PREVALENCE OF CHLAMYDIA TRACHOMATIS IgM IN STUDENTS OF FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE, NIGERIA

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CERTIFICATION

This dissertation was supervised by me and approved in accordance with the partial fulfilment of the requirements for the award of B.Sc. (Hons) degree in Microbiology, Federal University Oye Ekiti, Nigeria.

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DEDICATION

This project is dedicated to god almighty that is most gracious and most benevolent for he has made this work possible and he made it a success.

It is also dedicated to my parents for their tireless effort on me throughout my studies.

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TABLE OF CONTENTS

ABSTRACT

Chlamydia trachomatis infections are the most common bacterial cause of sexually transmitted diseases (STDs) in the world. This study was to investigate the prevalence of *Chlamydia trachomatis* infection amongst students of Federal university Oye-Ekiti, Ekiti State, Nigeria. Urine samples were collected from ninety four participants and were screened using the microwell ELISA *Chlamydia trachomatis* kit – Swab/Urine (Golden Bio Technologies Corp, USA). Out of 94 patients screened, 26 (27.7%) was positive for *Chlamydia trachomatis* IgM, higher percentage was recorded in males (80.8%) compared to females (19.2%). The age group prevalence was as follows 15 – 19 years (50.0%), 20 – 24 years (46.1%), 25 years and above (3.9%). It was observed that participants that have no permanent sex partner were 76.9% of the total number of participants positive for Chlamydial infection, one sex partner were 19.2%, two partners were 0.0% and 3.9% for those with more partner. There seem to be an association between chlamydial infection and number of sex partners thus screening for *Chlamydia trachomatis* infection in asymptomatic patients is necessary to prevent the adverse consequences Chlamydial infection.

C. trachomatis infection if unchecked will continue to pose a threat to reproductive life with its established complications. Since asymptomatic cases are common in the population regular screening should be encouraged.

CHAPTER ONE

INTRODUCTION

Chlamydia trachomatis is a small, non-motile, Gram-negative bacterium that is an obligate intracellular parasite with a unique bipolar life cycle (Adams *et al.*, 2004). It is the most common bacterial sexually transmitted disease in the world.

Asymptomatic infection is common among both men and women, approximately 50% of infected males and 80% of infected females show no symptoms and to detect Chlamydial infections, health-care providers frequently rely on screening tests. The infection may cause a mucopurulent cervicitis in females and urethritis in males.

Chlamydiae are classified in the order *Clamydiales*, family *Chlamydiacae*, and genus *Chlamydia*. Within this genus four species are recognized currently, *C. pecorum*, *C. trachomatis*, *C. pittaci*, *C. pneumonae*. *Chlamydia trachomatis* is further classified on serological basis in 15 serotypes. Serotype l-1, l-2, l-3 causes lymphogranuloma venereum (LGV). Serotype- A, B, Ba and C cause trachoma, the remaining D, E, F, G, H, I, J, K serotypes are the causative agents in occulo-genital and sexually transmitted infections like epididymitis, new-born pneumonia and perinatal infections (Pipal *et al.*, 2017).

The bacteria can present in one of three ways: genitourinary (genitals), pulmonary (lungs), and ocular (eyes). Genitourinary cases can include genital discharge, vaginal bleeding, itchiness (pruritus), painful urination (dysuria), among other symptoms (Mishori *et al.*, 2012). Often, symptoms are similar to those of a urinary tract infection.

C. trachomatis genitourinary infection is more common in females compared to males, females aged 15–19 have the highest prevalence, followed by female aged 20–24, although the rate of

increase of diagnosis is greater for men than for women. Risk factors for genitourinary infections include unprotected sex with multiple partners, lack of condom use, and living in an urban area. Pulmonary infections can occur in infants born to women with active Chlamydial infections, although the rate of infection is less than 10% (Mishori *et al.,* 2012). Ocular infections take the form of conjunctivitis or trachoma, both in adults and children. Trachoma is the primary source of infectious blindness in some parts of rural Africa and Asia and is a neglected tropical disease that has been targeted by the World Health Organization for elimination.

Chlamydia has been identified as a cofactor in the transmission of HIV infection (Nwanguma *et al.,* 2010). It has also been proposed as an independent risk factor for development of cancer of the cervix (Anttila *et al.,* 2001). Silent untreated infection leads to dreaded consequences like pelvic inflammatory disease, infertility, ectopic pregnancy and chronic pelvic pain. It has been estimated that as much as 50% of salpingitis and infertility are caused by Chlamydia infection (Mehta *et al.,* 2001).

The prevalence of *Chlamydia trachomatis* varies, it depends on the characteristics of the study population and the different methods used for Chlamydia detection (Buvé *et al.,* 2001). The United States has an overall prevalence of 5%. In the United Kingdom, recent data suggest that rate of infection among young women may exceed 10% (Lee *et al.,* 2006). A systematic review by Lewis *et al.,* (2012) in Australia, reported an overall prevalence of 4.6% with a higher prevalence of 5.6% among adolescent and young adults.

Prevalence of genital *Chlamydia trachomatis* in parts of Africa varies considerably ranging from 3.78% in Cameroon (Ngandjio *et al.,* 2019) to as high as 68.25% in female sex workers in Niger Republic. Data from African countries suggest that prevalence is on the rise and may exceed that reported in developed countries (Nwanguma *et al.,* 2010, Esumeh *et al.,* 2009).

Reports from Nigeria vary as well; a low prevalence of 0.1% (1/140) in was observed in population sampled across three Western States of Nigeria (Adesiji *et al.,* 2015), 29.4% prevalence was reported by Ikeme *et al.,* (2011) while Mawak *et al.,* (2011) reported a prevalence of 56.1% among 164 women attending gynecology clinic in Jos, Plateau State. A prevalence of 51% was reported by Esumeh *et al.,* (2009) among pregnant and non-pregnant women and their spouses attending prenatal and antenatal clinic of College of Medicine of University of Lagos. Nwanguma *et al.,* (2010) in Owerri and Nsukka studied 102 Nigerians, and prevalence of 50% in HIV-positive volunteers and 17% in the HIV-negative group was reported, with an overall prevalence of 33% reported in the asymptomatic volunteers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy and Classification of *Chlamydia trachomatis*

Domain: Bacteria

Phylum: *Chlamydiae*

Class: *Chlamydiae*

Order: *Chlamydiales*

Family: *Chlamydiaceae*

Genus: *Chlamydia*

Species: *trachomatis*

Chlamydia trachomatis is further classified on serological basis in 15 serotypes. *C. trachomatis* strains are generally divided into three biovars based on the type of disease they cause. These are further subdivided into several serovars based on surface antigens recognized by the immune system (Elwell *et al.,* 2016). Serovars A through C causes trachoma. Serovars D through K infect the genital tract, causing pelvic inflammatory disease, ectopic pregnancies, and infertility. Serovars L1 through L3 cause an invasive infection of the lymph nodes near the genitals, called lymphogranuloma venereum (Elwell *et al.,* 2011).

C. trachomatis belongs to the, family *Chlamydiaceae* together with *Chlamydia muridarum* and *Chlamydia suis*. Other Chlamydiae infecting humans, *Chlamydophila pneumoniae* and *Chlamydophila psittaci*, are currently classified in a separate genus. However, this subdivision of the family into the two genera *Chlamydia* and *Chlamydophila* has been discussed controversially during the past decade. Recently, in the light of recent genomic data and in the

context of the unique biological properties of these microorganisms, it was proposed to classify all the eleven currently recognized *Chlamydiaceae* species in a single Chlamydial genus. Three *C. trachomatis* biovars comprising all 15 classical serovars and several additional serovars and genovars are recognised within the *C. trachomatis* species: the trachoma biovar (serovars A–C), the urogenital biovar (serovars D–K) and the LGV biovar (serovars L1–L3) (Elwell *et al.*, 2011).

2.2 Description of *Chlamydia trachomatis*

Chlamydia trachomatis is a gram-negative bacterium that can only replicate within a host cell. It is a small, non-motile bacterium. *C. trachomatis* life cycle consists of two morphologically distinct life stages: elementary bodies and reticulate bodies.

Elementary bodies are spore-like, they range from 200 to 400 nanometers across, and are surrounded by a rigid cell wall that allows them to survive outside of a host cell (Elwell *et al.*, 2011). The elementary body is the infectious form of *Chlamydia trachomatis*. They possess a rigid outer membrane that bind to receptors on host cells and initiate infection. Because of their rigid outer membrane, the Chlamydia inhibits the fusion of the endosome and the lysosome and therefore resist intracellular killing. Reticulate bodies are the non-infectious intracellular form, yet they are the metabolically active replicating form of Chlamydia. When the elementary body finds a host cell, it is engulfed through endocytosis and then becomes infected. A vacuole encloses the elementary body and the bacteria are now a reticulate body. It can then replicate itself through binary fission. After division, the reticulate body becomes the elementary body and is released trough reverse endocytosis (Price *et al.*, 2016).

2.3 Genome of *Chlamydia trachomatis*

The *C. trachomatis* genome is substantially smaller than that of many other bacteria at approximately 1.04 megabases, encoding approximately 900 genes. A number of important metabolic functions are not encoded in the *C. trachomatis* genome, and instead are likely scavenged from the host cell (Price *et al.*, 2016). In addition to the chromosome that contains most of the genome, nearly all *C. trachomatis* strains carry a 7.5 kilobase plasmid that contains 8 genes. The role of this plasmid is unknown, though strains without the plasmid have been isolated, suggesting it is not required for survival of the bacterium (Postma *et al.*, 2010).

2.4 Life Cycle of *Chlamydia trachomatis*

C. trachomatis has a life cycle consisting of two morphologically distinct forms. First, *C. trachomatis* attaches to a new host cell as a small spore-like form called the elementary body. The elementary body enters the host cell, surrounded by a host vacuole, called an inclusion (Wariso *et al.*, 2012). Within the inclusion, *C. trachomatis* transforms into a larger, more metabolically active form called the reticulate body. The reticulate body substantially modifies the inclusion, making it a more hospitable environment for rapid replication of the bacteria, which occurs over the following 30 to 72 hours (Ogbu *et al.*, 2017). The massive numbers of intracellular bacteria then undergo transition back to resistant elementary bodies, before causing the cell to rupture and being released into the environment. These new elementary bodies are then shed in the semen or released from epithelial cells of the female genital tract, and attach to new host cells (Witkin *et al.*, 2017).

