One phagolysosome usually produces 100 to 1000 elementary bodies (Clark n.d.). The entire intracellular life cycle of Chlamydia occurs within the endosomes. Intracytoplasmic inclusions formed after 4 to 8 hours, conversion of EB into RB after 8
to 12 hrs, conversion of RB into EB after 30 to 40 hrs and release of EB via cell lysis or exocytosis after 48 to 72 hrs. The entire development cycle of Chlamydia lasting from 48 to 72 hrs. (Dimitrkov 2002; Mayer 2005; Belland et al. 2003).

**Growth & Metabolism**

Chlamydia require an intracellular habitat, because they are unable to synthesize ATP and depend on the host cell for energy requirement. Chlamydia are aerobic, grow in cultures of a variety of eukaryotic cell lines. McCoy cells treated with cycloheximide are commonly used to isolate chlamydia. *C. trachomatis* can proliferate in embryonated eggs, particularly in the yolk sac. The cell wall lacks muramic acid. It instead contains cysteine-rich proteins. This allows for the intracellular division and extracellular survival (Meillissa’s Microbe 2005).

For metabolism, *Chlamydia trachomatis* has a glycolytic pathway and a linked tricarboxylic acid cycle. Glycogen synthesis and use of glucose derivatives plays a supporting role in chlamydial metabolism (Stephens 1998).

The replication of chlamydia can be inhibited by many antibacterial drugs. Inhibitors of protein synthesis (tetracycline, erythromycin) are effective in most clinical infections. *C. trachomatis* synthesize folates and are susceptible to inhibition by sulfonamide (eds Brooks et al. 2007).

**Structure & Chemical Composition**

In chlamydia, the outer cell wall resembles the cell wall of gram negative bacteria. It has relatively high lipid content. It is rigid but does not contain a typical bacterial
peptidoglycan. Penicillin binding protein occurs in chlamydia, N-AcetylMuramic acid absent from chlamydial cell walls. Both DNA and RNA are present in elementary and reticulate bodies. The RBs contain about four times as much RNA as DNA, whereas the EBs contain about equal amounts of RNA and DNA. In EBs, most DNA is concentrated in the electron-dense central nucleoid. Most RNA exists in ribosomes. The circular genome of chlamydia (MW $7 \times 10^8$) is similar to that of bacterial chromosomes (eds Brooks et al. 2007).

**Genome structure**

*Chlamydia trachomatis* have a double stranded DNA genome that consists of 1,042,519 nucleotide base pairs and has approximately 894 protein coding sequences (Stephens et al. 1998). The outer membrane contains several proteins including the major outer protein (MOMP) which differs between chlamydial species and has species, subspecies and serovar specific antigenic domain. The MOMP of *Chlamydia trachomatis* consists of four variable and five interspersed constant domains (Fielder et al. 1991). MOMP genes encode highly conserved protein structure that contains four evenly spaced variable domains (VDs) whose sequences vary on the different serovars. The VDs lead to multiple *C. trachomatis* serovars associated with different clinical manifestations of ocuculogenital infections (Jantos et al. 1997).

The major neutralizing and serotyping antigenic determinants are located on these VDs. VD III is smallest and least variable domain. Antigenic determinants have not been mapped to VDIII. VD IV is the largest of the domains, is the location of the subspecies, serogroup and highly conserved species specific antigenic determinant. VD1 and VDII are the locations of serovar specific determinants. The MOMP VDs are primarily responsible for antigenic differences between serovars and are associated with important
immunological and biological properties. Comparative amino acid sequence homologies of all four domains separated the serovars into three groups: group 1 serovars are B, Ba, D, E, L1 and L2; group 2 serovars are G and F; and group 3 serovars are A, C, H, I, J, K, and L3 (Yuan et al. 1989).

Most strains of *C. trachomatis* strains have an extrachromosomal plasmid, which was sequenced to be a 7493-base pair plasmid (Stephens et al. 2001). Interestingly, chlamydial plasmids are more closely related than the corresponding chromosomal DNA in their nucleotide sequence (Yuan et al. 1989).

The plasmid of *C. trachomatis* is a favored target for DNA-based diagnosis of *C. trachomatis* because there are approximately 7 to 10 copies of the plasmid present per chlamydial particle. Its sequence is highly conserved among different isolates of *C. trachomatis*. Some *C. trachomatis* strains lack these plasmids. Plaque purified *C. trachomatis* that do not contain the plasmids have unusual inclusion morphology, have no glycogen and show no alteration in antibiotic sensitivity. However, the fact that, the plasmid is not essential for *C. trachomatis* survival (Kalman et al.1999).

**Antigenic Structure of *C. trachomatis***

*C. trachomatis* is a strong immunogen that stimulates immunological processes of the host. At present, four antigen groups are distinguished within the *Chlamydia* genus. Group-specific antigen, shared by all *Chlamydia* species, the main component of it’s a thermostable polysaccharide complex, contains two constituents: lipopolysaccharide (LPS) and glycolipid (GLXA) (Stuart, Troidle & macdonald 1994). In chemical structure, molecular weight and function, the group-specific antigen resembles the LPS
antigen of certain Gram-negative bacteria and yeast-like fungi (Stefanow, Puchalska & Pucilo 2003).

Species-specific antigens, varying according to the *Chlamydia* species, have a protein structure and are thermolabile. They contain a few types of proteins of various molecular weight, of which the most important are the major outer membrane protein (MOMP) of 38–42 kDa, encoded by the *ompA* gene constituting 60% of all outer membrane proteins (OMP); 60–62 and 155 kDa proteins; and the so-called chlamydial heat shock proteins (C-HSP) of 10 and 60 kDa (Ward 1983). The 60-kDa cystein rich OMP2 (OmcB) is also highly conserved and could account for the osmotic stability of EBs by forming a disulfide cross links network with the periplasmic MOMP domains and other proteins. OmcB binds heparin and this may be related to mammalian host cell adhesion and entry (Everett & Hatch 1995; Stephens et al. 2001).

The third antigen group is of type-specific antigens, characteristic of the respective serotypes within the *C. trachomatis* species, most likely polypeptides of 30 kDa and thermolabile by nature.

The last type of antigen, also of polypeptide structure, refers to subspecies-specific antigens. Based on their presence, the respective serotypes of *C. trachomatis* have been categorized as two major subspecies, group B and group C (Wang and Grayston 1991; Stefanow, Puchalska & Pucilo 2003).

**Virulence Factors**
There are numerous factors that contribute to the pathogenicity of *Chlamydia trachomatis*. Colonization of Chlamydia begins with attachment to sialic acid receptors on the eye, throat or genitalia. It persists at body sites, in T-cells and B-cells, that are inaccessible to phagocytes. It also exists as different serotypes. These serotypes cause four major diseases in humans: trachoma, sexually transmitted disease (STD), inclusion conjunctivitis and lymphogranuloma venereum (LGV). Its unique cell wall structure is another virulence factor. Studies reveal that Chlamydia, because of its cell wall, is able to inhibit phagolysosome fusion in phagocytes (Hatch 1996).

The cell walls resemble those of gram-negative bacteria in that it contains an outer lipopolysaccharide (LPS) membrane but it does not have a typical peptidoglycan layer because it lacks muramic acid. However, it may contain a carboxylated sugar other than muramic acid. Its LPS is another virulence factor which causes damage to the host’s body due to the host’s immune response. It also contains cysteine-rich proteins (CRP) that may be the functional equivalent to peptidoglycan. This unique structure allows for intracellular division and extracellular survival (Levinson 2009; Hatch 1996).

Antigenic variation is also an important factor with different serotypes. The serovar or serogroup specific major outer membrane protein (MOMP) on the chlamydial cell surface is an important virulence factor (Zhang et al. 1987). Type 111 secretion system is another virulence factor identified recently (Yang n. d.). The *Chlamydia trachomatis* plasmid is a transcriptional regulator of chromosomal genes and acts as a virulence factor also (Carlson et al. 2008).

### 2.4 Pathogenesis and immunity
*Chlamydia trachomatis* genital and ocular infections are transmitted by direct contact. Transmission of *C. trachomatis* from the urogenital tract to the eyes and vice versa occurs via contaminated fingers, towels or other fomites and transmission in neonates by passage through an infected birth canal. These diseases appear in an epidemic form in populations with low standards of hygiene. *C. trachomatis* genital infections are sexually transmitted. (Becker n.d.).

EB enter the body via minute abrasions and lacerations. Chlamydiae have a hemagglutinin that may facilitate attachment to cells

↓

Infect non-ciliated columnar, cuboidal or transitional epithelial cells (urethra, endocervix, endometrium, fallopian tube, anorectum) and macrophages in LGV

↓

LGV biovars replicate in mononuclear phagocytes in lymphatic system

↓

Down regulation of Class I MHC

↓

Infection stimulates a severe inflammatory response (infiltration of neutrophils, lymphocytes and plasma cells).

↓

Submucosal lymphocytic infiltration leads to formation of lymphoid follicle

↓
Formation of granuloma, abscesses or sinus tracts in lymph nodes draining the site of primary infection

↓

Tissue damage, fibrosis and cicatrization within the affected organs.

↓

Disease results from destruction of cells and host immune response.

No long-lasting immunity after infection

Re-infection induces a vigorous inflammatory response with subsequent tissue damage (blindness and sterility) (Mayer 2009).

The pathogenesis of *C. trachomatis* infection has not been elucidated but pathophysiology has been focused on antigens i.e. major outer membrane protein (MOMP), heat shock protein (HSP60) and lipopolysaccharide (LPS). The MOMP, HSP60 and LPS are weak inducers of the acute immune response which may explain why lower genital tract infection with *C. trachomatis* is often asymptomatic (Ingalls et al.1995).

