SEROPREVALENCE AND PCR DETECTION OF *CHLAMYDIA TRACHOMATIS* AMONG PREGNANT WOMEN AND GYNAECOLOGIC PATIENTS ATTENDING SOME TERTIARY HOSPITALS IN NORTH CENTRAL, NIGERIA

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Declaration

I declare that the work in this thesis entitled **'SEROPREVALENCE AND PCR DETECTION OF *CHLAMYDIA TRACHOMATIS* AMONG PREGNANT WOMEN AND GYNAECOLOGIC PATIENTS ATTENDING SOME TERTIARY HOSPITALS IN NORTH CENTRAL, NIGERIA'** has been performed by me in the Department of Microbology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or other Institution.

_____	_____	_____
Name of student	Signature	Date

Certification

This thesis entitled, '**SEROPREVALENCE AND PCR DETECTION OF *CHLAMYDIA TRACHOMATIS* AMONG PREGNANT WOMEN AND GYNAECOLOGIC PATIENTS ATTENDING SOME TERTIARY HOSPITALS IN NORTH CENTRAL, NIGERIA',** by Hassan Isa Doko MUHAMMAD meets the regulations governing the award of the degree of Doctor of Philosophy in Microbiology of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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Dedication

To my beloved dad, Alhaji Muhammad Isa Doko, my beloved late wife, Fatima Muhammad and my beloved brother, Dr Abubakar Muhammad Isa, all of whom I lost in the course of this study. May Allah continue to have mercy on them.

Acknowledgement

All praises are due to Allah Lord of the Worlds. May peace and blessings of Allah be upon the Noble Prophet, members of his house hold and his companions.

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Abstract

This research was undertaken to detect the presence of Chlamydia in Pregnant women and Gynaecologic patients in some Tertiary Health Care Centers in the North-Central Nigeria. Six hundred Blood and endocervical swab samples were collected and analysed by ELISA and PCR Techniques respectively. The six hundred blood blood samples were also screened for HIV infection. A structured questionnaire was used to obtain data on socio-demographic and risk factors of Chlamydia from the study participants. A sero-prevalence of 59.0% was recorded for the Hospitals sampled. The sero-prevalence was higher among the gynaecologic patients (62.0%) than the pregnant women (57.5%). The difference was statistically significant (P = 0.0001). Of the four Health Care Centers chosen for the study, the National Hospital, Abuja had the highest prevalence (84.7%), while the General Hospital, Minna, Niger State had the least (28.7%). The difference was also statistically significant (P<0.0001). The prevalence rate of HIV among the participants in the Study Centers was found to be 17.2%. The National Hospital, Abuja had the highest prevalence rate of 24.6%, followed by the Federal Medical Centre, Makurdi, 16.7%, then Federal Medical Centre Lokoja, 12.0% and finally, General Hospital, Minna, 4.7%. Chlamydia was found to be associated with HIV (p<0.0001). Teenage (P<0.0001, OR= 12.35 CI:3.48-43.73), Secondary School Educational level (P = <0.0001, OR=3.69 CI: 1.57-8.98), unskilled occupation (P= <0.0001, OR=4.22 CI: 2.19-8.12), single marital status (P = 0.0002, OR=4.96 CI: 1.98-12.43) and more than one life time partners (P = 0.02, OR=7.35 CI: 3.55-15.21) were the risk factors associated with chlamydial infection. Chlamydia was found to present asymptomatically more than with symptoms (P= <0.0001). Chlamydia was found to be associated with HIV in the study area and may have resulted in the zone emerging with the highest HIV prevalence in the Country. The chlamydial complications considered in this study (PID, ectopic pregnancy and infertility), were not found to be statistically associated

with Chlamydia. Future studies are required to investigate the genotypes of chlamydia in circulation in the study population and to investigate the role of *Chalmydia trachomatis* genotypes in disease manifestations.