Chlamydia resides in a membrane-bound vacuole known as the inclusion. Following invasion, Chlamydia alters the features of the internalized vacuole, which allows the bacteria to avoid

degradation through the endocytic pathway (Esumeh *et al.,* 2009). Throughout the course of infection the inclusion expands as the number of Chlamydial cells increase. The inclusion expansion has been suggested to be dependent on host cell lipid metabolism, host cell cytoskeletal proteins, vimentin and actin (Nwankwo and Sadiq, 2014), and secreted bacterial effectors proteins, which may manipulate processes in the host cytoplasm that are required for optimal inclusion expansion. It is possible that expansion of the Chlamydial inclusion is linked to bacterial replication, and in fact, inclusion area has been used to indirectly measure Chlamydial replication and growth (Witkin *et al.,* 2017).

However, a potential link between inclusion expansion and bacterial replication has not been proven experimentally or characterized under different growth conditions. Acquisition of host cell metabolites is critical for Chlamydial intracellular growth and development. Host cell glucose-6-phosphate (G-6P) appears to be important for *C. trachomatis* infectivity (Esumeh *et al.,* 2009).

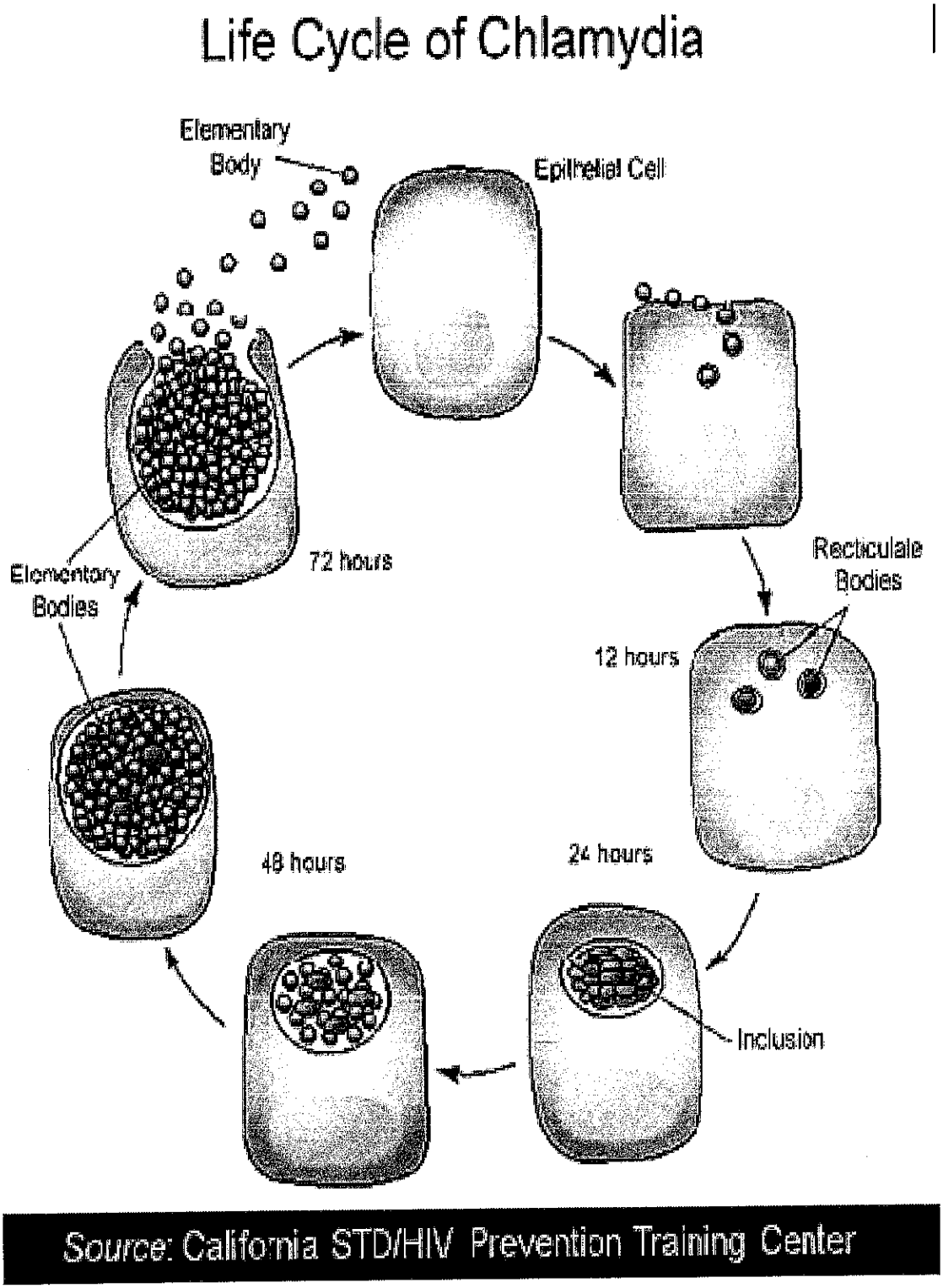


Figure 1: Life Cycle of *Chlamydia trachomatis*

2.5 Pathogenesis of *Chlamydia trachomatis*

C. trachomatis replicates preferentially on mucosal surfaces within columnar or transitional epithelial cells. The organisms stimulate a brisk infiltration of polymorphonuclear cells, especially early in infection. Submucosal lymphocytic infiltration is also impressive, leading to lymphoid follicle formation and fibrotic changes (Ige *et al.,* 2018).

Because of the inherent difficulties in acquiring human tissue samples for study, researchers have taken advantage of multiple animal models of Chlamydial infection to examine the nature and timing of the inflammatory response that occurs in the female genital tract after *in vivo* infection. Mousse and guinea pig models show that the response to primary Chlamydial infection occurs within 1.2 days of infection and is characterized by mucosal infiltration with neutrophils and modest numbers of monocytes. Neutrophils are recruited in large numbers to the site of infection and are capable of killing accessible elementary bodies. Later, T cells accumulate at the site of Chlamydial infection and play a critical role in controlling the infection. A consistent observation in the murine model is that tubal dilatation is a frequent end result of primary infection. Thus, the inflammatory process resulting from a single Chlamydial action may be sufficient to induce tubal damage and infertility. In contrast, in female guinea pigs, the host response to primary infection results in long-term tissue damage in a minority of infected animals. Analogous information on the proportion of women developing tubal damage after Chlamydial infection is not available, but most infected women do not appear to develop clinical complications. Taking into consideration reported complication rates together with the high prevalence of genital tract *C. trachomatis* infection in women, it appears that the guinea pig model may more closely approximate human disease. In female animals and women, the multidimensional set of immune

events that occurs at the time of infection ultimately results in an acquired immune response (Pipal *et al.,* 2017).

2.6 Virulent Factors of *Chlamydia trachomatis*

Unfortunately, the adaptive response induced by infection is not effective in preventing re-infection, and the hosts' oviducts remain vulnerable to repeated inflammatory insult (Nwanguma *et al.,* 2010).

Endocervical epithelial cells released interleukin (IL) after infection, and the induced pro-inflammatory cytokine cascade could be inhibited by specific anti IL-1a antibodies. Thus, IL-1a, released on epithelial cell lysis, may act to amplify the inflammatory response by stimulating additional cytokine production. These findings formed the basis for Richard Stephens's cellular paradigm of Chlamydial pathogenesis. Stephens theorized that the inflammatory processes of Chlamydial pathogenesis are elicited by infected host cells and are necessary and sufficient to account for chronic inflammation and the promotion of cellular proliferation, tissue remodeling and scarring the ultimate cause of disease sequelae (Pipal *et al.,* 2017).

2.7 Immunity

The cellular paradigm of Chlamydial pathogenesis states that the host response to *Chlamydiae* is initiated and sustained by epithelial cells that are the primary targets of Chlamydial infection. Infected host epithelial cells act as first responders, initiating and propagating immune responses. They secrete chemokines that recruit inflammatory leukocytes to the site of infection and cytokines that induce and augment the cellular inflammatory response and these mediators induce direct damage to the tissues. At the time of re-infection, host cell release of chemokines leads to recruitment of Chlamydia specific immune cells that rapidly amplify the response. The release of proteases, clotting factors, and tissue growth factors from infected host cells and

infiltrating inflammatory cells leads to tissue damage and eventual scarring the hallmark of chlamydia induced oviduct disease. The cellular paradigm makes no distinction between damage induced by professional innate immune cells (neutrophils and monocytes) and adaptive lymphocyte populations but assumes that both cell populations contribute to pathogenesis. Chronic Chlamydial infections are common and would lead to ongoing release of mediators that promote continued influx of inflammatory cells, damage to host epithelium, scarring, and ultimately, fibrosis and scarring. Because reinfection with chlamydiae is a frequent occurrence, repeated inflammatory responses may lead to repeated insult to the tissues and may promote tissue scarring. Studies using the murine model of *C. trachomatis* genital tract infection have established that resolution of genital Chlamydial infection is dependent on an influx of interferon (IFN)-producing CD4+ Th1 cells. The immunological paradigm for pathogenesis is based on the premise that T cell responses that are essential to host defense may also cause collateral tissue damage. It was speculated that host T cell responses induced on primary infection to a species specific antigen were increased with subsequent infections, promoting tissue damage and scarring (Witkin *et al.*, 2017).

Chlamydia heat shock protein 60 (Chsp60) has been investigated as a potential antigen responsible for induction of delayed type hypersensitivity induced disease. This molecule attracted attention as a candidate pathogenic antigen after studies in immune guinea pigs and monkeys suggested direct eye inoculation with this sensitizing antigen promoted heightened inflammation of the conjunctiva. However, residual Triton-X detergent contaminating the extracts proved to be the inducer of disease. Later studies conducted in the guinea pig model of trachoma revealed a protective role for vaccination with Chsp60, and although human studies have revealed detection of elevated titers of antibody to Chsp60 in those with more severe

disease, this may simply reflect increased exposure to *Chlamydia trachomatis* through chronic or repeated infection. A recent large prospective study of women with PID did not reveal a correlation of increased antibody titers to Chsp 60 with worse outcome. Furthermore, IFN-g production by peripheral blood T cells stimulated with Chsp60 predicts protection from incident infection in women at high risk of repeated infection. Despite the lack of evidence for a specific Chlamydial pathogenic antigen that primes anamnestic T cell responses, in the presence of chronic or repeated infection, an ongoing or augmented memory T cell response might heighten disease development.