The disease process and clinical manifestation of chlamydial infection probably represent the combined effects of tissue damage, chlamydial replication and inflammatory response to chlamydia and the necrotic material that are destroyed by host cells. Chlamydial LPS plays an important role in the pathogenesis of chlamydial infection. The inflammatory cytokine responses to chlamydia infection are mediated primarily through LPS. Pro inflammatory cytokines upregulate receptors for lymphocyte adhesion and receptors for MHC class 11 expressions (Ingalls et al.1995).

**Immunologically mediate pathogenesis hypothesis:**
Chlamydial disease result, in part, from hypersensitivity or it is a disease of immunopathology (Patton, Sweeney and Kuo 1994). The current prevalent theory is that much of the chlamydial disease is due to the delayed hypersensitivity reactions to specific Chlamydia heat shock protein (Morrison et al. 1989). These heat shock protein (HSP) are similar to the HSP of other organisms (Wager et al.1990). Chlamydial HSP60 contains specific antigenic sites but it has other antigenic sites that are also present in Mycobacteria and E. coli and even in eukaryotic cells. Antibodies formed against C. trachomatis cross react with antigens on the cells of the urethra, joints and uveal tract. Thus it is possible that, part of the chlamydial disease may be due to autoimmune reaction. (Toye et al.1993).

During primary infection, a serial infection of mucosal cells may occur. The infected and damaged epithelial cells secrete numerous proinflammatory chemokines and cytokines including interleukin IL-1, IL-6, IL-8, granulocyte macrophage colony-stimulating factor (GM-CSF), growth regulated oncogene and tumour necrosis factor (TNF)-α (Morton & Kinghorn 1999). The released cytokines cause vasodilation, increased endothelial permeability, activation and migration of neutrophils, monocytes and to lesser extent macrophages and T lymphocytes. It also causes elevated expression of adhesion molecules and stimulation of other cells to secrete cytokines. The local inflammatory reaction is intense on days 2 to 4 after onset of infection. IL-1, secreted by undamaged cells, has a special role to stimulate the secretion of other cytokines by non-infected adjacent cells and TNF-α release inductor, a strong initiator of the inflammatory process (Rassmusen et al. 1997).
*Chlamydia* passes via lymphatic vessels to local lymph nodes, which in many cases become enlarged. Some elementary bodies, released by decaying epithelial cells, are phagocytized by neutrophils, probably through the fusion of phagosomes and lysosomes. The bactericidal action of neutrophils is associated with the production of hydrogen peroxide and secretion of myeloperoxidase (Yong, Klebanoff & Kuo 1982).

T lymphocytes, mainly T helper (Th1) cells, have an important role during the early phase of the infection. They secrete IFN-γ, necessary for regression of infection, due to activation of *Chlamydial* antigen (Lampee et al. 1998). IFN γ increases the potential of various phagocytes especially monocytes and macrophages, to destroy *Chlamydia* and stimulates the secretion of other cytokines including IL-1. IL-1, in turn, stimulates the secretion of IL-2 by Th1 lymphocytes which causes increased replication of cytotoxic lymphocytes and natural killer cells (Witkin et al. 2000).

The production of secretory immunoglobulin A class antibodies also plays a role in the neutralization of primary infection. Locally produced antibodies limit the spread of chlamydial infection but do not eliminate the bacteria completely. In most cases, the immune response of the host to the primary infection is transitory and is not associated with tissue damage (Stefanow, Puchalska & Pucilo 2003).

**Chronic Infection, Recurrent Infection, Reinfection**

Recurrence and reinfection constitute another problem. Reinfection is due to repeated infection, while recurrence is caused by the presence of a Chlamydia reservoir in the lymph nodes and spleen. Reports on a large number of recurrences (20%–39%) observed both in men and in women with chlamydial infection a few months after
therapy. Macrophages, in which Chlamydia can persist even for 10 days, have a special role in disease recurrence. They circulate with macrophages round the body, finding a temporary shelter in the lymph nodes, spleen and serous cavities (Jones 1990; Stefanow, Puchalska & Pucilo 2003).

Chronic infection, associated with the persistence of the bacteria in host cells (reiterative infection) or reinfection, are much more dangerous. The inflammatory state develops in a considerably shorter time and is more intense which is associated with the presence of specifically sensitized Th1 lymphocytes. A long-term or recurrent stimulatory action of Chlamydia antigens results in a delayed hypersensitivity reaction or the more seldom type III hypersensitivity reaction (Arthus reaction) (Paavonen 1996).

In a type III reaction, immune complexes are formed in the circulation or tissues as a result of interactions of soluble antigen with a specific antibody. Processes which occur during these immune reactions lead to tissue damage, fibrosis and cicatization within the affected organs. The consequences can be severe and often irreversible, e.g. trachoma (conjunctiva cicatization, blepharon deformation, loss of sight), pelvic inflammatory disease (PID) leading to mechanical infertility, ectopic pregnancy, chronic pelvic pains and chronic urethritis (urethronnosis) (Cates & Wasserheit 1991).

Since chlamydial infection is frequently asymptomatic, the sequelae are common and cause serious health and economic problems. After a single episode of salpingitis, about 1 patient in 10 becomes infertile because of tubal occlusion. After two or three episodes, infertility ensues in about 35% to 70% cases. In chronic infections in women with salpingitis, Chlamydia can be more frequently detected in the material collected from the
salpinx or endometrium (40%–60%) (Stefanow, Puchalska & Pucilo 2003; Paavonen et al. 1987).

Chronic infection may result from lack of treatment or improper therapeutic management. Dietary factors, such as an insufficient supply of tryptophan, L-isoleucine and cysteine in the diet, as well as certain cytokines, e.g. interferon (IFN)-γ, TNF-α and transforming growth factor (TGF)-β play a significant role. They all lead to disorders in the growth cycle of the bacteria, including RB maturation, delay or inhibition of their differentiation into infectious EB and thus to the growth of atypical forms of Chlamydia. The atypical forms are larger, non-infectious, have reduced metabolic activity and do not replicate, yet remain alive. They display a different antigenic structure, characterized by a reduced number of MOMP and LPS and invariable amounts of the pathogenic C-HSP 60 kDa, a strong stimulator of immune reactions (Aultk et al. 1996; Beatty et al. 1993).

The role of the C-HSP 10 kDa, in the pathogenesis of complications in C. trachomatis infection has been emphasized (Stefanow, Puchalska & Pucilo 2003). Irreversible sequel occur mainly due to the delayed hypersensitivity reactions induced by this strong antigen or as a result of an autoimmune reaction associated with a similarity between the chlamydial HSP and human HSP (about 50% homology) (Witkin et al. 1994).

In patients with complications, a decrease in the production of anti- MOMP antibodies with protective properties and an increased production of pathogenic anti-C-HSP antibodies are frequently observed. Affected by IFN-γ, the bacteria are hypnotized in macrophages and their genetic material are transmitted into further generations of these cells. The anti-MOMP antibodies play a slightly protective role in reinfection, hence the
lack of efficacy of the vaccine, based on the major outer membrane antigens (Paavonen & Lethinen 1994; Cotter et al. 1995; Morton & Kinghorn 1999).

2.5 Clinical Syndromes

Most Chlamydial bacteria affect the epithelial cells where mucous membranous tissues are found. The most prevalent area of infection is the urogenital tract. Symptoms generally occur one to three weeks after contact with an infected individual (Houry 2004; Mayer 2005).

Urogenital infections

In females the infection is usually (80%) asymptomatic but symptoms of cervicitis, urethritis and salpingitis can include. The symptoms of Chlamydial urogenital infections in women are vaginal discharge, cramps or pain in the lower abdomen and burning sensation in micturation. Postpartum fever in infected mothers is common. If left untreated, Chlamydia can cause pelvic inflammatory disease, infertility, premature delivery and an increased rate of ectopic pregnancy due to salpingitis. Tubal pregnancy is the leading cause of pregnancy related deaths in first trimester (Hwang 1998; Houry 2004; Mayer 2005).

In males, the infection is usually (75%) symptomatic. After a 3 week incubation period, patients may develop urethral discharge, dysuria and pyuria. Approximately 35% to 50% of non-gonococcal urethritis is due to C. trachomatis (biovar: trachoma). Post-gonococcal urethritis also occurs in men infected with both Neisseria gonorrhoeae and C. trachomatis. The symptoms of chlamydial infection occur after treatment for gonorrhea because the incubation time is longer (Mayer 2005).
**PID**

Up to 40% of women with untreated chlamydia will develop pelvic inflammatory diseases and about 20% of these women will become infertile. Many untreated cases (about 18%) result in chronic pelvic pain. Women infected with chlamydia have a 3 to 5 fold increased risk of acquiring HIV (Mayer 2005).

**Reiter's syndrome**

Reiter's syndrome is a triad of symptoms that include conjunctivitis, polyarthritis and genital inflammation. The disease is associated with HLA-B27. Approximately 50% to 65% of patients have an acute *C. trachomatis* infection at the onset of arthritis and greater than 80% have serological evidence for *C. trachomatis* infection. Other infections (e.g. shigellosis or *Yersinia enterocolitica*) have also been associated with Reiter's syndrome. Infected women can pass Chlamydia to their newborn during birth and disease usually develops within the first ten days of life. Chlamydia can also result in premature birth (Hwang 1998; Mayer 2005).

**Inclusion conjunctivitis**

Inclusion conjunctivitis is caused by *C. trachomatis* associated with genital infections (serovars D to K). The infection is characterized by a mucopurulent discharge, corneal infiltrates and occasional corneal vascularization. In chronic cases, corneal scarring may occur. In neonates, infection results from passage through an infected birth canal and
becomes apparent after 5 to 12 days. Ear infection and rhinitis can accompany the ocular disease (Schachter & Aixander 1998; Mayer 2005).

**Infant pneumonia**

Infants infected with *C. trachomatis* (serovars: D to K) can develop pneumonia at birth. The children develop symptoms of wheezing and cough but not fever. The disease is often preceded by neonatal conjunctivitis (Preece, Anderson & Thompson 1989; Mayer 2005).