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List of Abbreviations

CDC: Centers for disease control and prevention

DFA: Direct fluorescent antibody

FVU: First void urine

EB: Elementary Body

ELISA: Enzyme linked immunosorbent assay

LCR: Ligase chain reaction

LGV: Lymphogranuloma venerum

LPS: Lipopolysaccharide

MIC: Minimum inhibitory concentration

MIF: Microimmunofluorescence

MMWR: Morbidity mortality weekly report

MOMP: Major outer membrane protein of *Chlamydia trachomatis*

NAAT: Nucleic Acid Amplification Test

NvCT: New variant *Chlamydia trachomatis*

OMP1: outer membrane protein 1 gene

PCR: polymerase chain reaction

PG: Peptidoglycan

PID: Pelvic inflammatory disease

PLT: Psittacosis lymphogranuloma venerum trachoma group

PROM: Premature rupture of membrane

PZ: Plasticity zone

RB: Reticulate body

RFLP: Restriction fragment length polymorphism

SDA: Strand displacement amplification

STD: Sexually transmitted diseases

STI: sexually transmitted infections

TAE: Tris-acetate EDTA

TMA: Transcription mediated amplification

TRIC: Trachoma inclusion conjunctivitis

TTSS: Type three secretion system

VS: variable segments

CHAPTER ONE
1.0 INTRODUCTION

Chlamydial infection is the most common curable bacterial sexually transmitted disease (CDC, 2006; WHO, 2011). It is caused by *Chlamydia trachomatis*, a coccoid bacillus closely related to Gram negative bacteria (Cheesbrough, 2000). In recent years, bacterial sexually transmitted diseases have been over shadowed by the growing epidemic of viral sexually transmitted diseases, in particular, HIV. In addition, the declining prevalence of some bacterial infections in the last twenty years might have suggested that these agents were diseases of the past, of diminishing importance and of limited interest outside specialist care. This has never been the case with chlamydial genital infections, especially, and is timely to review their importance, particularly in the light of recent developments (Moss, 2001). Chlamydia presents a major public health concern both in industrialized and developing countries and is of high economic importance (Gomes *et al*., 2007).

Chlamydia trachomatis is a coccoid bacillus, gram negative, non motile and obligatory intracellular organism living in man and animal cells because it requires host cell Adenosine triphosphate (ATP) for its life cycle (Posada *et al*., 1992; Presscott *et al*., 2005). It contains both DNA and RNA and has its own enzyme systems, which differentiate it from the viruses under which it was formerly classified (Cheesbrough, 2000). *Chlamydia trachomatis* belongs to the genus *Chlamydia* which includes organisms previously called the Psittacosis Lymphogranuloma venerum Trachoma group (PLT) or the TRIC (TRachoma Inclusion Conjunctivitis group) (Mackie & Mac Cartney, 1989).

Chlamydia trachomatis has 15 immunotypes (serotypes) viz; A-C which cause trachoma (chronic conjunctivitis endemic in Africa and Asia), D-K, which cause genital tract infection

and L1-L3 responsible for lymphogranuloma venerum (associated with genital ulcer disease in tropical countries) (Houry, 2006).

Chlamydia trachomatis serovars D-K cause curable sexually transmitted disease called Chlamydia (CDC, 2006). The disease which arises from the infection of the lower genital tract is one of the most prevalent sexually transmitted diseases in the world (Gerbase *et al*., 1998; Beagley and Timms, 2000). It is usually asymptomatic, in fact 50% of men and 80% of women are asymptomatic, and for this reason it is referred to as silent disease (Hopwood *et al*., 1990; Harry *et al*., 1994; Gaydos *et al*., 1998; CDC, 2006; Houry, 2006; Ward *et al*., 2007). In cases where symptoms are present they may last only a few days and may not be noticed or considered significant (Kidshealth, 2006). Clinical symptoms in women, where present include vaginal discharge, dysuria, easily induced endocervical bleeding, irregular menstruation or intermenstrual bleeding, dyspareunia, lower abdominal pain, genital itching, increased urination frequency and sore in the vagina (CDC, 2006; Al-Mutairi *et al*., 2007).