Monkey and guinea pig models of repeated infection indicate that CD4 and CD8 T cells infiltrate more rapidly and in larger numbers than do neutrophils during repeat oviduct infections, and this recurrent inflammatory reaction ultimately culminates in fibrosis and scarring of importance, because guinea pigs develop sufficient immunity after primary infection to significantly limit bacterial burden during a secondary vaginal infection, this indicates that very small amounts of Chlamydiae may be sufficient to induce an enhanced T cell response in the oviduct that culminates in disease. Although CD4 and CD8 T cells have been observed in both of these models of repeated infection, with CD8 T cells being predominant in the monkey model, no data exist on the specific role of CD8 T cells in pathogenesis in humans. Because the ultimate goal of Chlamydia control programs is to prevent reproductive tract complications, a more complete understanding of how *C. trachomatis* infection leads to sequelae is needed. The cellular paradigm of pathogenesis does not invoke professional innate or adaptive immune cells as being more or less responsible for disease development. Instead, the central player of pathogenesis is assumed to be the host epithelial cell that drives the inflammatory response through its recognition of Chlamydial infection.

Epithelial cells possess surface and intracellular innate immune receptors that enable them to recognize conserved Chlamydial ligands and initiate inflammation. Thus, the infected epithelial cell serves as a key innate responder cell. Therefore, a determination of genetic polymorphisms that result in heightened innate inflammatory responses to *C. trachomatis* may serve to identify persons at high risk of disease development and in need of increased levels of screening and treatment (Odusolu *et al.,* 2016).

IL-1 receptor antagonist to the cultures completely eliminates tissue destruction induced by infection, indicating a direct role for this cytokine in pathogenesis. *In vitro* infection of Fallopian tube epithelium also results in the production of tumor necrosis factor (TNF) and increased expression of adhesion molecules on oviduct endothelial cells. This milieu could easily lead to activation and recruitment of first innate and, later, adaptive immune cells to effect resolution of infection. However, subsets of these responses may also induce collateral damage to genital tract tissue (Price *et al.,* 2016). Proinflammatory chemokines and cytokines have been documented in murine and guinea pig models of genital tract Chlamydial infection. The up regulation of integrins in the murine genital tract is coincident with the onset of infection. Detection of the neutrophil chemokine macrophage inflammatory protein-2 (MIP-2), a chemokine analogous to IL-8 in humans, in genital tract secretions of infected mice coincides with a rapid influx of neutrophils into the lower genital tract.

Many patients with *C. trachomatis* infections show hyperimmunoglobulinemia with polyclonal activation and secretion of IgM, IgG and IgA in tears and serum. Although neutralizing antibody plays a protective role in experimental infection, antibodies are not totally protective since disease persists as long as re-inoculation continues. The antibody's role *in vivo* may be to limit the extent of Chlamydial multiplication rather than to affect a cure (Lanjouw *et al.,* 2015).

2.8 Clinical Signs and Symptoms of *Chlamydia trachomatis*

Urogenital infections: Symptoms and signs in women

- 70–95% asymptomatic
- Mucopurulent cervicitis with or without contact
- Bleeding
- Cervical friability
- Cervical oedema
- Endocervical ulcers
- Urethritis
- Dysuria
- Vaginal discharge
- Postcoital bleeding and intermenstrual bleeding
- Poorly differentiated abdominal pain or lower abdominal pain

Symptoms and signs suggestive of pelvic inflammatory disease (PID) (Lanjouw *et al.*, 2015)

- Lower abdominal tenderness and pain – usually bilateral
- Cervical motion tenderness on bimanual vaginal examination
- Adnexal tenderness on bimanual vaginal examination
- Deep dyspareunia – particularly of recent onset
- Abnormal bleeding – intermenstrual bleeding, post coital bleeding and menorrhagia can occur secondary to associated cervicitis and endometritis
- Abnormal vaginal or cervical discharge – as a result of associated cervicitis, endometritis or bacterial vaginosis
- Fever (>38˚C) – in moderate to severe PID

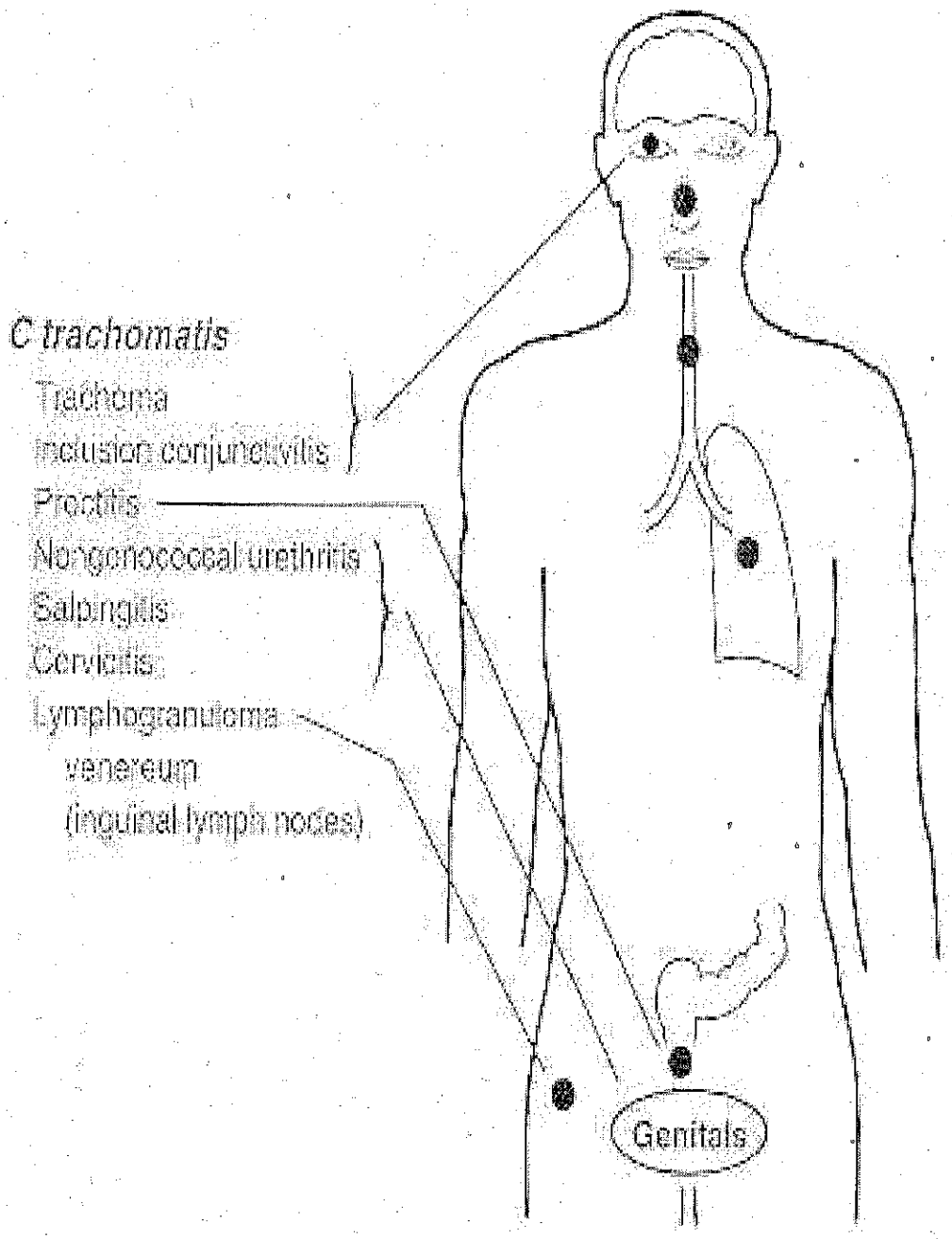
- PID (endometritis, salpingitis, parametritis, oophoritis, tuboovarian abscess and/or pelvic peritonitis)
- Chronic pelvic pain
- Tubal infertility
- Ectopic pregnancy
- Sexually acquired reactive arthritis (SARA) (<1%)
- Fitz-Hugh-Curtis syndrome (PID and perihepatitis)

Symptoms and signs in men (may be so mild that they are not noticed)

- Usually more than 50% (25–100%) asymptomatic
- Urethritis
- Dysuria
- Urethral discharge
- Epididymitis
- Testicular pain

Complications in men

- SARA (<1%)
- Epididymitis, epididymo-orchitis



Source: (Ogbu *et al.*, 2017)

Figure 2: Complications caused at each body part

- Trachoma: Chronic infection or repeated reinfection with *C. trachomatis* (biovar: trachoma) results in inflammation and follicle formation involving the entire conjunctiva. Scarring of the conjunctiva causes turning in of the eyelids and eventual scarring, ulceration and blood vessel formation in the cornea, resulting in blindness. The name trachoma comes from 'trakhus' meaning rough which characterizes the appearance of the conjunctiva. Inflammation in the tissue also interferes with the flow of tears which is important antibacterial defense mechanisms. Thus, secondary bacterial infections occur (Lanjouw *et al.*, 2015).

- Inclusion conjunctivitis: Inclusion conjunctivitis is caused by *C. trachomatis* (biovar: trachoma) associated with genital infections (serovars D - 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 - 12 days. Ear infection and rhinitis can accompany the ocular disease (Lanjouw *et al.*, 2015).
- Infant pneumonia: Infants infected with *C. trachomatis* (biovar: trachoma; serovars: D - K) at birth can develop pneumonia. The children develop symptoms of wheezing and cough but not fever. The disease is often preceded by neonatal conjunctivitis. Ocular lymphogranuloma venereum infection with the LGV serovars of *C. trachomatis* (biovar: LGV) can lead to oculoglandular conjunctivitis. In addition to the conjunctivitis, patients also have an associated lymphadenopathy.

- Urogenital infections: In females, the infection is usually (80%) asymptomatic but symptoms can include cervicitis, urethritis, and salpingitis. Postpartum fever in infected mothers is common. Premature delivery and an increased rate of ectopic pregnancy due to salpingitis can occur. In the United States, tubal pregnancy is the leading cause of first-trimester, pregnancy-related deaths.