### 2.6 Diagnosis of *C. trachomatis* urogenital infection

**Clinical**

**A) History**

History should be taken regarding risk factors including unprotected sexual intercourse, multiple partners, commercial sexual transaction, other STDs in self or in partner. Teenage girls and young women are at increased risk of getting infection (CDC 2007). History should also include previous Chlamydia exposure, treatment history particularly incomplete treatment, partner's sexual history and previous pelvic inflammatory disease.

**B) Clinical manifestations**

70% to 80% women and up to 50% men are infected with *C. trachomatis* do not experience symptoms If symptoms do occur, they usually appear within 1 to 3 weeks after exposure (Stamm and Cole 1986).
In women, who have symptoms might have an abnormal vaginal discharge or a burning sensation when urinating, lower abdominal pain, low back pain, nausea, fever, pain during intercourse or bleeding between menstrual periods. Chlamydial infection of the cervix can spread to the rectum.

Men with signs or symptoms might have a discharge from their penis or a burning sensation when urinating or burning and itching around the opening of the penis.

Men or women who have receptive anal intercourse may acquire chlamydial infection in the rectum, which can cause rectal pain, discharge or bleeding. Chlamydia can also be found in the throats of women and men having oral sex with an infected partner (CDC 2007).

**Laboratory**

The laboratory diagnostic procedures are:

1. **Microscopy (Cytological examination):**
   Detection of inclusion bodies by-
   1. Giemsa staining technique
   2. Iodine staining technique

2. **Isolation procedure:**
   1. Isolation in cell culture
   2. Yolk sac isolation

3. **Immunological test**
   Antigen detection:
   1. Direct Fluorescence Antibody (DFA) test
   2. Enzyme Linked Immunosorbent Assay (ELISA)
3. Point of care EIA
4. ICT (Immunochromatographic Test)

Antibody detection:
1. Enzyme Linked Immunosorbent Assay (ELISA)
2. Recombinant Enzyme-Linked Immunosorbent Assay.
3. Microimmunofluorescence (MIF).
4. Whole-inclusion immunofluorescence test (WIF)
5. Complement fixation test (CFT)
6. Time resolved fluorometric immunoassay (TRFIA)

*Newer non-culture test (Molecular diagnostic test)*:
1. NAAT (Nucleic Acid Amplification Test)
2. NAH (Nucleic Acid Hybridization)
3. RNA Amplification by Nucleic Acid Sequenced-Baesd Amplification (NASBA)
   (Mayer 2007; Mahony et al. 2001).

**Microscopy**

**Iodine staining technique**

Among the various laboratory methods for diagnosing *C. trachomatis*, iodine staining is rapid, simple less-time consuming technique when specimen can be preserved indefinitely. Demonstration of typical intracytoplasmic inclusions in direct smear can be useful in diagnosing of *C. trachomatis* infection. Glycogen containing inclusions of *C. trachomatis* in cytoplasm of epithelial cells are stained brown with iodine. Inclusion bodies are highly sensitive in conjunctival infection (exceeds 90%) whereas in genital infections it is relatively insensitive (Gordon et al. 1969; eds Joklik et al. 1992; Chernesky 2005).
**Giemsa staining technique**

Direct cytological examination by Giemsa staining of fixed smear also provides a rapid laboratory diagnosis of chlamydial infection. It detects typical intracytoplasmic inclusions of infected epithelial cells obtained from involved sites. It can also be applied to monolayer of chlamydia infected McCoy cells. Giemsa stain is an alcohol based Romanowsky stain that requires dilution in buffered water or buffered physiological saline. Dilution in buffered water often gives a cleaner background to the preparation. It is simple to perform, inexpensive and provides a permanent preparation (eds Joklik et al. 1992; Chun and Yin 1998; Fedorko and Smith 1991).

**Isolation**

**Isolation in cell culture**

Definitive diagnosis can be made by isolating the organism in tissue culture and by NAA testing in selective circumstances. Cell culture was considered the ‘gold standard’ for diagnosis of genital *C. trachomatis* infection before the development of NAAT (Lees, Newnan and Garland 1988) because of its sensitivity and specificity were thought to be close to 100%. Problem associated with cell culture isolation of chlamydia includes technical complexity an long turnaround time and stringent requirements related to collection, transport and storage of specimens. These have driven the development of commercially available noncultural methods that have found wide spread application in many routine laboratories. With the advent of antigen detection methods, it became clear that the sensitivity of culture was substantially lower than previously thought, most
probably due to the presence of nonviable chlamydiae that died during transport and processing. Culture for detection of chlamydiae in clinical specimens is generally now performed only in specialized laboratories. Culture is strongly recommended in treatment failures cases and in cases related to possible sexual assault for medicolegal reasons ((Black 1997; Johnson et al. 2002).

**Susceptible cell lines** for *C. trachomatis* (Madhavan 1992)

1. McCoy - most commonly used for *C. trachomatis*
2. HeLa229 - commonly used for *C. trachomatis*
3. Hep-2
4. HL
5. BHK29
6. L929
7. Buffalo green monkey kidney cell (BGMK)
8. Vero cells.

McCoy cell lines, a continuous cell lines, tissue of origin is mouse fibroblast cells, commonly used for isolation of *C. trachomatis* (Schachter and Stamm, 1995). Culture is performed by inoculating a confluent monolayer of human epithelial cells and incubating for 48 to 72 hours. When the infected cells develop characteristic intracytoplasmic inclusions, that contain *C. trachomatis* EBs and RBs, are detected by staining with fluorescent-conjugated monoclonal antibody, specific for MOMP of *C. trachomatis* (Lees, Newnan and Garland 1988). The infected cells can be examined for the presence of iodine-staining inclusion bodies. Iodine stains glycogen in the inclusion bodies. The
The presence of iodine-staining inclusion bodies is specific for *C. trachomatis* since the inclusion bodies of the other species of chlamydia do not contain glycogen. The sensitivity of this test is about 80% but specificity is 100% (eds Brooks et al. 2007).

**Embryonated Egg Yolk Sac inoculation:**

Prior to 1950s, the standard host for virus propagation was the embryonated egg. A biovariety of embryonic cell types and routes of inoculation are available so that most of the viruses known at that time could be grown in the developing eggs. It is still used for the isolation avian, influenza viruses and for vaccine production. The generally acceptable criteria for the positive isolation are the presence of EBs in the impression smear, serially transmissible egg mortality, the presence of group antigens in the yolk sac and the absence of contaminating bacteria. It is not often used for evaluation of clinical specimen but still employed for preparing antigens for the microimmunofluorescence (MIF) test (Schachter and Stamm 1995).

**Immunological test**

**Antigen detection**

**Direct Fluorescence Antibody (DFA) Test**

Fluorescent dyes can be covalently attached to antibody molecules and made visible by UV light in the Fluorescence microscope (FM). Such “labeled” antibody can be used to identify antigens. The Immunofluorescence(IF) reaction is direct when known labeled antibody interacts directly with unknown antigen.
The DFA technique adds the considerable advantage of chlamydia specific antibody staining to direct examination of specimens and remains one of the most useful diagnostic techniques available. Like culture, the DFA test has very high specificity due to its dependence on visualization of the distinctive morphology and staining characteristics of chlamydial inclusions and elementary bodies. The DFA test is the only diagnostic test available that permits simultaneous assessment of specimen adequacy by visualization of cuboidal columnar epithelial cells. The DFA test is relatively rapid and does not require refrigeration of specimens during transport.

These tests involving monoclonal antibodies are based on detecting elementary bodies in smears (Cles et al.1988). Several DFA assays are commercially available and use monoclonal antibodies directed against MOMP or LPS. Monoclonal antibodies to the LPS will stain all chlamydiae but the specimen may be more difficult to read because of the uneven distribution of LPS on the chlamydial particle. The anti-MOMP monoclonal antibodies are prepared against *C. trachomatis*; therefore, they are species-specific. The quality of fluorescence is better because MOMP is evenly distributed on the chlamydial particle.

The immunofluorescence technique is now widespread use in clinical medicine. The DFA tests may be another alternative for testing endocervical swabs when a NAAT is not available (Johnson et al. 2002).

**Principles of Immunofluorescence**
Immunofluorescence is a combination of histochemical and immunological methods which detects specific antigen-antibody complexes in tissue sections or cellular smears with the help of fluorochrome tagged antibody. The principal parts of the technique are fluorochromes and ultraviolet(UV) light sources. Fluorochrome is substances which emit light i.e. visible radiation when excited by light of a short wavelength. Fluorescence is the emission of light of one color, while substances are irradiated with light of different color. The emitted wave length is at a lower energy level than the incident or absorbed light. Fluorochrome such as rhodamines or fluorescein used in clinical laboratories has characteristic absorption and emission spectra. Fluorescein isothiocyanate (FITC) is a chemical form of fluorescein that rapidly binds covalently to proteins at high pH primarily through ε-amino residue of lysine and terminal amino groups. Its absorption is maximam at 490-495 nm and it emits its characteristics green color at 517 nm. Consequently, different excitation and barrier filter must be used to visualize the characteristics green or red color of these fluorescence dyes. Generally one wants to achieve an exciting wavelength nearly equal to that of the excitation maximam of the dye. Similarly the barrier filter should remove all but the omitted wavelength spectrum. In practice, the actual brightness of fluorescence observed by the eye depends on three factors:

1. Efficiency with which the dye converts incident light into fluorescent light;
2. The concentration of the dye in the tissue specimen and
3. The intensity of exciting (absorbed) radiation.

( Parslaw et al. 2001)
Microscope used for visualizing immunofluorescece specimen is modification of standard transmitted light microscope. This test is recommended when NAAT is not available or not economical (Johnson et al. 2002).

**Characteristics of Immunofluorescece:**

1. **Sensitivity**

The sensitivity of this test is also high. With the use of monoclonal antibody reagents specific for the MOMP of *C. trachomatis*, the sensitivity of DFA is 80 to 90% relative to culture (Chernesky 1986; Quin 1987; Smith 1987).