The main burden of the disease falls upon women (Thompson *et al*., 1983; Keyhani *et al*., 2006). In females, *Chlamydia trachomatis* causes cervicitis, urethritis, ectopic pregnancy, pelvic inflammatory disease (PID), tubal factor infertility and chronic pelvic pain (Morre *et al*., 2000). Studies have also associated chlamydial infection with cervical and ovarian cancer as well as increase in HIV infectivity (Luostarinen *et al*., 2004).

Antibiotics play a major role in treating chlamydial infection. Azithromycin and doxycycline are considered as first line drugs (Workowski and Berman, 2010). Though the efficacy of these drugs is high, many researchers have reported the problem of recurrent infections and treatment failures (Wang *et al*., 2005). Recent studies have also indicated the emergence of antibiotic resistance in chlamydia which is feared to create severe problems in the treatment of the disease (Bhengraj *et al*., 2012).

1.1 Statement of Problem

The incidence of chlamydial infections in women has increased dramatically from 79 to 467 per 100,000 between 1987 and 2003 (Sexually Transmitted Disease Surveillance, 2003). According to the World Health Organization (WHO, 2011), 101 million chlamydial infections are detected annually worldwide. In the U.S. the Centres for Disease Control and Prevention (CDC) estimates that 2.8 million people are infected each year (CDC, 2006). In some parts of the third-world countries, more than 90 per cent of the population is infected (Gomes *et al*., 2007).

Chlamydia is one of the non – ulcerative sexually transmitted infections which elicit localised inflammations and immune responses characterised by the infiltration and accumulation of immune cells expressing CD4 surface proteins essential for the binding of HIV prior to entry, thus also facilitating the entry of HIV (Altes *et al*., 2002; Joyee *et al*., 2005; Wodarz *et al*., 2007). In a study conducted in the South- Eastern part of Nigeria by Nwaguma *et al*. (2009), it was found that the prevalence of *C. trachomatis* infection observed in the HIV – seropositive subjects (50%) was much higher than the prevalence in the HIV-seronegative subjects (17.6%).

Chlamydia, if left untreated can persist for at least 15 months (Mc Cormack *et al*., 1979) and can have potentially serious lifetime consequences which include pelvic inflammatory disease (PID) (Stamm *et al*., 1984, Land *et al*., 2010). In USA, approximately 20-30 per cent of PID cases have been attributed to *C. trachomatis* (Soper, 2010). The incidence rates of PID in Nigeria vary between 0.28 and 4.4% of deliveries (Iloabachi, 1990). Sequelae of PID include ectopic pregnancy and tubal infertility (Buchan *et al*., 1993). The risk of developing sequelae is dependent on the number of PID episodes (Westrom, 1994).

In terms of economic cost, both PID and its sequelae have been projected to exceed $10 billion by the year 2000 (Washington *et al*. 1993). Infertility related to genital *Chlamydia trachomatis* infection is of public health importance in sub-Saharan Africa and the treatment of infection and associated sequelae absorb a sizeable part of health care resources in developing countries (Moss, 2001).

1.2 Justification

Generally, sexually transmitted infections (among which is chlamydia) are poorly recognised and inadequately treated in Nigeria despite the fact that they constitute a major risk factor for sexual transmission of HIV infection (Kehinde *et al*., 2005). Data on the prevalence of Chlamydial infection in patients with tubal infertility are scarce especially in the developing countries due to unavailability and high cost of facilities necessary for diagnosing the infection (Tukur *et al*., 2006). In the same vein, there is lack of sufficient surveillance data on the prevalence of chlamydial infection in ectopic pregnancy patients which has made it difficult to assess the contribution of the infection to the PID and ectopic pregnancy epidemics (Moss, 2001).

In the U.S. studies have shown that intervention based on the screening for chlamydial infection has reduced the incidence of PID and ectopic pregnancy by more than 50% and 20% respectively (Hillis *et al*., 1995). Swedish data also indicated that the screening for genital chlamydia rapidly reduces the incidence of ectopic pregnancy among 20-24 years olds (Egger *et al*, 1998).

A reliable epidemiological data is needed to determine the prevalence rate of the disease in the populations which will help in devising an effective chlamydia control program.