  In males, the infection is usually (75%) symptomatic. After a 3 week incubation period patients may develop urethral discharge, dysuria and pyuria. Approximately 35 - 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. Up to 40% of women with untreated (undiagnosed) *C. trachomatis* will develop pelvic inflammatory diseases and about 20% of these women will become infertile. Many untreated cases (18%) result in chronic pelvic pain. Women infected with *C. trachomatis* have a 3 - 5 fold increased risk of acquiring HIV up to 75% of patients show symptoms such as urethral discharge, dysuria and pyuria (Lanjouw *et al.*, 2015).

- 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 - 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 (Shigellosis or *Yersinia enterocolitica*) have also been associated with Reiter's syndrome. Lymphogranuloma venereum (*C. trachomatis* biovar: LGV). The primary lesion of LGV is a small painless and inconspicuous vesicular lesion

that appears at the site of infection, often the penis or vagina. The patient may also experience fever, headache and myalgia. The second stage of the disease presents as a marked inflammation of the draining lymph nodes. The enlarged nodes become painful 'buboes' that can eventually rupture and drain. Fever, headache and myalgia can accompany the inflammation of the lymph nodes. Proctitis is common in females; lymphatic drainage from the vagina is perianal. Proctitis in males results from anal intercourse or from lymphatic spread from the urethra. The course of the disease is variable but it can lead to genital ulcers or elephantiasis due to obstruction of the lymphatics (Khan, 2011).

2.9 Laboratory Diagnosis

2.9.1 Culture

Culture is the most specific method for diagnosis of *C. trachomatis* infections. Specimens are added to cultures of susceptible cells and the infected cells are examined for the presence of iodine-staining inclusion bodies. Iodine stains glycogen in the inclusion bodies. The presence of iodine-staining inclusion bodies is specific for *C. trachomatis* since the inclusion bodies of the other species of *C. trachomatis* do not contain glycogen and stain with iodine.

Chlamydia being obligate intracellular parasites cannot be cultured on bacteriologic media. However, they can be grown in yolk sac of embryonated egg and in number of cell culture monolayers. Successful isolation has been done with monkey kidney, HeLa and Mc Coy cells. Mc Coy and HeLa cells are most commonly used. After incubation at 35°C for 48-72 hours, the inoculated monolayer is stained with fluorescent antibodies (which can be genus or species specific) or with iodine, which detects *C. trachomatis* only (Chakrabarty 2003).

Recently Culture was considered as gold standard test for detection of *C. trachomatis*. It has specificity of 100%, but its sensitivity is as low as 50%. Cell culture was long the gold standard for diagnosis of *C. trachomatis* infections because of its absolute specificity. However, due to its labor-intensive methodology, turnaround time, cost, and requirements for infrastructure and technical expertise, cell culture facilities were limited to specialized research laboratories only. Other non-culture tests, such as enzyme immunoassay (EIAs), serological tests, etc., lack the high sensitivity and specificity needed for accurate diagnosis of ongoing infection (Ogbu *et al.*, 2017).

2.9.2 Cytology

C. trachomatis infections of conjunctiva, urethra or cervix can be diagnosed by demonstrating typical intracytoplasmic inclusions on cytological examination. Giemsa stain was most frequently used in the past, but the more sensitive immunofluorescence techniques have now largely replaced Giemsa Stain. Staining of cell with Giemsa reagent to detect Chlamydial infection provides a rapid laboratory diagnosis. Among the various laboratory methods for diagnosing *C. trachomatis*, Giemsa staining is rapid, simple, less time consuming technique but this method is not as sensitive as other methods (Khan, 2011).

2.9.3 Serology

Serologic tests are generally not useful in the diagnosis of genital tract infections caused by *C. trachomatis*. This is because antibodies elicited by *C. trachomatis* infection are long lived and a positive antibody test will not distinguish a previous from a current infection. The use of convalescent phase serum specimens can be helpful, since a fourfold increase in antibody titer is predictive of an active infection; however, it can take up to 1 month or longer for antibody titers to rise, and the delay is not appropriate to the time frame necessary for therapy and management

of patients. The presence of IgM antibodies is an unreliable marker of acute infection in adolescents and adults since IgM is often not present, presumably because the person has been infected previously with *C. trachomatis* or possibly with another Chlamydial species such as *C. pneumoniae* and is generating an anemnestic response to the most recent exposure. In spite of test manufacturers claims, no currently available serologic test of a single serum specimen will provide conclusive evidence of current infection with *C. trachomatis* (Aladeniyi *et al.*, 2017). Serologic tests that have been developed for detection of antibodies to *C. trachomatis* for diagnostic purposes are the CF test, the MIF test, and tests based on EIAs.

2.9.4 ELISA

Serological diagnosis of Chlamydial infections in the past has relied heavily on the CF and MIF tests. The lack of sensitivity of the CF test and the difficulty in performing the MIF test has fostered the search for new serological tests for the measurement of Chlamydial antibodies. A simple, rapid enzyme-linked immunosorbent assay (ELISA) has been developed for the measurement of IgG and IgM antibodies to *C. trachomatis*. Wells of microtiter plates were coated with renografin-purified elementary bodies (serotype L2) grown in cycloheximide-treated McCoy cells, and serum antibody was detected with peroxidase-labeled goat antihuman IgG and IgM antibody (Price *et al.*, 2016).

Diagnostic tests based on immunochemical detection of LPS genus-specific antigen were developed during the late 1980s, and this technology has produced the greatest number of commercial tests available today. In direct EIAs, enzyme-labeled antibodies that recognize all species of *Chlamydia* bind to LPS extracted from elementary bodies in the specimen. In indirect EIAs, primary anti-LPS antibody (usually murine IgG) is used as the detector reagent, followed by a secondary enzyme-linked, usually antimurine IgG antibody. LPS is used with this

technology since it is more abundant and more soluble than MOMP. The enzyme component of specifically bound antibodies converts a colorless substrate to a colored product that is detected by a spectrophotometric reader. Alternatively, the conjugated enzyme may convert a fluorescence-generating substrate to a signal detected by a fluorescence reader. Total processing time for manual EIAs is 3 to 4 hours. A disadvantage of the EIA is that antibodies to LPS may cross-react with the LPS of other gram-negative bacteria to produce false-positive results. To improve the specificity, some manufacturers have developed blocking assays that are used to verify positive EIA results. The blocking test is performed by repeating initially positive EIAs in the presence of monoclonal antibodies specific for Chlamydial LPS. The monoclonal antibody competitively inhibits, or blocks, the LPS epitope bound by the detector antibody; thus, a reduced signal with the use of blocking antibody is interpreted as verification of the initial positive test result. The first developed and most extensively evaluated commercial EIA is Chlamydiazyme. Another widely used and more recently developed EIA for *C. trachomatis* is the Microtrak EIA (Khan, 2011).

ELISA has high false positive rate and low sensitivity. When compared with Culture only, the sensitivity is 83% and specificity 98%. The amplified DNA tests Polymerase chain reaction and ligase chain reaction are more sensitive than tissue culture and EIA.

2.9.5 Antigen detection

2.9.5.1 Direct Fluorescence Antibody Test (DFA)

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. With the use of monoclonal antibody reagents specific for the MOMP of *C.*

trachomatis, the sensitivity of DFA is 80 to 90% and the specificity is 98 to 99% relative to culture when both are performed optimally.

2.9.5.2 Immunochromatographic Test (ICT)

C. trachomatis was researched for lipopolysaccarid antigen (LPS) by immunochromatographic method. Immunochromatographic screening test which used monoclonal antibodies for detection of lipopolysaccharide antigen extracted from Chlamydiae. Among the various laboratory methods for diagnosing *C. trachomatis*, Immunochromatographic method is rapid, simple, less time consuming technique (Khan, 2011).

2.9.5.3 Rapid kit tests

Rapid tests, also called point-of-care or near-to-patient tests, for *C. trachomatis* employ EIA technology in formats based primarily on membrane capture or latex immunodiffusion. Rapid tests are performed in physician offices, do not require sophisticated equipment, and can be completed in about 30 min. Results are read visually and are thus qualitative.

Nucleic acid detection method: Three tests based on nucleic acid probes are available. These tests are sensitive and specific and may replace culture as the method of choice.

2.10 Transmission

Transmission of *C. trachomatis* usually takes place by direct mucosal contact between two individuals during sexual intercourse (vaginal, anal or oral sex) or at birth through an infected cervical canal. It is difficult to estimate the risk of sexual transmission. One transmission dynamic mathematical modeling study provided estimates (Lanjouw *et al.,* 2015), based on data from a cross-sectional heterosexual partnership study in clinical attendees. The model estimated a median transmission probability of around 10% for a single act of vaginal coitus and around

55% over the course of a partnership in a population that has two partnerships in a six month period. Partners of people with *C. trachomatis* infection are very likely to be infected themselves, so contact notification and subsequent treatment are very important (Lanjouw *et al.*, 2015).

2.11 Epidemiology

C trachomatis is the most prevalent cause of bacterial sexually transmitted infections (STI), with an estimated 100 million cases reported annually throughout the world (Hashemi *et al.*, 2007). In 1999, World Health Organization (WHO) estimated that 92 million new Chlamydial infections occur worldwide annually, with the largest proportion (43 million) of these infections being contracted in South and South-east Asia, a region that is mostly resource limited with respect to infectious disease diagnosis and management (Khan, 2011). 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 and 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 (CDC, 2016).

In random community based samples study, *Trichomonas vaginalis* was the most commonly found STI (5%), followed *by Neisseria gonorrhoeae* (3-4%), *Chlamydia trachomatis* and herpes simplex virus type 2 (HSV2) (1-1.4%) and *Treponema pallidum* (Syphilis) being the least prevalent about 0.3% (Khan, 2011).

Chlamydial genital infection is distributed worldwide affecting both genders but especially affects sexually active adolescents and younger adults in the 15 to 24 year-old age group. Underreporting is substantial because most people with *Chlamydia* are not aware of their

infections and do not seek testing. Women are frequently re-infected if their sex partners are not treated (Postma *et al.*, 2010).