2. **Specificity:**

The antibody with which the fluorochrome is conjugated is a protein having a high specific reactivity for antigen. Immunofluorescece staining with this antibody is thus highly specific and combines only the antigen for which the antibody has been prepared. The test has 98% to 99% specificity compared with culture (Chernesky 1986; Quinn 1987; Smith 1987).

3. **Rapidity:**

The procedure offers rapid diagnosis, taking only 30 minutes to perform (Chernesky 2005).

**EIA (Enzyme Immuno Assay)**

A number of commercial EIAs are available for the detection of chlamydial antigens in clinical specimens. These products use either monoclonal or polyclonal antibodies to detect chlamydial LPS, which is more soluble than the MOMP. Most EIAs take several hours to perform and are suitable for batch processing. The sensitivity profile of the commercially available *C trachomatis* EIAs ranges from 65% to 75% compared with
NAA assays. Without confirmation, the tests have a specificity of 97%. Therefore, they are not amenable to screening low-prevalence populations because of the low predictive value of a positive result in such groups. To address this problem, confirmatory tests have been developed in which all tests giving positive results are repeated in the presence of a monoclonal antibody directed against the group-specific epitope on the LPS. This blocks the specific reactions but not the false positive reactions. The appropriate application of confirmatory tests increases the specificity to approximately 99.5%. Another approach to confirmation involves testing the specimen by a second test based on a different principle (e.g., a DFA test based on MOMP detection to confirm an LPS based EIA) (Chernesky 1986; Moncada et al. 1990).

**Antibody detection**

Enzyme Linked Immunosorbent Assay (ELISA) detects serum antibodies much more commonly and are higher than trachoma because of relatively greater antigenic mass of chlamydia in genital tract infection. In genital secretions, antibody can be detected during active infections and directed against the infecting immunotypes (serovars) (eds Brooks et al. 2007).

The classical complement fixation (CF) test is rarely performed today. The MIF test, developed by Wang and Grayston (1974), is the current method of choice for the serodiagnosis of chlamydial infection. The commonly used serological assay includes EIA to detect immunoglobulin M (IgM), IgG, IgA or total classes of antibodies with family, species or serotype specificity. The surface associated chlamydial
macromolecules include MOMP, OmcB and LPS may induce strong antibody response in infected individuals (Chernesky 2005).

Other assays include the whole-inclusion immunofluorescence test (WIF), EIA using EBs, RBs or infected cells and a recombinant enzyme-linked immunosorbent assay to LPS. Other serological tests include indirect hemagglutination, neutralization, precipitation, gel diffusion, enzyme-linked fluorescence, immunoperoxidase and immunoelectrophoresis. Most of these assays employ in-house methods, although a few have been commercialized and are being used by clinical laboratories. Total antibody determination by CF or WIF has been useful in identifying patients with tubal factor infertility (Richmond and Caul 1975; Conway et al. 1984).

**EIA**

To overcome the problems associated with the MIF testing such as technically demanding, time consuming and less useful for higher volume testing, EIAs have been developed that offer a more automated workflow and objective end points for serodiagnosis of chlamydial infections. EIAs based on synthetic peptides from the variable domains 4 (VD4) of the *C. trachomatis* MOMP have been marketed for detection of *C. trachomatis* specific IgG and IgA antibodies. These assays performs well as the MIF assay in a few studies; however little is known regarding how long specific antibodies may persist in individuals with resolved infections (Morre et al. 2002).

**Molecular diagnostic test**
NAAT (Nucleic Acid Amplification Test)

Several Nucleic Acid Amplification (NAA) methods are currently used for detecting of *C. trachomatis* in clinical specimen: polymerase chain reaction (PCR), ligase chain reaction (LCR), transcription mediated amplification (TMA) and strand displacement amplification (SDA). All the assays appear to be highly specific if problems with cross contamination of reactions are avoided. Clinical evaluations of the amplification methods have demonstrated higher sensitivity than culture and the other non-culture methods (microscopy, EIA, DFA and NAH) (Johnson et al. 2002). In many evaluations NAATs detected 20% to 30% more positive specimens than could be detected by earlier technologies. The specificity of this method is close to 100%. However, the actual sensitivity with the clinical specimen is lower (90% to 96%) because of sampling variability and inhibition of amplification reactions by factors in the specimen. Because of these manoeuvres are costly, time consuming and require validation, these manoeuvres are not recommended at this time on a routine basis (Chernesky et al. 2005).

Nucleic Acid Hybridization (NAH) tests

Nucleic Acid Hybridization (NAH) tests for *C. trachomatis* infection have been used in some parts of the world extensively in laboratory handling large numbers of specimen on a daily basis. One commercially available probe test (PACE 2, Gene Probe) uses DNA-RNA hybridization in an effort to increase the sensitivity by detecting chlamydial RNA. Available data suggest that the probe test is relatively specific and provides sensitivity similar to that of the antigen detection and cell culture methods. Another NAH test, the Hybrid Capture II, for *C. trachomatis* uses a signal amplification component to increase
sensitivity to approximately 90% of NAA assays. The overall sensitivity and specificity of this method are about 85% and 98 to 99% respectively (Clarke et al. 1993; Schachter, Hook & McCormack 1999; eds Brooks et al. 2007).

In DNA probe method, cloned or synthetic genes are used as ‘probes’ that bind to parts of the nucleic acid of an organism. A lab scientist is then able to identify the organism. They are rapidly performed but high cost of the equipment needed to process samples and detect infectious agent. This test is recommended when NAAT is not available or not economical (Johnson et al. 2002).

2.7 Treatment and Prevention

2.7.1 Treatment

Medication  (Struble et al. 2010)

Treatment of genitourinary chlamydial infection is clearly indicated when the infection is diagnosed or suspected. Treatment is also indicated for sex partners of the index case if the time of the last sexual encounter was within 60 days of onset and it should be considered for longer periods for the last sexual partner.

Chlamydial species are susceptible to the tetracycline, macrolide and fluoroquinolone classes of antibiotics. The most active of these are doxycycline, erythromycin, azithromycin, ofloxacin, levofloxacin are usually used to treat chlamydial infections.

Azithromycin

Azithromycin is a relatively new member of the macrolide family of antimicrobials. It is considered by many to be the treatment of choice of C. trachomatis genitourinary
infection because it may be administered as a single dose treatment which improves adherence to treatment. If patient vomits within one to two hours of taking the medication, an alternative treatment should be considered.

**Pregnancy**

Fetal risk not confirmed in studies in humans but has been shown in some studies in animals.

**Precautions**

Generally not recommended for routine use during pregnancy but can be used as an alternative if failure occurs after treatment with erythromycin or amoxicillin.

**Doxycycline**

Well absorbed tetracycline antimicrobial. When administered for 1 week, appears to be as effective as single-dose azithromycin for genitourinary chlamydial infections. Although the course is longer (7 days versus 1 dose) than azithromycin, the cost is less and it has been used in clinical practice for a much longer time.

**Pregnancy**

Fetal risk shown in humans; causes breakthrough bleeding and increased risk of pregnancy, used only if benefits outweigh risk to fetus.

**Precautions**

Photosensitivity may occur rarely; use during tooth development (last half of pregnancy through age 8 years) can cause permanent discoloration of teeth.
**Erythromycin**

Macrolide antimicrobial agent that generally is considered the recommended treatment for chlamydial genitourinary infection during pregnancy.

**Pregnancy**

Fetal risk not confirmed in studies in humans but has been shown in some studies in animals.

**Ampicillin**

Amoxicillin is considered a recommended treatment for genitourinary chlamydial infection only in pregnant women.

**Pregnancy**

Fetal risk not confirmed in studies in humans but has been shown in some studies in animals.

**Treatment of Chlamydial infection in adolescence and adults** (CDC MMWR 2006)

**Recommended Regimens**

Azithromycin 1 gram orally in a single dose

OR

Doxycycline 100 mg orally twice a day for 7 days
Alternative Regimens

Erythromycin base 500 mg orally four times a day for 7 days
OR
Erythromycin ethylsuccinate 800 mg orally four times a day for 7 days
OR
Ofloxacin 300 mg orally twice a day for 7 days
OR
Levofloxacin 500 mg orally once daily for 7 days

Medical care

To maximize compliance with recommended therapies, medications for chlamydial infections should be dispensed on site and the first dose should be directly observed therapy (DOT). To minimize transmission, persons treated for chlamydia should be instructed to abstain from sexual intercourse for 7 days after single dose therapy or until completion of a 7-day regimen. To minimize the risk for reinfection, patients also should be instructed to abstain from sexual intercourse until all of their sex partners are treated (Struble et al. 2010).

Follow-up

It is an essential part of management of chlamydial infections. These include follow up of partner, health education, reassurance, microbiological test of cure and exclusion of reinfection (Schachter & Alexander 1998).

A high prevalence of *C. trachomatis* infection is observed in women who were treated for chlamydial infection in the preceding several months. The majority of post treatment infections result from reinfection, frequently occurring because the patient’s sex partners were not treated or because the patient resumed sex with a new partner infected with *C.*
trachomatis. Repeat infections confer an elevated risk for PID and other complications when compared with the initial infection. Therefore, recently infected women are a major priority for repeat testing for *C. trachomatis*. Clinicians and health-care agencies should consider advising all women with chlamydial infection to be retested approximately 3 months after treatment. Providers also are strongly encouraged to retest all women treated for chlamydial infection whenever they next seek medical care within the following 3 to 12 months, regardless of whether the patient believes that her sex partners were treated (CDC MMWR 2006).