1.3 Research Questions

i. How prevalent is Chlamydia in the North Central Geopolitical zone of Nigeria?

ii. Does Chlamydia aid the spread of HIV/AIDS in the study area?

iii. What are the risk factors associated with Chlamydia in the study population?

iv. Has Chlamydia any link with PID, infertility and ectopic pregnancy?

1.4 Aim and Objectives

Aim

To detect the presence of Chlamydia in Pregnant women and Gynaecologic patients in the North-central geopolitical zone of Nigeria

Objectives

1. To determine the prevalence of Chlamydia among pregnant women and gynaecologic patients in the North- Central part of Nigeria
2. To check the rate of occurrence of Chlamydia – HIV co-infection
3. To detect *Chlamydia omp* gene in some ELISA positive samples using PCR
4. To determine the risk factors associated with chlamydial infections
5. To assess the association between chlamydial infection and its complications

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Chlamydia trachomatis*

2.1.1 Taxonomy and Classification

The chlamydia organisms belong to the family Chlamydiaceae in order Chlamydiales (Everett *et al.,* 1999). According to the Approved List of Bacterial Names, published in 1980, the family Chlamydiaceae contained one genus (Chlamydia) which contained just two species, *Chlamydia trachomatis* and *Chlamydia psittaci* (Skerman *et al.,* 1980). The genus *Chlamydia* was characterised by its unique developmental cycle and the two species were separated based on characteristics such as host range, accumulation of glycogen within the chlamydial inclusion and sensitivity to sulfadiazine (Beagley and Timms, 2000).

In 1990s, the application of DNA-based classification methods contributed to the recognition of two new species, an emerging human pathogen, *C. pneumoniae* (Grayston *et al*., 1990) and a pathogen of ruminants, *C. pecorum* (Fukushi and Hirai, 1992), both of which were previously sub-groupings within *C. psittaci*.

The expanded use of molecular methods, particularly gene sequencing of isolates, has revealed many more phylogenetic groupings within the Chlamydiaceae family. Everett *et al*. (1999), therefore, proposed the use of 16SrRNA and 23SrRNA gene sequence analysis to divide the Chlamydiaceae family into 2 genera (*Chlamydia* and *Chlamydophila*) and nine species. In this proposal, *C. pneumoniae, C. psittaci, and C. pecorum* were placed into the new genus; *Chlamydophila*. However, this taxonomy has not been generally accepted and was opposed by a large group of researchers in the field, who argued that the new genus designation ignore the unique, highly conserved biology shared by these organisms when they were in a single genus (Schachter *et al*., 2001). This debate resulted in simultaneous use

of the former and the new nomenclature in the literature (Essig, 2007). *C. trachomatis* and *C. pneumoniae* are the two chlamydial species pathogenic to humans, whereas the other species occur mainly in animals and birds (Schachter *et al*., 2001).

According to the recent review of the Bergey's Manual by Garrity *et al.* (2004) and Krieg *et al.* (2010), the family Chlamydiaceae contained two genera *Chlamydia* and *Chlamydophila.* The genus *Chlamydia* has six species namely; *Chlamydia trachomatis C. muradirum, C. pecorum, C. pneumoniae, C. suis* and *Chlamydia psittaci*.

C. trachomatis was initially classified into 15 different serovars. These serovars include A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2, and L3. The proposal of four new subtypes of earlier recognized serovars has been made, i.e. Da, Ga, Ia, and L2a. Based on biological characteristics, the different serovars have been grouped into biovars: lymphogranuloma venereum (LGV, four serovars) and trachoma (including all remaining ones). There is no strong evidence for a virulent advantage of any of the serovars (Mardh, 2005) and the relationship between certain *C. trachomatis* serovars and clinical manifestations has been controversial (Takahashi *et al*., 2007).

Serovars of *C. trachomatis* have been identified by micro-immuno-fluorescence (MIF) tests using panels of polyclonal and monoclonal antibodies. MIF test depends on type-specific epitopes residing on the major outer membrane protein (MOMP). The subdivision by MIF fitted well with the clinical spectrum of infections caused by the various serovars and with subdivision obtained by genetic sequence (Cevenini *et al*., 2002; Caldwell *et al*., 2003; Mardh, 2005).