2.11.1 Epidemiological statistics in Nigeria: The lowest prevalence of Chlamydia was reported to be 0.1% (1/140) in a population sampled across three Western States of Nigeria (Adesiji *et al.*, 2015), a report among patients attending Family Planning and Obstetrics and Gynaecology clinics from selected Hospitals in Southern part of Nigeria (Osun state, Edo state and Oyo state) to have prevalence 0.7% (Adesiji, 2016), In Southern West Nigeria the 7.30% among infertile women asymptomatic for genital infection at University College Hospital, Ibadan. Oyo state (Ajani *et al.*, 2017), 9.6% among patients attending infertility and sexually transmitted diseases clinic (STD) in Kano, North Western Nigeria (Nwankwo and Sadiq, 2014), 26% among Women of reproductive age group in a Tertiary Hospital in Kaduna State, Northern Nigeria (Ige *et al.*, 2018), 11% among Female Undergraduates of the University of Port Harcourt (Wariso *et al.*, 2012), 38.6% in Infertile Women in Calabar (Odusolu *et al.*, 2016), 75.0% among infertile women in Garki hospital in Abuja (Ogbu *et al.*, 2017) the highest prevalence in Nigeria was 91.2% reported in symptomatic patients attending clinics in South West Nigeria (Okoror *et al.*, 2014)

2.12 Prevalence of *Chlamydia trachomatis*

Three times as many women as men are diagnosed with genitourinary *C. trachomatis* infections. Women aged 15–19 have the highest prevalence, followed by women aged 20–24, although the rate of increase of diagnosis is greater for men than for women. Risk factors for genitourinary infections include unprotected sex with multiple partners, lack of condom use, and living in an urban area.

Pulmonary infections can occur in infants born to women with active Chlamydial infections, although the rate of infection is less than 10% (Mishori *et al.*, 2012). Ocular infections take the form of conjunctivitis or trachoma, both in adults and children. Trachoma is the primary source of infectious blindness in some parts of rural Africa and Asia and is a neglected tropical disease that has been targeted by the World Health Organization for elimination by 2020.

2.13 Prevention and Control of *Chlamydia trachomatis*

C. trachomatis is a completely preventable disease; this should be the focus of health care and research facilities.

a. Primary prevention strategies

These are efforts to prevent Chlamydial infection. Primary prevention of *C. trachomatis* can be accomplished in two general ways:

Behavioral changes that reduce the risk of acquiring or transmitting infection should be promoted (e.g., using condoms during sex, partner selection, delaying the age of first intercourse, monogamy, discussing sexual history with partners, and educating yourself about sexually transmitted diseases and enlightening your friends). Efforts to effect behavioral changes are not specific to *Chlamydia trachomatis* prevention but are also critical components in preventing sexual transmission of the human immunodeficiency virus (HIV) and other sexually transmitted diseases (STDs).

Identify and treat persons with genital Chlamydial infection before they infect their sex partners and for pregnant women before they infect their babies. Efforts to detect

Chlamydial infection are essential to Chlamydia prevention. Identifying and treating Chlamydial infections require active screening and referral of sex partners of infected persons, since infections among women and men are usually asymptomatic (Emele *et al.*, 2015).

b. Secondary Prevention Strategies

Secondary prevention strategies are efforts to prevent complications among persons infected with Chlamydia. The most important complication to be prevented is salpingitis and its potential sequelae (i.e., ectopic pregnancy, tubal infertility, and chronic pelvic pain). Secondary prevention of Chlamydial salpingitis can be accomplished by

a) Screening women to identify and treat asymptomatic Chlamydial infection;
b) Treating the female partners of men with infection;
c) Recognizing clinical conditions such as mucopurulent cervicitis (MPC) and the urethral syndrome, and then applying or using appropriate *C. trachomatis* diagnostic tests and treatment, as appropriate.

Target Population: Chlamydial infection is especially prevalent among adolescents. Furthermore, PID occurs more commonly after Chlamydial infection among adolescent females than among older women. Therefore, efforts to prevent *C. trachomatis* should be directed toward young women. All sexually active adolescents and young adults are at high risk for Chlamydia, the infection is broadly distributed geographically and socioeconomically. Therefore, Chlamydia prevention programs should target all sexually

active adolescents and young adults. All private and public health-care providers should be involved in these prevention efforts.

Specific Strategies: Specific strategies for the prevention of Chlamydia are grouped into two categories. Those categories are community-based strategies, and health-care provider strategies.

I. Community-Based Strategies: Since the prevalence of Chlamydia is consistently high among adolescents and young adults regardless of socioeconomic status, race, or geographic location, prevention efforts should be implemented communitywide.

- Public Awareness. Community-based strategies should increase public awareness of Chlamydia, its consequences, and the availability and importance of diagnosis and treatment. Groups at high risk for Chlamydial infection and the persons who educate and care for them (e.g., parents, teachers, and health-care providers) must be informed about the high rate of genital Chlamydial infection and its sequelae among sexually active adolescents and young adults (CDC, 2016).
- STD Risk Reduction Programs. Programs designed to reduce the risk of sexual transmission of HIV and other STDs by means of behavioral changes should emphasize the especially high risk of Chlamydial infection. In addition, Chlamydial infection may be a sentinel for unsafe sexual practices. Concern about Chlamydia may provide additional motivation for persons to delay initiation of sexual activity, limit the number of sex partners, avoid sex partners at increased risk for STDs, and use condoms.

- Schools. Because of their access to adolescents, educators have an important role to play in Chlamydial prevention programs. Chlamydia-specific material should be integrated into educational curricula that address HIV and other STDs. In addition, school programs should assist students in developing the social and behavioral skills needed to avoid Chlamydial infection, HIV, and other STDs (CDC, 2016).
- Although most school health education curricula address HIV, fewer discuss other STDs (including Chlamydia). Information that should be provided through school health education programs are listed below:

Rates of Chlamydial infection among adolescents Adverse consequences of Chlamydia (e.g., PID and infertility) Symptoms and signs of Chlamydial infection (and other STDs) Asymptomatic infection Treatment for sex partners Where and how to obtain health care (including locations, telephone numbers {e.g., STD hot-line number}, costs, and issues of confidentiality) (CDC, 2016).

Some schools may offer more than classroom instruction (e.g., access to health care for infected persons and screening programs to identify asymptomatic Chlamydial infection). Personnel in school-based clinics that perform pelvic examinations should use the opportunity to test for Chlamydial infection. However, tests also are needed to identify asymptomatic males (e.g., by screening urine collected during sports physical examinations or other health-screening programs). The leukocyte esterase test (LET) of urine is one possible method for testing males, but the accuracy of the test requires additional evaluation. Such approaches to identifying and treating young men with

asymptomatic Chlamydial infection may prove important since these persons may account for most of the transmission of *Chlamydia trachomatis* to young women.

- Out-of-school Adolescents. The prevalence of Chlamydia may be even greater among adolescents who have dropped out of school. Therefore, organizations serving these adolescents (e.g.; Job Corps, vocational training centers, detention centers, community-based recreational programs) should offer health care that addresses Chlamydial infection as part of STD/HIV risk reduction programs.

II. Health-Care Provider Strategies

- Reducing the high prevalence of Chlamydial infection requires that health-care providers be aware of the high prevalence of Chlamydia and recognize Chlamydial illness, screen asymptomatic patients, arrange for the treatment of sex partners, and counsel all sexually active patients about the risks of STD infections. Medical providers should be trained to recognize and manage the following conditions that may be caused by Chlamydia: MPC, PID, urethral syndrome (women), and urethritis and epididymitis (men).
- Screening: The screening of women for Chlamydial infection is a critical component in a Chlamydia prevention program since many women are asymptomatic, and the infection may persist for extended periods of time. Many women of reproductive age undergo pelvic examination during visits for routine health care or because of illness. During these examinations, specimens can be obtained for Chlamydia screening tests.
- Treatment of Sex Partners. Treatment of sex partners of infected persons is an important strategy for reaching large numbers of men and women with asymptomatic

Chlamydial infection. Also, if partners are not treated, re-infection may occur. In addition, treating the male partners of infected women is critical since this is the principal way to eliminate asymptomatic infection among males. If *Chlamydia trachomatis* screening is widely implemented, the number of infected women identified may exceed the capacity of some public health systems to notify, evaluate, and treat partners. Therefore, health department personnel should assist health-care providers in developing cooperative approaches to refer partners for treatment. Where possible, health-care providers who treat female patients for *C. trachomatis* should offer examination and treatment services for the patients' male sex partner(s), or should arrange the appropriate referral of such partners.

- Risk Reduction Counseling. In addition to screening, treatment, and referral of sex partners(s) of persons with Chlamydial infection, health-care providers should:
- Educate sexually active patients regarding STDs, Assess the patients' risk factors for infection, Offer at-risk patients advice about behavior changes to reduce the risk of infection,
- Preventing Chlamydial infection during Pregnancy. To prevent maternal postnatal complications and Chlamydial infection among infants, pregnant women should be screened for *C. trachomatis* during the third trimester, so that treatment, if needed, will be completed before delivery. The screening criteria already discussed can identify those at higher risk for infection. Screening during the first trimester prevents transmission of the infection and adverse effects of *C. trachomatis* during the pregnancy. However, the evidence for adverse effects during pregnancy is minimal. If

screening is performed only during the first trimester, a longer period exists for infection before delivery (CDC, 2016).

- Infants with Chlamydial infections respond readily to treatment; morbidity can be limited by the early diagnosis and systemic treatment of infants who have conjunctivitis and pneumonia caused by Chlamydial infection (secondary prevention strategies). Further, the mothers of infants diagnosed with Chlamydial infection and the sex partner(s) of those mothers should be evaluated and treated.

2.14 AIM OF STUDY

The aim of this research work is to provide information on the prevalence of *Chlamydia trachomatis* among students of Federal university Oye-ekiti which will help in controlling the transmission of *C. trachomatis* as there is no past information on the prevalence of Chlamydia among this study population.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study population: Students of Federal University Oye-Ekiti.

3.2 Study period

This cohort study was conducted in July 2018. Included in the study were samples taken from randomly selected students aged between 15 to 30 years among a population of students of Federal University, Oye-Ekiti.

3.3 Sample size: 93

3.4 Study area: Federal University Oye-Ekiti, Ekiti State.

3.5 Type of study: Cross sectional study.

3.6 Specimen:

Mid Stream Urine samples were collected from each of the consented participants.

3.7 Collection of specimen:

Brief lecture and demonstration on when and how to properly collect samples was done by a male and a female for the male and female participants group respectively. Each participant was given a universal bottle which was used to collect the mid-stream urine samples aseptically.