**Management of Sex Partners**

Patients should be instructed to abstain from sexual intercourse until they and their sex partners have completed treatment. Abstinence should be continued until 7 days after a single-dose regimen or after completion of a 7-day regimen. Timely treatment of sex partners is essential for decreasing the risk for reinfecting the index patient. Sex partners should be evaluated, tested and treated if they had sexual contact with the patient during the 60 days preceding onset of symptoms in the patient or diagnosis of chlamydia. The most recent sex partner should be evaluated and treated, even if the time of the last sexual contact was >60 days before onset of symptom or diagnosis (CDC MMWR 2006).

**Special Considerations**

**Pregnancy**

Doxycycline, ofloxacin and levofloxacin are contraindicated in pregnant women. Repeat testing 3 weeks after completion of therapy with the following regimens is recommended
for all pregnant women to ensure therapeutic cure, considering the sequelae that might occur in the mother and neonate if the infection persists. The frequent gastrointestinal side effects associated with erythromycin might discourage patient compliance with the alternative regimens.

**Recommended Regimens**

(Low, Welch & Radcliffe 2004; CDC MMWR 2006)

Azithromycin 1 g orally in a single dose

OR

Amoxicillin 500 mg orally three times a day for 7 days

**Alternative Regimens**

Erythromycin base 500 mg orally four times a day for 7 days

OR

Erythromycin base 250 mg orally four times a day for 14 days

OR

Erythromycin ethylsuccinate 800 mg orally four times a day for 7 days

OR

Erythromycin ethylsuccinate 400 mg orally four times a day for 14 days

The lower dose 14-day erythromycin regimens may be considered if gastrointestinal tolerance is a concern.

**HIV Infection**

Patients who have chlamydial infection and also are infected with HIV should receive the same treatment regimen as those who are HIV negative.

**Antibiotic resistance**
Antibiotic resistance of *C. trachomatis* has not been shown and is not currently a clinical problem. Persistent forms are also slightly antibiotic-sensitive which seems to be associated with reduced MOMP count and thus decreased transport of antibiotics to the cell. Therefore, in the case of chronic infections, therapy frequently results in failure (Schachter & Alexander 1998).

### 2.7.2 Prevention and Control

Primary prevention starts with changing sexual behaviors that increase the risk of contracting chlamydial STD. Health-care providers have a unique opportunity to provide education and counseling to their patients. As part of the clinical interview, health-care providers should routinely and regularly obtain sexual histories from their patients. One approach to eliciting information concerning five keys- the five Ps: Partners, Prevention of Pregnancy, Protection from STDs, Practices, Past History of STDs.

Secondary prevention consists of standardized detection and treatment of the infection. (Kane et al. 2004).

The CDC guidelines for the prevention and control of Chlamydia STD are based on five major concepts (CDC STD 2006):

**Concepts for Prevention**

1. Education and counseling on safer sexual behavior in persons at risk
2. Identification of asymptomatic infected persons and of symptomatic persons unlikely to seek diagnostic and treatment services
3. Effective diagnosis and treatment of infected persons
4. Evaluation, treatment and counseling of sex partners of persons infected with an STD

5. Pre-exposure immunizations for vaccine-preventable STDs

Clinical prevention guidance recommended by CDC Sexually Transmitted Diseases Treatment Guidelines 2006 is as follows (CDC MMWR 2006):

- Prevention Counseling
- Prevention Methods
- Partner Management
- Reporting and Confidentiality

**Prevention Counseling**

Effective delivery of prevention messages requires client-centered counseling and education regarding specific actions that can reduce the risk for chlamydia transmission e.g., abstinence, condom use, limiting the number of sex partners, modifying sexual behaviors and vaccination. Prevention messages should be individually delivered and based on stages of patient development and understanding of sexual issues. Performing counseling and discussing behavioral interventions have been shown to reduce the likelihood of Chlamydia STD and reduce risky sexual behavior (Shain et al. 2004; Shrier et al. 2001; CDC 2006).
Prevention Methods

Client-initiated interventions to reduce sexual transmission of chlamydial STD and unwanded pregnancy:

1. Abstinence and reduction of number of sex partners

The most reliable way to avoid transmission of chlamydial STDs is to abstain from sex i.e., oral, vaginal or anal sex. Counseling that encourages abstinence from sexual intercourse is crucial for persons who are being treated for an STD or whose partners are undergoing treatment and for persons who want to avoid the possible consequences of sex completely e.g. unintended pregnancy. Screening before initiating sex might reduce the risk for future transmission of asymptomatic chlamydial infection.

2. Condoms

When used consistently and correctly, Latex male condoms are highly effective in preventing the sexual transmission of chlamydial infection and can reduce the risk for chlamydia.

Partner Management

Partner notification (“contact tracing”)

Health care providers or public health authorities learn from persons with STDs about their sex partners and help to arrange for the evaluation and treatment of sex partners either directly or with assistance from state and local health departments by partner services. Many persons individually benefit from partner notification. When partners are
treated, index patients have reduced the risk for re-infection. Partner notification can disrupt networks of chlamydia transmission and reduce disease incidence. Therefore, providers should encourage their patients with chlamydia to notify their sex partners and urge them to seek medical evaluation and treatment.

**Reporting and Confidentiality**

The accurate and timely reporting is integrally important for assessing morbidity trends and assisting local health authorities in partner notification and treatment. Reports should be kept strictly confidential.

**Chlamydia Screening Recommendations**

(CDC 2006)

The CDC recommends routine Chlamydia testing for-

1. All women with cervical discharge containing both mucus and pus;
2. All sexually active women under the age of 25;
3. Those have a history of sexually transmitted disease within the last year, regardless of age;
4. Those have partners who have had multiple partners within the last year, regardless of age;
5. Those have new or multiple sexual partners, regardless of age;
6. Those who do not consistently use barrier contraceptives.

Test all pregnant women at least once regardless of age including those who plan to terminate the pregnancy. Re-screen all women who tested positive, especially adolescents, 3 to 4 months after treatment due to the high incidence of re-infection.
The U.S. Preventive Services Task Force (USPSTF) strongly recommends that all women 25 years and younger receive routine screening for Chlamydia (Cook et al. 2002).

**Prophylaxis**

Treatment of patient eliminates infection and therefore provides prophylaxis against the complications. Treatment of women during pregnancy or immediately post delivery will eliminate infection and may protect against post partum fever and endometritis. Simultaneously sexual partner must be treated to prevent re-infection. Treating mothers and partners prior to delivery can prevent chlamydial disease of infants effectively. Topical erythromycine or tetracycline is prophylactic for eye infection at birth (Schachter & Alexander 1998).

**Immunization:**

No immunization for occulogenital disease is available (Schachter & Alexander 1998).

**Current research on immunization**

Using the major outer membrane protein (MOMP) of *Chlamydia trachomatis* antibody-based diagnostics as well as recombinant vaccines are being developed. DNA priming followed by protein boosting of MOMP of *C. trachomatis* has been shown to be promising in developing a vaccine for *C. trachomatis* (Zhang et al. 2000).
The current research uses a live-attenuated form of the influenza A virus to provide viral vector for a vaccine against *C. trachomatis*. This vaccine is tested to be used intra-nasally. In the experiment, mice were intra-nasally immunized with influenza A viral recombinants. The result was a very strong immune T helper 1 response against intact *Chlamydia trachomatis* elementary bodies. The genital secretions in the mice showed high levels of specific Th1 cells and elevated immunoglobulin G2a which indicates a possibility of long term protective immunity. This study, using *C. trachomatis*, is very important because it indicates that live attenuated vaccines of the influenza virus could be a new and reliable approach to preventing the spread of sexually transmitted disease (He et al. 2007).
3. Materials and Methods

108 sexually active women were selected from in patient and out patient department of Obstetrics and Gynaecology, Mymensing Medical College Hospital (MMCH) from January 2009 to December 2009.

3.1 Study design: Cross sectional observational study.

3.2 Study period: From January 2009 to December 2009.

3.3 Study place: Specimens were collected from in patient and out patient department of Obstetrics and Gynaecology, MMCH. The laboratory works were performed at the department of Microbiology, Mymensingh Medical College (MMC).

3.4 Study population: Adult women (aged 15 to 45 years) attending at out patient and in patient department of Obstetrics and Gynaecology, MMCH during the study period.

3.5 Sample size: 108

3.6 Inclusion criteria: Symptomatic and asymptomatic sexually active women.

3.7 Exclusion criteria: History of recent (within last 15 days) antibiotic treatment.

3.8 Data collection, recording and analysis:

All the relevant information and data was systematically recorded in predesigned data sheet (appendix-1) used for each patient. The data was analyzed manually.

3.9 Ethical consideration:
The study protocol was approved by protocol approval committee of the department of Microbiology and Ethical Review Committee of MMC.

### 3.10 Collection of sample

#### 3.10.1 Collection Endocervical swab (ECS)

The sample was collected from each of the patient with the help of a female gynaecologist following standard operating procedures (Black 1997):

The informed written consent of the patient was taken. Afterwards filling the questionnaire form, the patient was placed in the dorsal recumbent position on the examining table, the limbs and lower abdomen draped with a sheet. The cervix was exposed properly under sufficient light. A Cusco’s vaginal speculum was used to provide a clear sight of the cervix. With a sterile moistened (moistened with sterile saline) swab (cleaning swab), the marginal area of the cervix and surrounding mucosa was cleaned, removing any excess mucus or inflammatory exudates. This swab was discarded. The first sterile sampling swab (specimen collection swab) was inserted 1 to 2 cm into the cervical os past the squamocolumnar junction and rubbing it by rotating several (more than 2) times clockwise for 15 to 30 seconds against the wall of endocervix to ensure adequate sampling. The swab was withdrawn carefully avoiding contact with vaginal surfaces and secretions. Similarly another sample was collected by the second sampling swab. The speculum was removed. Both the swabs were kept in dry sterile test tubes.

The sample was labeled with the patient name, ID number and date of collection. The specimen was transported to the Department of Microbiology, MMC.
3.10.2 Collection of Blood

Venous blood was collected aseptically. The punctured area was disinfected with iodine and 70% alcohol. With a sterile disposable syringe, 3ml whole blood was collected in a clean dry test tube by venepuncture and allowed to clot at room temperature for one hour. The blood was centrifuged for separation of serum. The serum was stored at -20°C until performing the test (Cheessbrough 2000).