Recently, molecular techniques have come into use to identify serovars and partially replaced the laborious and less sensitive serotyping techniques. Many studies have shown the feasibility of deducing the serotypes of *C. trachomatis* clinical isolates using polymerase

chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) or sequencing of the amplified omp1 gene, which encodes the MOMP. Genotyping methods exploiting genome variations, e.g. multilocus sequence and variable-number tandem repeat analysis, have been used recently to discriminate among strains. Typing of *C. trachomatis* is used to identify high-risk groups and track sexual networks (Gao *et al*., 2007).

2.1.2 Reproductive Cycle

Key for understanding the pathophysiology of chlamydial disease is an appreciation of the chlamydial developmental cycle (Fredlund *et al*., 2004)**.** Members of the genus *Chlamydia* are obligate intracellular energy parasites of eukaryotic cells, which supply them with ATP, GTP and UTP. Chlamydiae do encode functional glucose-catabolizing enzymes, which can be used for generation of ATP (Hammerschlag, 2002). In addition, proteomic analysis of *C. trachomatis* showed that all of the glycolytic enzymes were readily detectable. However, chlamydiae with mammalian hosts appear to have lost the genes for the F1 ATPase (ATPase that uses energy released by transport of protons across the bacterial cell membrane to synthesise ATP) during evolution. It is therefore likely that these ATPase components are not involved in energy generation (Skipp *et al*., 2005).

Although chlamydiae are classified as bacteria, they have a unique biphasic developmental cycle (Figure 1), involving an extracellular form, the elementary body (EB), which is infectious but metabolically inactive and an intracellular form, the reticulate body (RB) which is non infectious but metabolically active (Mardh, 2005). The EBs multiply by binary fission in a cytoplasmic vacuole (termed the inclusion) surrounded by a membrane with similarities to the host cell's cytoplasmic membrane (Cevenini *et al*., 2002).

The EB adheres to the host cell with the aid of heparin-sulphate-like glucosaminoglycan molecules which are required for chlamydiae to enter a host cell. A number of substances are

known to enhance contact between EBs and host cells, i.e., diethylaminoethyldextran and iodoxyuridine, which have been utilized in in-vitro cultures (Beagley and Timms, 2000; Mardh, 2005).

After eclipse phase of approximately 12 hours, the EB differentiates into the larger reticulate body (RB) which replicates inside the vesicle 200–500-fold by binary fission. The EB has a diameter of 200-300 nm, whilst that of the RB is 1000-1500 nm. During the synthesis of substances by the chlamydiae cells, the metabolism of the host cell is depressed through influence of the chlamydiae parasites. Eighteen to 24 hours after the EB has attached, the RBs undergo reorganization into EBs. Within another 6 hours, the EBs are released and the host cell dies. In vivo, the developmental cycle is longer than in in vitro cultures. Its exact time may differ by prevailing circumstances but may be up to a week (Beagley and Timms, 2000; Mardh, 2005).