3.8 Storage and stability of the specimen:

The specimen was kept in refrigerator at (2-8°C) until when the specimens were examined.

3.9 Principle:

Purified *Chlamydia trachomatis* antigen is coated on the surface of micro-wells. Diluted participant urine is added to wells, and the *Chlamydia trachomatis* IgM specific antibody, if present, binds to the antigen and all unbound materials are washed away.

After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

3.10 Preparation for assay

- ✓ 1x washing buffer was prepared by adding distilled water to 20x wash concentrate to a final volume of 1 liter.
- ✓ All samples and reagents were brought to room temperature (20-25°C) prior to testing.

3.11 Assay procedure

- ✓ 1:40 dilutions of the test samples, negative control, positive control and calibrator were prepared by adding 5µl to 200µl of absorbent solution. This was mixed properly.
- ✓ 100µl of diluted sera, calibrator and controls were dispensed into the appropriate wells. For reagent blank, 100µl absorbent solution was dispensed in 1A well position (the holder was gently tapped to remove air bubbles from the liquid and was mixed properly). This was incubated for 30mins at room temperature.
- ✓ Liquid from each well was removed and repeated washing were carried out three times with washing buffer.
- ✓ 100 µl of TMB Chromogenic Substrate was dispensed into each well and incubated for 15 minutes at room temperature.
- ✓ 100 µl of stop solution was added to stop reaction.
- ✓ O.D was read at 450nm with spectrophotometer.

3.12 Data analysis

The data obtained was analyzed using the SPSS and Microsoft Excel worksheet (Office suite 10). It was used to calculate the percentage of the gender and sex and other distributions of the results and to plot the graph representing the distributions.

CHAPTER FOUR

RESULTS

4.1 Distribution of Participants by Age and Chlamydial Infection.

Table 4.1 below revealed the age of participants and the prevalence of Chlamydial infection. Participants aged 15-19 years were positive for *Chlamydia trachomatis* by 50%, age group 20-24 years by 46.1% and age group 25 years and above by 3.9% these follow the same pattern for those that observed the negative outcomes of Chlamydial infection.

4.2 Distribution of Participants by Sex and Chlamydial Infection.

Figure 3 below revealed the sex of participants and the prevalence of Chlamydial infection. It was observed that male participants were mostly positive of *Chlamydia trachomatis* by 80.8% and female by 19.2% compare to those that were negative of *Chlamydia trachomatis*.

4.3 Distribution of Participants by Number of Sex Partner and Chlamydial Infection.

Figure 4 below revealed the number of sex partner of participants and the prevalence of Chlamydial infection. It was observed that participants that have no permanent sex partner (0 sex partner) were 76.9% of the total number of participants positive for Chlamydial infection, one sex partner were 19.2%, two partners were 0.0% and 3.9% for those with more partner.

4.4 Distribution of Participants by Condom Use and Chlamydial Infection.

Figure 5 below revealed the condom use of participants and the prevalence of *Chlamydia trachomatis*. It was observed participants that uses condom and were positive for Chlamydial infection were 34.6%, those that did not use condom and were positive for *Chlamydia*

trachomatis were 61.5% and those that use condom occasionally were 3.9% compare to those that were negative of *Chlamydia trachomatis*.

4.5 Distribution of Participants that Travel out of school and Chlamydial Infection.

Table 4.2 below revealed participants that travel out of school and the prevalence of *Chlamydia trachomatis*. It was observed that participants that did not travel out of school were 63.2% of not contracting Chlamydial infection and those that travelled out of school by 36.8% compare to those that were positive of *Chlamydia trachomatis*.

Table 4.1: Distribution of Participants by Age and Chlamydial Infection.

Background characteristics	Chlamydial Infection		
	Negative	Positive	TOTAL
Age			
15 – 19 years	34 (50.0)	13 (50.0)	**47 (50.0)**
20 – 24 years	30 (44.1)	12 (46.1)	**42 (44.7)**
25 – 30 years	4 (5.9)	1 (3.9)	**5 (5.3)**
Total	**68 (100.0)**	**26 (100.0)**	**94 (100.0)**

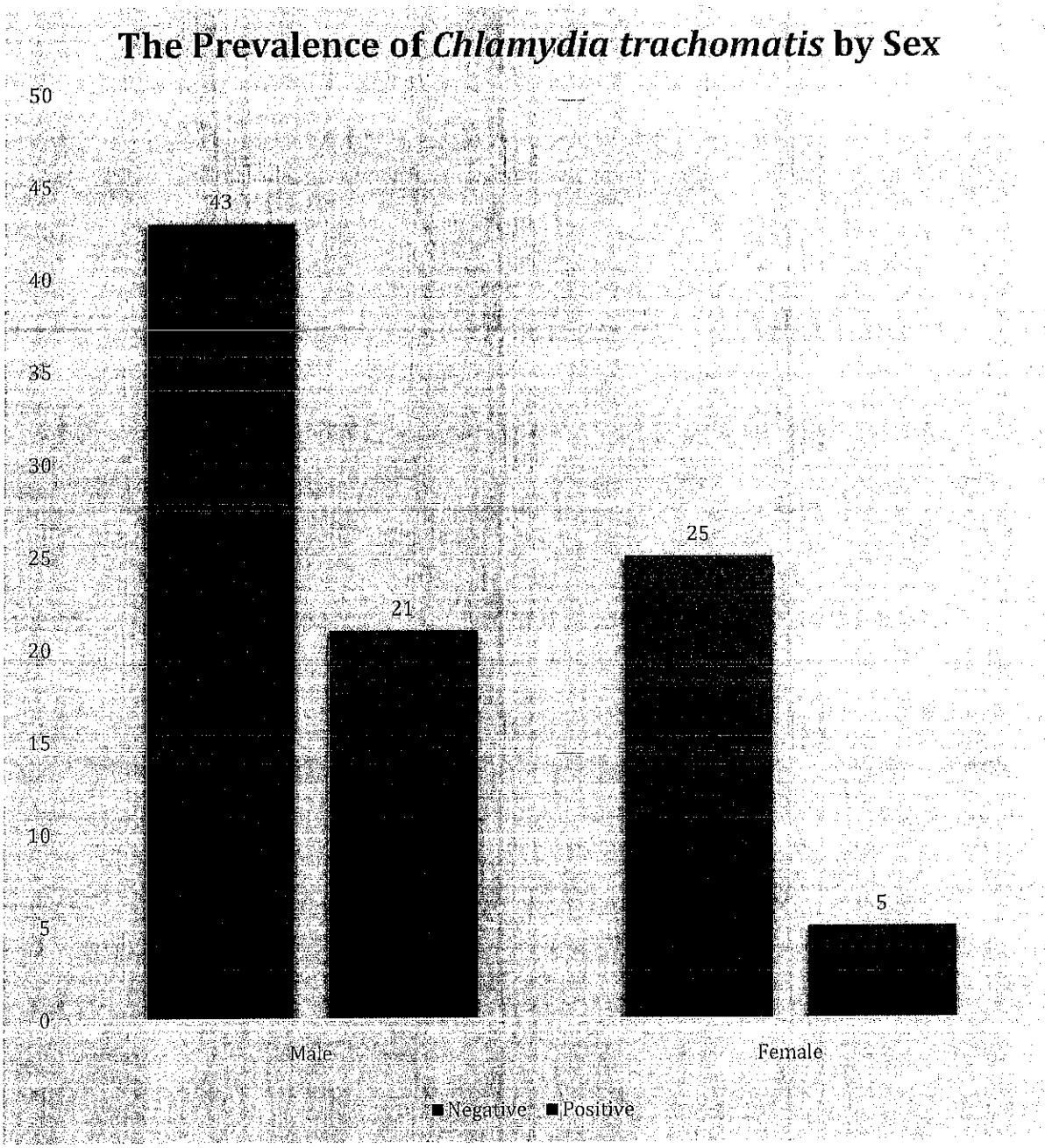


Figure 3: The prevalence of *Chlamydia trachomatis* by sex.