3.11 Storage of slides

Fixed smeared slides for Direct Fluorescent Antibody Test (DFAT) were stored at -20°C until staining. Fixed smeared slides for Giemsa and Iodine stain were preserved at room temperature.

3.12 Laboratory procedures

3.12.1 Microscopy
Detection of inclusion bodies

3.12.1.1 Giemsa staining technique to detect *C. trachomatis* inclusion bodies

**Specimen:** Endocervical swab

Preparation, fixation of smear and staining procedure were performed according to standard procedure (Cheesbrough 2000).

**Materials required** – Giemsa stain, Phosphate buffer saline (PBS) (appendix-III).

**Preparation of smear:**

The swab was rolled on a slide. The smear was spread evenly covering an area of 15 to 20 mm diameter on the slide and allowed to air dry.

**Fixation of smears:**

The dried smear was fixed by covering it with methyl alcohol for 3 minutes.

**Staining Procedure:**

The slide was placed in a petri dish; smear downwards, supported on each side by a thin piece of stick. The diluted Giemsa stain was poured into the petri dish and covered with a lid. The smear was left to stain for 2 hours. The stain was washed and rinsed with buffered water (PBS). The back of the slide was wiped and placed in a draining rack for the smear to air dry.

The smear was examined microscopically with the 40X objectives and with the oil immersion lens.

**Results and Interpretation-**

Looked for inclusion bodies (IB) varying in size from 20 µm to 200 µm, located in the host cell cytoplasm nearer the nucleus. *C. trachomatis* inclusion body appeared as a blue-
mauve stained mass consisting of closely packed reticulate bodies and as less dense mass consisting of mauve-red staining elementary particles. The nucleus of the host cell stained pink-mauve (Cheesbrough 2000).

### 3.12.1.2 Iodine staining technique to detect inclusion bodies

**Specimen:** Endocervical swab  

**Materials required** – Iodine stain (appendix-III).

**Procedure:**

Five to six drops of iodine stain solution were taken in a test tube. Endocervical swab was immersed into it. Waiting for 5 minutes, the swab was squeezed firmly against the test tube wall and then the swab was discarded. One drop of fluid was taken by a dropper and placed over a microscopic slide covering with a cover slip to examine microscopically by 40X magnifications.

**Interpretation:**

Glycogen containing inclusions of *C. trachomatis* in cytoplasm of epithelial cells was stained brown with iodine.

### 3.12.2 Direct Fluorescence Antibody (DFA) Test

**Detection of Antigen**
3.12.2 Detection of Antigen by Direct Fluorescence Antibody (DFA) Test

**Specimen:** Endocervical swab

**Principle:**

Fluorescent dyes can be covalently attached to antibody molecules and made visible by UV light in the Fluorescence microscope. Such labeled antibody can be used to identify antigens. The Immunofluorescence reaction is direct when known labeled antibody interacts directly with unknown antigen.

**Materials required (appendix):**

DFA reagent, mounting fluid, PBS, micropipette, fluorescence microscope (FM), incubator and microscope slides with 6 to 8 mm diameter wells.

**Preparation of smear:**

Endocervical swabs were allowed to air dry, smears were prepared immediately after specimen collection. The swab was rubbed on the cleaned slide making sure that the specimen was evenly dispersed and allowed to air dry. The slide was laid flat and 0.5 ml of methanol was added and left it at room temperature. Slides were stored at -20°C until staining was done.

**Test procedure:**

Slides were processed in the laboratory according to the manufacturer’s instructions. One positive control was included with each batch of the patient specimen as a quality control for Fluorotect chlamydia reagent.
25 µL fluorescence labeled monoclonal antibody (Fluorotect Chlamydia reagent; Omega Diag. Ltd. UK) was dispensed on to the fixed specimen smear covering the smear and positive control slide. The slide was incubated at 37°C in a moist dark chamber for 30 min. The slide was not allowed to dry, as this could cause non specific antibody binding. Slides were rinsed gently in bath of phosphate buffer saline for approximately 1 minute and excess moisture was removed with absorbent tissues. One drop of mounting fluid was added and a cover slip was placed on top of the drop and air bubble was removed. The specimen was scanned using fluorescence microscope at 400X magnification. Reading was taken immediately or the slides were stored at 2°C-8°C in the dark up to 24 hours or -20°C up to 7 days.

**Results and Interpretation:**

Specimens usually exhibit elementary bodies (EB) which appear as bright apple green fluorescent pin point smooth edged disc shaped bodies 0.2 µm to 0.4 µm in diameter. They were seen against background of counterstained cells. Reticulate bodies (size 0.6 µm to 1 µm) were also observed. Any material which was distinguished from chlamydial form or fluoresce other than apple green was disregarded. The control slide was used for comparison.

A positive diagnosis was made when fixed stained specimen showed at least 10 chlamydial bodies; this number is required in order to reduce false positive results due to misinterpretation of non-specific fluorescence.

A negative result was made when fixed stained smears was free of Chlamydial organisms. Irregular shaped fluorescent material that was different in size from Chlamydial bodies described above or fluoresced white, red or yellow was considered non-specific staining.
3.12.3 Enzyme-Linked Immunosorbent Assay (ELISA)

Detection of Antibody

3.12.3.1 Enzyme-Linked Immunosorbent Assay (ELISA) to detect *C. trachomatis* specific IgG antibody

*C. trachomatis* specific IgG antibody in serum was detected by IgG ELISA kit manufactured by DRG instruments GmbH, Germany, (EIA-3462). Test procedure was followed as per kit manual.

3.12.3.2 Test principle:

In this test, microtiter wells as a solid phase were coated with recombinant *C. trachomatis* antigen. Then the patient’s serum and controls were incubated after dispensing these into the wells. *C. trachomatis* specific antibodies of positive specimen and control were bound to the immobilized antigens. After a washing step to remove unbound sample and control material, horseradish peroxidase conjugated anti-human IgG antibodies were dispensed into the wells and incubated again. The anti-IgG conjugate binds specifically to IgG antibodies resulting the formation of enzyme linked immune complexes. After a second washing step to remove unbound conjugate, the immune complexes formed in positive results were detected by development of blue color after incubation with tetramethylbenzidine (TMB) substrates. The blue color turned into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of the color is directly proportional to the amount of *Chlamydia trachomatis* specific IgG antibodies in the patient serum. Absorbance at nm s read using an ELISA microtiter plate reader.
3.12.3.3 Preparation of reagents and specimen dilution:

All the reagents, samples, controls and strips were allowed to reach room temperature before starting the test run.

**Dilution of wash solution**

Wash Solution was diluted 1:19 with fresh and germ free redistilled water (e.g. 10 mL Wash Solution + 190 mL redistilled water). Diluted Wash Solution was prepared by adding 19 volume of fresh and germ free redistilled water to one volume of concentrated washing buffer.

**Specimen dilution**

A total of 100 serum samples were evaluated by ELISA. Each of the samples was diluted 1:100 with sample diluents (e.g. 10 μL of specimen + 1 mL of *Sample Diluent*), mixed well and allowed to stand for 15 minutes.

**Assay procedure**

Required numbers of microtiter wells (coated with *chlamydia trachomatis* antigen) were selected including A1 for the substrate blank, B1 for the Negative control, C1 and D1 for the cut-off control (CO) and E1 for the positive control. Microtiter strips were inserted into the holder. Left A1 for substrate blank.100 μL of Negative Control into well B1, 100 μL of Cut-off Control into wells C1 and D1, 100 μL Positive Control into well E1 were dispensed with new disposable tips. 100 μL of each diluted sample were dispensed with new disposable tips into appropriate (selected) wells. Then it was incubated at 37°C
for 60 minutes covering wells with foil. The contents of the wells were briskly shaked out. Then the wells were washed 5 times with diluted Wash Solution (300 µL per well). The wells were stroked sharply on absorbent paper to remove residual drops. Then 100 µL Enzyme Conjugate was dispensed into each well except A1. It was incubated again at room temperature for 30 minutes covering the wells with foil. Washed again 5 times with diluted wash solution. 100 µL substrate solutions were added to each well. Finally incubated again 15 minutes at room temperature in the dark covering the wells in foils. The reaction was stopped by adding 100 µL Stop Solution to each well. Optical density (OD) was read immediately at 450 nm with a microtiter ELISA plate reader. The absorbance value of all wells and the absorbance values for each control and patient sample were recorded in the distribution and identification plan. Averaging the two cut-off control, the mean absorbance value of Cut-off Control (CO) was calculated. Samples with OD values more than 10% above CO (Mean OD Patient >1.1×CO) were regarded as positive as per kits instructions.
Results

Incidence of genital chlamydia is common both in pregnant and non-pregnant adult women in Bangladesh. Seroepidemiologic study by IgG ELISA also showed high prevalence of chlamydia. The role of microscopy for diagnosing genital chlamydia by Giemsa staining and Iodine staining technique to detect inclusion bodies (IB) was found nearly insensitive procedures.

Out of 108 study cases of sexually active women, 49 (45.3%) cases were found infected with the *Chlamydia trachomatis* (CT) diagnosed by Direct Fluorescence Antibody Test (DFAT). The statistically significant number of positive cases (65%) were in the age group 15 to 25 years ($\chi^2 = 10.02$ at d.f. 2 $p < 0.05$). The age range of the study cases was 18 to 45 years with a mean age of 29.3 SD 6.8 years (Table 01).

The economical status of the subjects was distributed according to the monthly income of the family members. Majority of the study cases (58.3%) were in the lower income group, the highest percent of positive cases (52.3%) were in the same income group also. The next majority of positive cases (36.5%) were in the middle income group (Table 02).