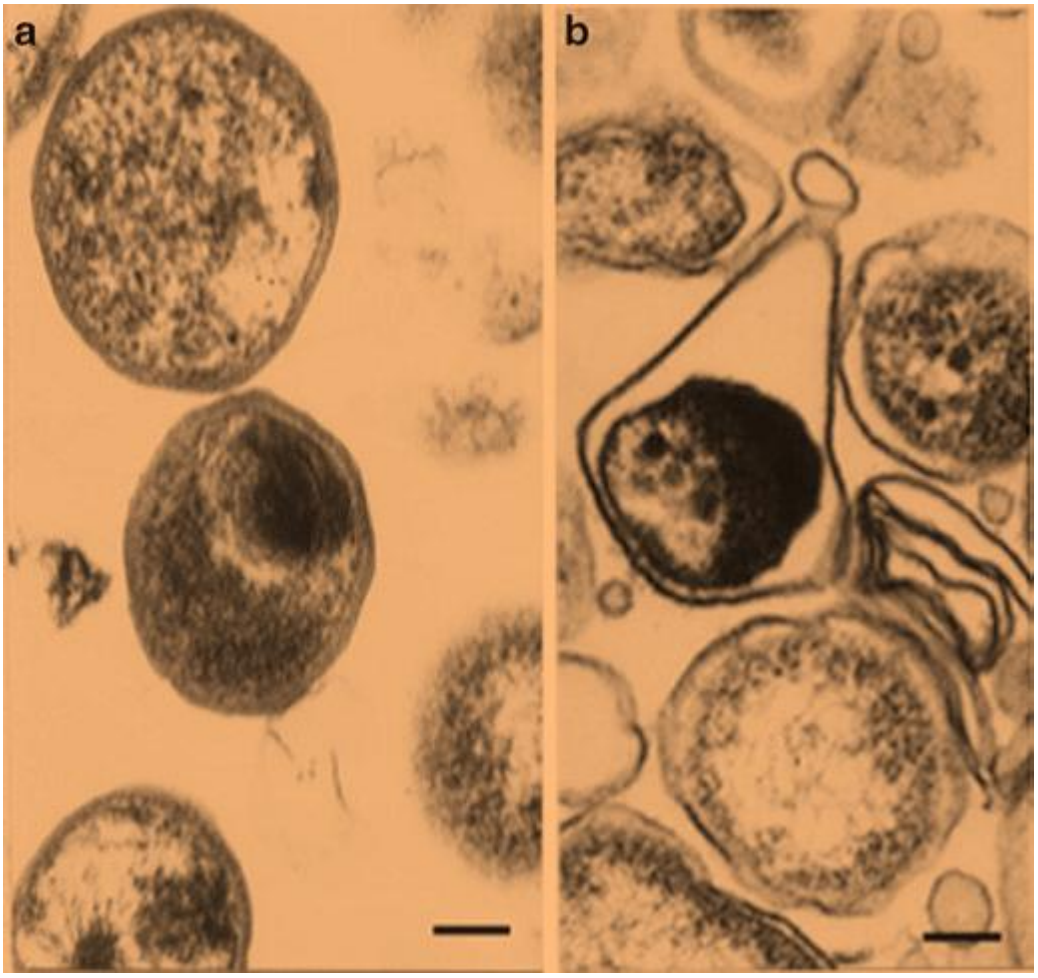


Figure 1: Elementary Body Form of Chlamydiae

The elementary body form of chlamydiae is round, except for *Chlamydia pneumoniae* which is pear-shaped. (a) *Chlamydia trachomatis* serovar B, strain TW-5, (b). *Chlamydia pneumoniae* strain TW-183. The reticulate body is round for all species. Bars = 0.1 mm. (Source: Grayston *et al*., 1989).