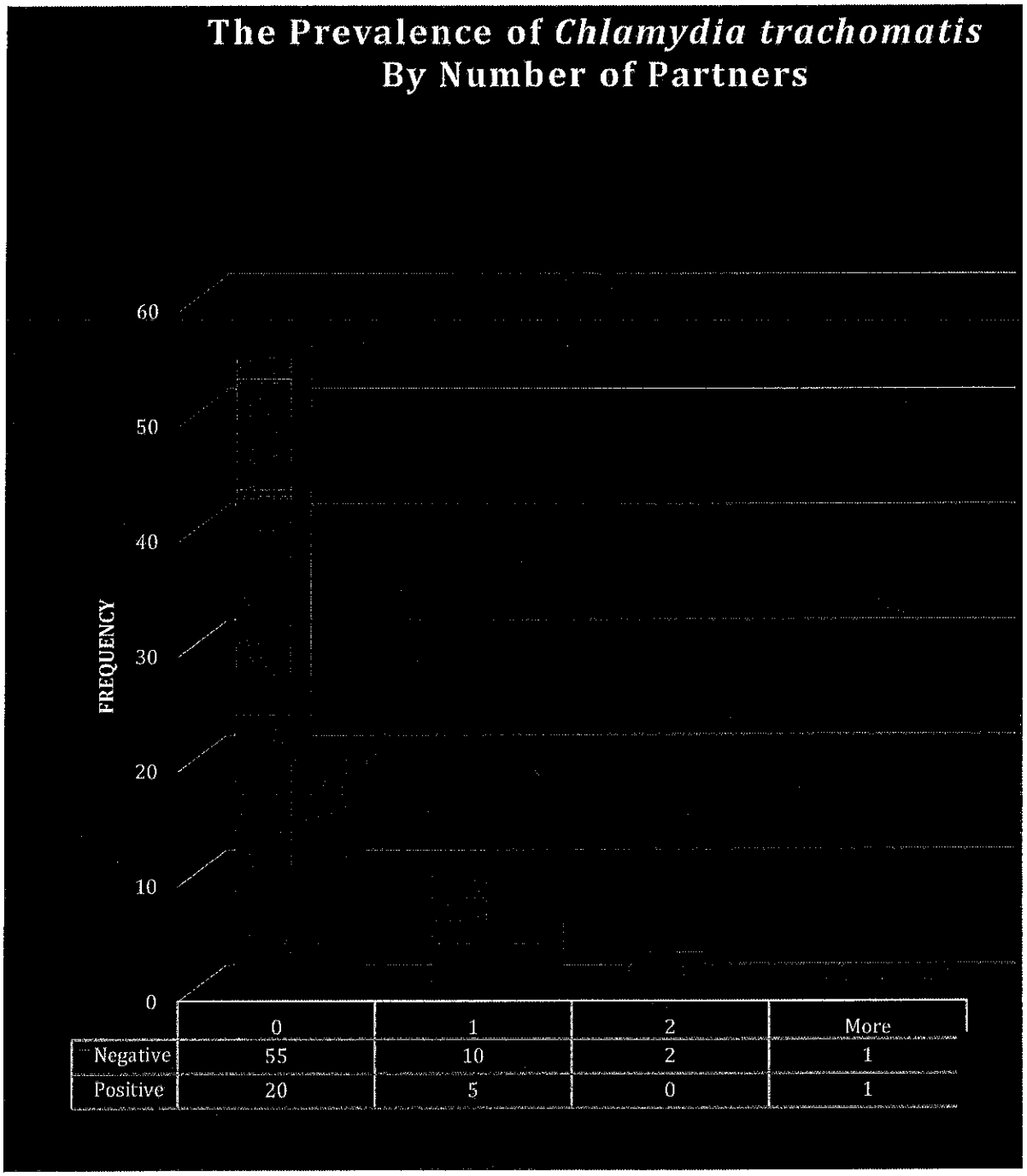


Figure 4: The Prevalence of *Chlamydia trachomatis* by number of partners

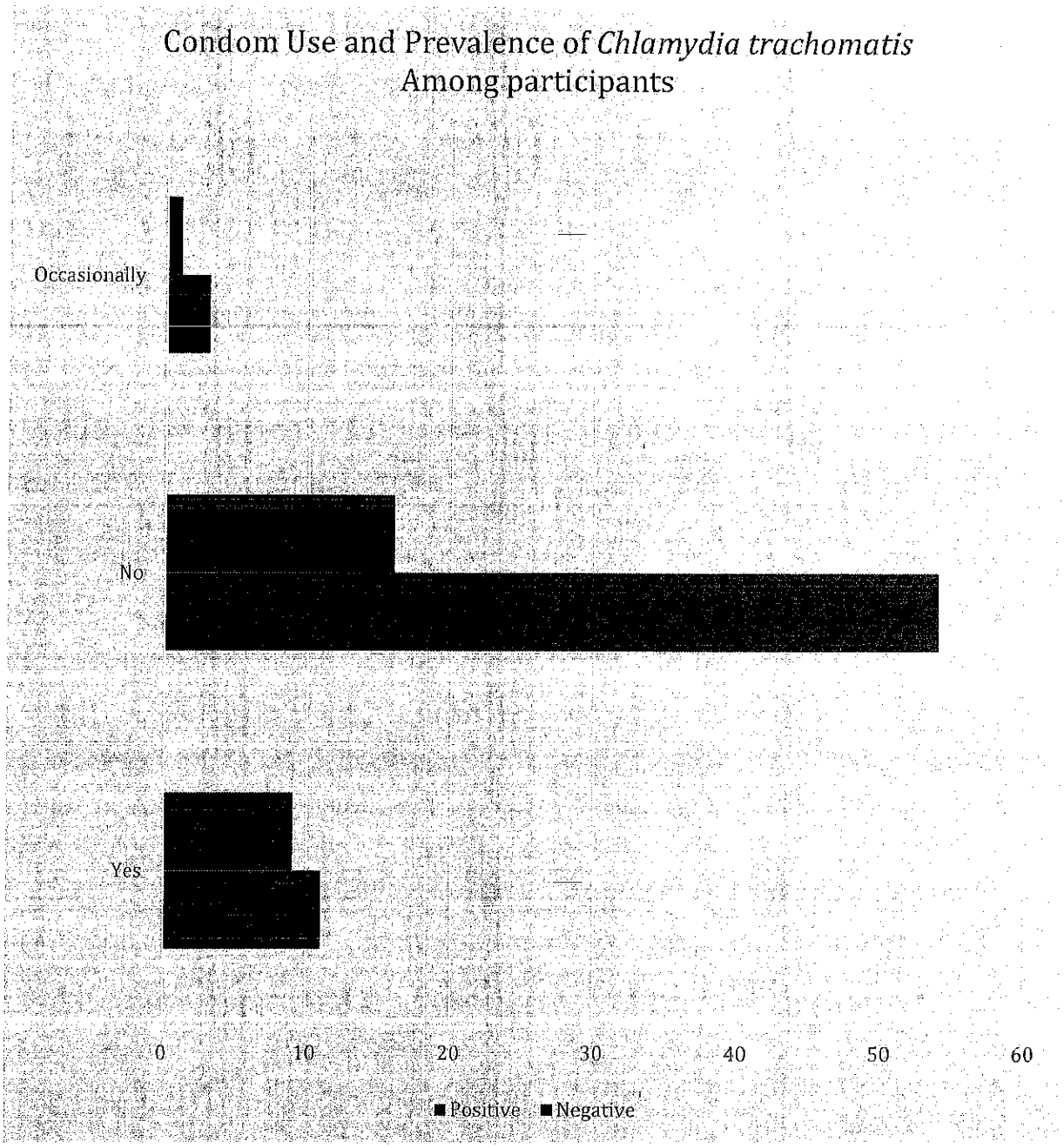


Figure 5: Condom use and prevalence of *Chlamydia trachomatis* among participants

Table 4.2: Distribution of Participants that Travels out of school and Chlamydial Infection.

Background characteristics	Chlamydial Infection		Total
	Negative	Positive	
Do you always travel out of school?			
Yes	25 (36.8)	9 (34.6)	**34 (36.2)**
No	43 (63.2)	17 (65.4)	**60 (63.8)**
Total	**68 (100.0)**	**26 (100.0)**	**94 (100.0)**

CHAPTER 5

5.1 DISCUSSION

Chlamydia trachomatis is an obligate intracellular gram negative bacterium which is the most prevalent cause of bacterial sexually transmitted infections (STI) worldwide (Hashemi *et al.*, 2007). WHO reported that, 91.98 million (27%) among 340 million new curable STDs (syphilis, gonorrhoea, and trichomoniasis) were due to Chlamydial infection. The infection is asymptomatic as commonly seen in both men and women, and Chlamydial infections can only be detected by laboratory diagnosis (Ikeme *et al.*, 2011). In many developed countries, screening programs for *Chlamydia trachomatis* have been set up to reduce transmission. The Centers for Disease Control and Prevention recommend annual screening of all sexually active women aged 25 or less (Khan, 2011), Chlamydial genital infection is the most frequently reported infectious disease, and the prevalence is highest in persons aged <25 years.

This study consisted of 94 students of Federal University Oye-Ekiti, Ekiti state, Nigeria. This study revealed that 26 (27.7%) out of 94 subjects were positive for *C. trachomatis* which is similar to the result of Ige *et al.*, 2018 (26% prevalence) in Kaduna State, Ikeme *et al.*, 2011 (29.4% prevalence in residents of Enugu, Nigeria), 33.33% prevalence which was reported in residents of Owerri and Nsukka South Eastern Nigeria.

However, the findings in this study showed higher Chlamydia IgM prevalence compared with Adesiji *et al.*, (2016) who reported prevalence of 0.7%, in Southern part of Nigeria (Osun state, Edo state and Oyo state), Ajani *et al.*, (2017) (7.30% among infertile women asymptomatic for genital infection) in Oyo state, Nwankwo and Sadiq, 2014 (9.6% prevalence) in Kano, 11% among Female undergraduates of the University of Port Harcourt (Wariso *et al.*, 2012).

The result of the this study is however lower to the 38.6% prevalence in infertile women in Calabar (Odusolu *et al.*, 2016), 75.0% among infertile women in Garki Hospital in Abuja (Ogbu *et al.*, 2017) and 91.2% reported in symptomatic patients attending clinics in South West Nigeria (Okoror *et al.*, 2014).

This is however in contrast to the observation from some countries such as United States of America where approximately 4 million cases of Chlamydial infection are reported annually with an overall prevalence of 5% and in Ethiopia 5.9% (Nwankwo and Sadiq, 2014) 58.33% was reported among pregnant women in India (Pipal *et al.*, 2017). These observed differences in prevalence rates could be due to study population and laboratory methods of identification.

The prevalence rate of Chlamydia (80.8%) in male samples was higher than that of females (19.2%), of all 25 females tested 5(20%) were positive for Chlamydia compared to male where 21(48.8) out of 43 were positive, this sex specific prevalence might be due to multiple sex partners of the males.

The highest rate of infection was recorded in subjects between the age group 15 – 19 years with a prevalence rate of 34 (50.0%) which is close to prevalence of 50% observed in the 17 – 24 years age group by Nwanguma *et al.*, (2010), in Enugu and Imo state and higher than the 33.3% in age group 16-18 reported by Wariso *et al.*, (2012) in Port Harcourt and (40.8%) among age group 15-19 years, reported by Ogbu *et al.*, (2017) while age group 20 – 24 years have a prevalence of 12 (46.1%). The age groups (15-24) have little or no information of safe sex and have high sexual activity; this may be responsible for high prevalence observed.

The least rate of infection was recorded in subjects in the age group 25 years and above with a prevalence rate of 5.9% which is lower compared to 17.1% prevalence reported in age group

prevalence 24-29 yrs in Kano (Nwankwo and Sadiq, 2014) and 90% reported in age group 25-45 in infertile Women in Calabar (Odusolu *et al.*, 2016).

In this study, high rate of *Chlamydia trachomatis* among subjects may be due to the fact that majority (75) of 94 screened had more than one sexual partner. Also, majority (94.7%) of the subjects were within the range of 15–19 and 20–24 years of age. This finding is similar to that reported by Nwankwo *et al.*, (2014) in Kano State. These age groups are of high sexual activity and this may be responsible for high prevalence observed in this study.

5.2 CONCLUSION AND RECOMMENDATIONS

Chlamydia trachomatis IgM antibody which is a marker of recent Chlamydial infection was detected among students of FUOYE. The overall prevalence rate was approximately 27.7%. The study showed the evidence of acute Chlamydial infection among the students, albeit a relatively high prevalence rate.