The comparison of *C. trachomatis* (CT) infection among the study population in relation to pregnancy status of the cases showed the higher number of infections (47.4%) in pregnant women group than the non-pregnant group (42.8%) (Table 03).
Table 01: Age distribution among the study population

<table>
<thead>
<tr>
<th>Age group in years</th>
<th>Number of study cases</th>
<th>Number of positive cases</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-25</td>
<td>40</td>
<td>26</td>
<td>65.0%</td>
</tr>
<tr>
<td>26-35</td>
<td>48</td>
<td>17</td>
<td>35.4%</td>
</tr>
<tr>
<td>36-45</td>
<td>20</td>
<td>6</td>
<td>30%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>49</td>
<td>45.3%</td>
</tr>
</tbody>
</table>
Table 02: Distribution of the economic status of the subjects

<table>
<thead>
<tr>
<th>Income group*</th>
<th>Number of study cases</th>
<th>Number of positive cases</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower</td>
<td>63</td>
<td>33</td>
<td>52.3%</td>
</tr>
<tr>
<td>Middle</td>
<td>41</td>
<td>15</td>
<td>36.5%</td>
</tr>
<tr>
<td>High</td>
<td>04</td>
<td>01</td>
<td>25.0%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>49</td>
<td>45.3%</td>
</tr>
</tbody>
</table>

*Income group is characterized into three classes according to monthly income of family members.

Lower = monthly income <10,000 Taka

Middle = monthly income 10,000 - 20,000 Taka

High = monthly income >20,000 Taka
Table 03: Comparison of *C. trachomatis*(CT) infection among the study cases in relation to pregnancy status

<table>
<thead>
<tr>
<th>Pregnancy status</th>
<th>Number of study cases</th>
<th>CT infection by DFA Test</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>59</td>
<td>28</td>
<td>47.4%</td>
</tr>
<tr>
<td>Non-pregnant women</td>
<td>49</td>
<td>21</td>
<td>42.8%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>49</td>
<td>45.3%</td>
</tr>
</tbody>
</table>
The comparison of *C. trachomatis* infection among the study subjects in relation to their presenting symptoms shows that out of 74 symptomatic cases, 35 (47.2%) was detected as positive by Direct Fluorescence Antibody Test (DFAT) which was higher than the asymptomatic cases (Table 04).

Result of the Iodine preparation of the endocervical swabs for detection of intracytoplasmic inclusion bodies (IB) in relation to symptom presentation shows that among the symptomatic cases, 05 (6.7%) was IB positive which was higher than the asymptomatic cases (2.9%)(Table 05).

Result of Iodine stained preparation of the endocervical swabs for detection of intracytoplasmic inclusion bodies (IB) in relation to pregnancy status of the cases shows that the number of IB positive cases was 04 (6.7%) in pregnant women which was higher than the non-pregnant group (4%)(Table 06).

The result of Giemsa stained preparation of the endocervical swabs to detect intracytoplasmic inclusion bodies (IB) in relation to presenting symptoms of the cases shows that the number of IB positive cases was higher in the asymptomatic cases (17.6%)( Table 7).

The result of the Giemsa stained preparation of the endocervical swabs to detect inclusion bodies (IB) in epithelial cell cytoplasm in relation to pregnancy status of the cases shows that the number of IB positive cases (16.9%) was higher in pregnant women than non-pregnant group (8.1%)(Table 8).
Table 04: Comparison of *C. trachomatis* infection among the study cases in relation to presenting symptoms of the cases.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Study population</th>
<th>Number of positive cases</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic cases</td>
<td>74</td>
<td>35</td>
<td>47.2%</td>
</tr>
<tr>
<td>Asymptomatic cases</td>
<td>34</td>
<td>14</td>
<td>41.1%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>49</td>
<td>45.3%</td>
</tr>
</tbody>
</table>
Table 05: Result of Iodine preparation of the endocervical swabs to detect inclusion bodies (IB) in relation to presenting symptoms of the cases

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number of study cases</th>
<th>Number of inclusion body (IB) positive cases</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic cases</td>
<td>74</td>
<td>05</td>
<td>6.7%</td>
</tr>
<tr>
<td>Symptomatic cases</td>
<td>34</td>
<td>01</td>
<td>2.9%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>06</td>
<td>5.5%</td>
</tr>
</tbody>
</table>
Table 06: Result of Iodine stained preparation of the endocervical swabs to detect inclusion bodies (IB) in relation to pregnancy status of the cases

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number of study cases</th>
<th>Number of inclusion body (IB) Positive cases</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>59</td>
<td>04</td>
<td>6.7%</td>
</tr>
<tr>
<td>Non-pregnant women</td>
<td>49</td>
<td>02</td>
<td>4.0%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>06</td>
<td>5.5%</td>
</tr>
</tbody>
</table>
Table 07: Result of Giemsa preparation of the endocervical swabs to detect inclusion bodies (IB) in relation to presenting symptoms of the cases

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number of study cases</th>
<th>Number of inclusion body (IB) positive cases</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic cases</td>
<td>74</td>
<td>08</td>
<td>10.8%</td>
</tr>
<tr>
<td>Asymptomatic cases</td>
<td>34</td>
<td>06</td>
<td>17.6%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>14</td>
<td>12.9%</td>
</tr>
</tbody>
</table>
Table 8: Result of the Giemsa stained preparation of the endocervical swabs to detect inclusion bodies (IB) in relation to pregnancy status of the cases

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number of study cases</th>
<th>Number of inclusion body (IB) positive cases</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>59</td>
<td>10</td>
<td>16.9%</td>
</tr>
<tr>
<td>Non-pregnant women</td>
<td>49</td>
<td>04</td>
<td>8.1%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>14</td>
<td>12.9%</td>
</tr>
</tbody>
</table>
*C. trachomatis* specific IgG antibody was detected by Enzyme Linked Immunosorbent Assay (ELISA) from serum. The number of seropositive cases (44%) was approximately four times higher in pregnant women than in the non-pregnant group (10.2%). The test is statistically highly significant. The overall seropositivity rate was 28.7% (Table 9).

Comparison between the test positivity for *C. trachomatis* infection of different methods shows that, antigen detection by DFA test from endocervical swabs was 45.3%, inclusion body detection from endocervical swabs was 5.5% by Iodine staining and 12.9% by Giemsa staining technique and *C. trachomatis* specific IgG antibody was 28.7% in serum (Table 10).

The measures of validity of iodine staining technique for detection of IB from endocervical swabs comparing with Direct Fluorescence Antibody Test as ‘gold standard’ showed 8.1% sensitivity and 96.1% specificity (Table 11).

The sensitivity and specificity of Giemsa staining to detect IB from endocervical swabs was found 16.2% and 89.1% respectively comparing with comparing with Direct Fluorescence Antibody Test (DFAT) (Table 12).

The measures of validity of ELISA for detection of *C. trachomatis* specific IgG antibody comparing with Direct Fluorescence Antibody Test as ‘gold standard’ shows that the sensitivity of IgG ELISA was 44.8% and the specificity was 84.7% (Table 13).
Table 9: Seropositive (IgG positive) *C. trachomatis* cases by ELISA in relation to pregnancy status

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number of study cases</th>
<th>Number of seropositive cases</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>59</td>
<td>26</td>
<td>44.0%</td>
</tr>
<tr>
<td>Non-Pregnant women</td>
<td>49</td>
<td>05</td>
<td>10.2%</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>31</td>
<td>28.7%</td>
</tr>
</tbody>
</table>

($\chi^2 = 150.51$, at d.f. 1 $p<0.001$). The test is highly significant.
Table 10: Comparison between the test positivity for *C. trachomatis* infection by different methods (n=108)

<table>
<thead>
<tr>
<th>Tests</th>
<th>Number of positive tests</th>
<th>Percent of test positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFAT for Antigen</td>
<td>49</td>
<td>45.3%</td>
</tr>
<tr>
<td>Iodine staining for IB</td>
<td>06</td>
<td>5.5%</td>
</tr>
<tr>
<td>Giemsa staining for IB</td>
<td>14</td>
<td>12.9%</td>
</tr>
<tr>
<td>ELISA for IgG antibody</td>
<td>31</td>
<td>28.7%</td>
</tr>
</tbody>
</table>
Table 11: Comparison of Iodine staining technique with DFAT to detect inclusion bodies (IB)

<table>
<thead>
<tr>
<th>Tests results</th>
<th>Disease positive</th>
<th>Disease negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine staining</td>
<td>04 (a)</td>
<td>02 (b)</td>
<td>8.1%</td>
<td>96.1%</td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine staining</td>
<td>45 (c)</td>
<td>57 (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49 (a+c)</td>
<td>59 (b+d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 12: Comparison of Giemsa staining technique with DFAT to detect inclusion bodies.

<table>
<thead>
<tr>
<th>Tests results</th>
<th>Disease positive</th>
<th>Disease negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giemsa positive</td>
<td>08 (a)</td>
<td>06 (b)</td>
<td>16.26%</td>
<td>89.1%</td>
</tr>
<tr>
<td>Giemsa negative</td>
<td>41 (c)</td>
<td>53 (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49 (a+c)</td>
<td>9 (b+d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 13: Sensitivity and specificity of IgG ELISA to detect *C. trachomatis* specific IgG antibody in serum

<table>
<thead>
<tr>
<th>Tests results</th>
<th>Disease positive (diagnosed by DFAT)</th>
<th>Disease negative (negative by DFAT)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA positive</td>
<td>(a) True positive = 22</td>
<td>(b) False positive = 09</td>
<td>44.8%</td>
<td>84.7%</td>
</tr>
<tr>
<td>ELISA negative</td>
<td>(c) False negative = 27</td>
<td>(d) True negative = 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>(a+c) = 49</td>
<td>(b+d) = 59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Genital chlamydial infections are the most common sexually transmitted diseases worldwide. Cervicitis, urethritis and pelvic inflammatory diseases (PID) are common among the genital chlamydia infections. Maternal chlamydial infections have been considered as significant factors in the causation of poor pregnancy outcome and complications like ectopic pregnancy and tubal infertility. Moreover chlamydial infection during pregnancy causes a variety of perinatal complications like delivery of Low Birth Weight (LBW) babies, premature labor, spontaneous abortion and perinatal death (Numazaki & Black 1998). Barlow et al. (2001) detected chlamydial DNA in 71% of patients with tubal infertility. Because of these risks, screening of pregnant women at the first prenatal visit is recommended by the Centers for Disease Control and Prevention (CDC) and US preventive Services Task Force.

The greatest challenge to control chlamydial diseases is that, 70%-80% of women who are infected do not experience any symptoms. As a result, these infections often go untreated which can lead to various complications. Individuals with asymptomatic genital infections are an important reservoir of infections for others also (Stamm & Cole 1986). On the other hand, virtually all the cases can be treated successfully and inexpensively with antibiotic by early and rapid diagnosis of the infection in symptomatic and asymptomatic patients. So, successful treatment and prevention of the spread of chlamydia depends on the ability to diagnose infections accurately and rapidly.

In the present study, the highest number of positive cases (65%) was in the age group 15 to 25 years followed by 26 to 35 years (35.4%). The youngest and oldest positive cases
were in the age 18 and 45 years respectively (Table 01). A study in the same institute by Shamsuzzaman, Parveen and Hossain (2003) showed the higher number of genital Chlamydia trachomatis (CT) infection in the age group 20 to 30 (82.3%) and 31-40 years (46.1%) respectively. Yasodhara et al. (2001) showed that majority of the women were between 20 to 30 years. Another study by Agrawal et al. (2003) showed the peak incidence (70%) in the age group 21 to 25 years, the youngest and oldest patient was in the age 20 and 55 years respectively. Martin et al. (1982); Frommell et al. (1979) have linked the female genital Chlamydia trachomatis (CT) infection in younger age group. Chernesky et al. (1986) reported the higher rate of positivity among men and women in the age group 20 to 30 years. Findings of mentioned studies support our findings. The younger group is more sexually active than elders which in turn elevate the chance of spread of infection.

In this study, majority of the cases (58.3%) were in the lower income group, the highest percent of positive cases (52.3%) were in the same income group also (Table 02). The rate of positivity was more than double in the lower income group than that in the other group. Sachacher and Alexander (1998) also found increased frequency of genital chlamydial infection in individuals of lower socioeconomic status. A study by Malenae, Joshi and Mathur (2006) showed the higher incidence of CT infection in poor socioeconomic group. Agrawal et al. (2003) also showed higher incidence in unemployed and low income group also. Martin et al. (1982); Frommell et al. (1979) have linked CT infection in lower socioeconomic status. Lower socioeconomic group may have poor sex education and social structure which may promote sexually transmitted diseases including Chlamydia. Due to insufficient sample size, only one
patient was available from the high income group. So, no conclusive comment can be drawn regarding this group.

In the present study, we found 45.3% CT infection among the study populations by Direct Fluorescence Antibody (DFA) test (Table 03, 04). Shamsuzzaman, Parveen and Hossain (2003) found 58.3% genital CT infection in female in the same study place. The higher detection rate in their study may be explained by the reason that their study was conducted on symptomatic cases only after selection by clinical examination rather than random selection. The incidence of chlamydia varies in different geographical regions and societies. In 1999, World Health Organization(WHO) reported that, the new cases of chlamydia infections in adults were 4.2% in North America, 5.6% in Western Europe, 3.4% in North Africa and middle Europe, 6.5% in Eastern Europe & central Asia, 17.2% in Sub-Saharan Africa, 5.7% in East Asia and Pacific, 0.3% in Australia and New Zealand, 10.1% in Latin America and Caribbean, 46.6% in South and South East Asia (WHO 2001). A study by Agrawal et al. (2003) among male patients from India, demonstrated 36.6% CT infection. Molano et al. (2003) showed 35% CT infection among females in Jamaica. In another study in India Joyee et al. (2003) reported 25% genital CT infection among the sexually transmitted diseases (STD). A study from Iran reported 27.2% CT infection (Jenab et al. 2009). Another study from Netherlands reported 28.8% prevalence rate of \textit{C. trachomatis} (Morre et al. 2002). Our study showed relatively higher infections than the previous study abroad and relatively lower than that of the home but correlates the overall incidence \((46.6\%\) of South and South East Asia.

In our study, \textit{Chlamydia trachomatis} infection (CT) was 47.4% in pregnant women which was higher than in the non pregnant women \((42.8\%\) (Table 03). In prevalence
study from India reported 29.8% CT infection during pregnancy (Yasodhora et al. 2001). Another study among pregnant women from India detected 19% positive CT antigen (Malenie, Joshi and Mathur 2006). Rastogi, Kapur & Salhan (1999) from India reported 21.3% of pregnant women were infected with *C. trachomatis* in their study. Reports from developing countries show the prevalence in pregnant women ranging from 7% to 45% (Rowel 1994).

In our study, CT infection was 47.2% in symptomatic and 41.1% in asymptomatic women (Table 04). An Indian study among symptomatic and asymptomatic pregnant women found 26.4% symptomatic and 15.1% asymptomatic positive cases for *C. trachomatis* (Malenie, Joshi and Mathur 2006). Another study from Iran reported 18.9% CT infection in symptomatic and 27.2% in asymptomatic women (Jenab et al. 2009). The results of our study correlate with the Indian study. There is no statistically significant difference between symptomatic and asymptomatic positive cases.

In the present study, result of the Iodine preparation of the endocervical swabs for detection of inclusion bodies (IB) was 5.5% positive among the study population (Table 05, 06). Zapta, Chernesky & Mahony (1984) reported only 10% positive cases positive cases by Iodine staining in cell line culture. All inclusions do not possess matrix or some contain so little that they are likely to escape detection by the iodine (Dark 1955). This limitation perhaps reflected in the current study.

Initial body inclusions may not be stained with iodine at all (Thygeson 1938) because glycogen is present only for limited period in the developmental cycle. So iodine stain may fail to detect IB if the specimen does not contain the material during the developmental period. The primary usefulness of iodine staining is in the rapid staining
of tissue culture monolayer used for the isolation of *C. trachomatis* from clinical specimens (eds Joklik et al. 1992).

The result of Giemsa stained preparation of the endocervical swabs to detect intracytoplasmic inclusion bodies (IB) of our study cases shows that the number of IB positive cases was only 12.9% (Table 07, 08).

Though Giemsa staining is most useful in the diagnosis of neonatal inclusion conjunctivitis, it has limited to no value in the diagnosis of genital tract infections (eds Joklik et al. 1992).

Nicholas et al. (1963) reported 14% positive result for inclusion body in Giemsa staining of 106 trachoma cases. However, recently, procedure of seeking chlamydia inclusions in epithelial cells from the genital tract by using giemsa staining technique was shown insensitive (Chernesky 2005; Schachter & Dawson 1977).

In our study, we found 28.7% seropositivity of IgG among the 108 study cases (Table 09). Morre et al. (2002) from Netherlands reported 35% seroprevalence of *C. trachomatis* for IgG. Mazara et al.(1989) found 50.2% Anti-CT IgG by ELISA in cases of genital non gonococcal infection in a Italian study among 115 men and 116 women. A study from Iran found 29.4% IgG to CT in serological screening which is in accordance with our result (Jenab et al. 2009).

The seropositivity in pregnant women (44%) was four times higher than the non pregnant (10.2%)(Table 09). Sawhney and Batra (2003) detected 45.8% and 10% seropositivity in pregnant and in non pregnant women respectively which shows very close correlation with our study. The differences in seropositivity rates between the
pregnant and non pregnant women is statistically highly significant (p<.001). Higher seropositivity in pregnant women group due to increased susceptibility to pregnancy for *Chlamydia trachomatis* infection and increased proliferation of the organism due to decreased immunity by physiological immunosupresion.

In our study, Relationship of *C. trachomatis* specific IgG ELISA test with DFA test of the study cases shows, 22 (44.8%) cases true positive, 09 false positive, 27 false negative and 50 true negative cases. The IgG ELISA is found statistically significant (P<.001) (Table 13). In the present study, we calculated the measures of validity of CT specific IgG ELISA comparing with Direct Fluorescene Antibody (DFA) Test as ‘gold standard’ (Table 13). The sensitivity and specificity for IgG-ELISA was found 44.8% and 84.7% respectively. A study showed 29.4% sensitivity and 100% specificity of the IgG ELISA comparing with PCR as gold standard (Jenab et al. 2009). Similar studies from United Kingdom(UK) revealed 73.8%, 59.8 %, 55.5%, and 45.7 %, sensitivities and 97.6%, 99%, 96% and 97.2% specificity respectively (Wills et al.2009).

In the present study, a patient was considered infected with *C. trachomatis* when the presence of organisms was demonstrated by DFA test (Tabrizi 2009). We detected 45.3% positive cases infected with *C. trachomatis* in sexually active women. Comparing the test positivity for *C. trachomatis* from endocervical swabs by different methods, DFA test was found more sensitive although its test validity could not be evaluated due to lack of cell culture facilities (Table 10). We did not perform IgM specific ELISA to differentiate acute infection from the past.
Conclusions and Recommendations

Analyzing the findings of the present study, it can be concluded that the incidence of genital chlamydia is common both in pregnant and non-pregnant adult women in Bangladesh. Seroepidemiologic study by IgG ELISA also shows high prevalence of chlamydia. The sensitivity of microscopy for diagnosing genital chlamydia by Giemsa staining and Iodine staining techniques to detect inclusion bodies (IB) is very low.

Screening of chlamydial infection should be done on routine basis by suitable tests in sexually active symptomatic and asymptomatic women including pregnant women to prevent complications. Further study should be done on large scale to find out the actual situation at both community and hospital level by more specific tests like PCR (polymerase chain reaction).
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