As a recommendation, Chlamydial infection among the students' needs to be studied in further details incorporating a larger population size; infected students should be subjected to treatment, public awareness should be done so that each student will know their status and will know how to treat/prevent themselves.

This study is based on IgM which shows recent Chlamydial infection, treatment is recommended for the infected people so as to minimize the transmission of *C. trachomatis* in the population.

In addition, use of condom and not having more than a sexual partner should be encouraged among students.

REFERENCES

Adams, E. J., LaMontagne, D. S., Johnston, A. R., Pimenta, J. M., Fenton, K. A., Edmunds, W. J. (2004). Modelling the healthcare costs of an opportunistic chlamydia screening programme. *Sex Transm Infect, 80,* 363–370. https://doi.org/10.1136/sti.2004.009654

Adesiji, Y. O., Iyere, S. I., and Ogah, I. J. (2015). Low Prevalence Of *Chlamydia trachomatis* Infection In Women From Southern Nigeria. *Nitte University Journal of Health Science, 5*(1), 4–8.

Adesiji, Y. O., and Ogah, J. (2016). Low Prevalence Of *Chlamydia trachomatis* Infection In Women From Southern Nigeria. *Nitte University Journal of Health Science,* (April), 1–6.

Ajani, T. A., Fayemiwo, S. A., Oluwasola, T. A. O., Chinenye, G., Ajani, M. A., and Bakare, R. A. (2017). Prevalence of Asymptomatic Genital *Chlamydia trachomatis* Infection Among Infertile Women. *Indian Journal of Medical Research and Pharmaceutical Sciences, 4,* 13–24. https://doi.org/10.5281/zenodo.893890

Aladeniyi, O. B., Bodunwa, O. K., and Sonde, M. (2017). Statistical Analysis of Reported Cases of Sexually Transmitted Diseases. *African bioscience 7*(3), 186–191.

Anttila, T. Pekka, S., Pentti, K., Aini, B. B. (2001). Serotypes of *Chlamydia trachomatis*. In *Chlamydia Trachomatis* and Risk of Cervical Carcinoma *American Medical Association* 285, pp. 47–51.

Buvé, A. Weiss, M., Laga, E., VanDyck, R., Musonda, L., Zekeng, M., Kahindo, S., Anagonou, L., Morison, N., Robinson, R. (2001). The epidemiology of gonorrhoea , Chlamydial infection and syphilis in four African cities. *Health Technology Assessment, 2,* 2000–2002.

Centers for Disease Control and Prevention (2016). *Recommendations for the prevention and management of Chlamydia trachomatis infection.* Retrieved from *https://www.cdc.gov/std /chlamydia/stdfact-chlamydia.htm*

Elwell, C. A., Jiang, S., Kim, J. H., Lee, A., Wittmann, T., Melancon, P., and Engel, J. N. (2011). *Chlamydia trachomatis* Co-opts GBF1 and CERT to Acquire Host Sphingomyelin for Distinct Roles during Intracellular Development. PLoS Pathog 7(9).

Elwell, C., Mirrashidi, K., and Engel, J. (2016). Chlamydia cell biology and pathogenesis. *Nat Rev Microbiol, 14*(6), 385–400. https://doi.org/10.1038/nrmicro.2016.30.

Emele, F. E., Enweani, I. B., Agbakoba, N. R., and Manafa, P. O. (2015). Relationship between Chlamydia seropositivity and presence of symptoms of Sexually Transmitted Infections among students of Nnamdi Azikiwe University , Awka. *Journal of Biomedical Investigation 2008; 6* (2): 29–31. https://doi.org/10.4314/jbi.v6i2.65834

Esumeh, F. I., Agbonlahor, D. E., and Okoror, L. E. (2009). Chlamydia activity in North East zone of Nigeria, *African Health Sciences, 14,* 10–16.

Ige, O. T, Ige, S. O., Olayinka, A. T. (2018). Prevalence of *Chlamydia Trachomatis* Infection among Women of Reproductive Age Group in a Tertiary Hospital in Northern. *8 Annals of*

Tropical Pathology, *9*, 17–21. https://doi.org/10.4103/atp.atp

Ikeme, A. C., Ezegwui, H. U., Ikeako, L. C., Agbata, I., and Agbata, E. (2011). Seroprevalence of *Chlamydia trachomatis* in Enugu , Nigeria. *Nigerian Journal of Clinical Practice*, *14*(2), 176–180. https://doi.org/DOI: 10.4103/1119-3077

Khan, E. R. (2011). Prevalence of genital *Chlamydia trachomatis* infection by ICT and PCR in endocervical swab samples., 1–101.

Lanjouw, E., Ouburg, S., Vries, H. J. De, Stary, A., and Radcliffe, K. (2015). 2015 European guideline on the management of *Chlamydia trachomatis* infections. *International Journal of STD and AIDS*, *0*(0), 1–16. https://doi.org/10.1177/0956462415618837

Lee, V., Tobin, J. M., and Foley, E. (2006). Relationship of cervical ectopy to chlamydia infection in young women. *J. Fam Plann Reprod Health Care*, *6*(2), 104–107.

Lewis, D., Newton, P., Danielle, C. Rebecca, J., and Hammad, A. (2012). The prevalence of *Chlamydia trachomatis* infection in Australia : a systematic review and meta-analysis. *BMC Infectious Diseases*, *12*, 113.

Mawak, J. D., Dashe, N., Agabi, Y. A., Panshak, B. W. (2011). Prevalence of Genital *Chlamydia trachomatis* Infection among Gynaecologic Clinic Attendees in Jos, Nigeria. *E-Medical Journal*, *12*(2), 113.

Mehta, S., Rothman, R., Kelen, G., Quinn, T., and Zenilman, J. (2001). Unsuspected Gonorrhea and Chlamydia in Patients of an Urban Adult Emergency Department : A Critical Population for STD Control Intervention. *Sexually Transmitted Diseases. 28*(1)33-39

Mishori, R., Mcclaskey, E. L., and Winklerprins, V. J. (2012). *Chlamydia trachomatis* Infections: Screening, Diagnosis, and Management, *American family physician* 1127–1132.

Ngandjio, A., Clerc, M., Fonkoua, M. C., Bianchi, A., Thonnon, J., and Be, C. (2019). Communication Restriction endonuclease patterns of the ompl gene of reference *Chlamydia trachomatis* strains and characterization of isolates from Cameroonian students, *Journal of Medical Microbiology*, (2004), 47–50.

Nwanguma, B., Kalu, I., and Ezeanyika, L. (2010). Seroprevalence of anti-*Chlamydia trachomatis* IgA antibody in a Nigerian population : diagnostic significance and implications for the heterosexual transmission of HIV . *The Internet Journal of Infectious Diseases*, (January 2016), 1–6.

Nwankwo, E. O., and Sadiq, M. N. (2014). Prevalence of *Chlamydia trachomatis* infection among patients attending infertility and sexually transmitted diseases clinic (STD) in Kano , North Western Nigeria. *African Health Sciences*, *14*(3), 672–678.

Odusolu, P. O., Edet, E. E., Emechebe, C. I., Agan, T. U., Okpe, A. E., and Etuk, S. J. (2016). Prevalence of *Chlamydia trachomatis* Immunoglobulin G Antibody in Infertile Women in Calabar. *African Journal of Medical and Health Sciences*, *16*, 74–79.

Ogbu, G. I., Anzaku, S. A., and Aimakhu, C. (2017). Burden of *Chlamydia trachomatis* infection amongst infertile women compared with pregnant controls in North-central Nigeria. *International Journal of Research in Medical Sciences*, *5*(9), 3819–3826.

https://doi.org/http://dx.doi.org/10.18203/2320-6012.ijrms20173954

Okoror, E. L., Cyril, O., and Tolulope, E. (2014). Prevalence and risk of *Chlamydia trachomatis* in symptomatic patients attending clinics in South. *Archives of ClinicAl Microbiology*, *5*, 1–11. https://doi.org/10.3823/285

Pipal, V. R., Pipal, R., Pipal, D., Yadav, S., Kamal, M., and Singariya, G. (2017). Seroprevalence of *Chlamydia trachomatis* infection among pregnant women and their outcome Original Research Article Seroprevalence of *Chlamydia trachomatis* infection among pregnant women and their outcome. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, *6*(3), 1016–1020. https://doi.org/10.18203/2320-1770.ijrcog20170576

Postma, M. J., Land, J. A., Bergen, J. E. A. M. Van, and Morre, S. A. (2010). Epidemiology of *Chlamydia trachomatis* infection in women and the cost- effectiveness of screening. *Human Reproduction Update*, *16*(2), 189–204. https://doi.org/10.1093/humupd/dmp035

Price, M. J., Soldan, K., Welton, N. J., Macleod, J., Simms, I., Deangelis, D., and Horner, P. J. (2016). Health technology assessment. *Health Technology Assessment*, *20*(22). https://doi.org/10.3310/hta20220

Wariso, K. T., Odigie, J., Eyaru, S., Harcourt, P., and Harcourt, P. (2012). Prevalence of *Chlamydia trachomatis* Infection among Female Undergraduates of the University of Port Harcourt Using Strand Displacement and Amplification [SDA] Technique. *The Nigerian Health Journal*, *12*(2), 35–38.

Witkin, S. S., Minis, E., Athanasiou, A., Leizer, J., and Linhares, I. M. (2017). Chlamydia trachomatis:the persistent pathogen. *Clin Vaccine Immunol.*, *24*(10), 1–9.

APPENDICES

Materials used:

- ✓ Microwell strips: *Chlamydia trachomatis* antigen coated wells.
- ✓ Absorbent solution.
- ✓ Calibrator: Factor value (f) stated on label.
- ✓ Negative Control
- ✓ Positive Control
- ✓ Washing Concentrate
- ✓ Enzyme Conjugate
- ✓ TMB Chromogenic Substrate
- ✓ Stop Solution
- ✓ Hand gloves
- ✓ Universal bottles

QUESTIONNAIRE

1 Age.....

2 Sex.....

3 Location in oye-ekiti

4 Number of sex partner?

a. 0 ○

b. 1 ○

c. 2 ○

d. More ○

5 Do you use condom?

a. Yes ○

b. No ○

c. Occasionally ○

6 Do you travel out of school?

a. Yes ○

b. No ○