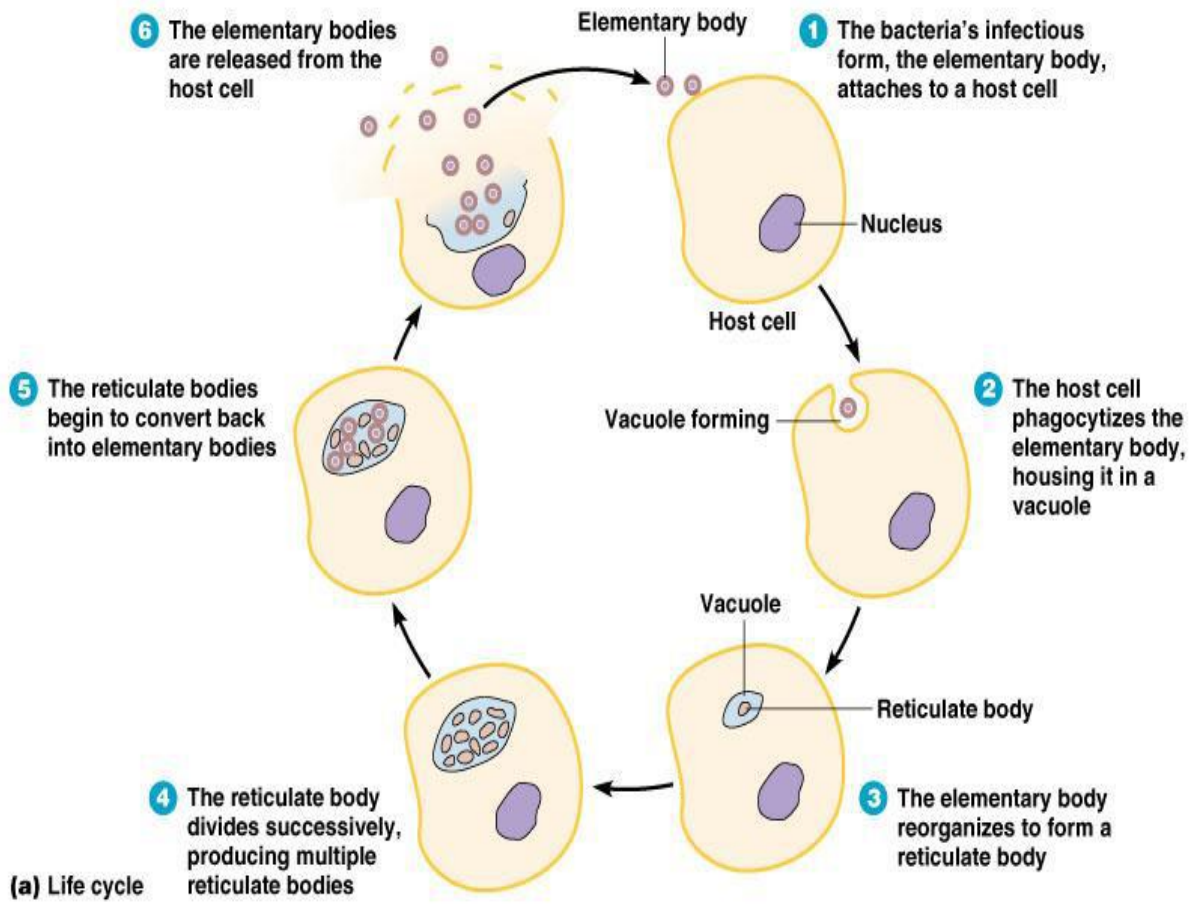


Figure 2: The Intracellular Developmental Cycle of Chlamydiae

(Abdel-Rahman and Belland, 2005).

At the end of the reproductive phase, the chlamydial inclusion may be so large that it displaces the nucleus of the host cell. This phenomenon may give the impression that the inclusion more or less surrounds the nucleus, like an overcoat. This once gave the organisms their name "chlamydia", which in Greek means "overcoat" (Mardh, 2005).

Deviations from the typical developmental cycle have been experimentally induced by a variety of stimuli, including IFN-γ, antibiotics, and nutrient deprivation**.** These stimuli, particularly IFN-γ can alter chlamydial growth and facilitate persistent or chronic infection (Fredlund *et al*., 2004).

There are many aspects of chlamydiae biology that indicate that these intracellular bacteria are usurping cellular processes. In addition to exploiting host cell functions for chlamydial attachment and uptake, including subversion of the cytoskeleton to facilitate intracellular redistribution of newly internalized EBs, the inclusion is nonfusogenic with endosomal and lysosomal compartments. It therefore avoids intracellular destruction and intercepts cholesterol- and sphingomyelin-containing vesicles originating from the Golgi body. The chlamydiae can modulate programmed cell death pathways (apoptosis) to promote their survival in infected host cells. Early, these intracellular pathogens inhibit apoptosis to ensure intracellular survival for the duration of the developmental cycle, and upon completion of the cycle, they induce cell death- may be through an apoptosis-like mechanism- to release chlamydial progeny and down-regulate inflammation. The cellular immune response is also modulated as major histocompatibility complex (MHC) expression is markedly down-regulated in chlamydiae-infected cells. Altogether, these indicate that chlamydiae occupy a unique niche within the cell **(**Byrne and Ojcius, 2004; Peters *et al*., 2007; Kleba and Stephens, 2008; Heuer *et al*., 2009; Sharma and Rudel, 2009).

The molecular mechanisms by which chlamydiae elicit various host responses to establish an environment favourable for their proliferation throughout the developmental cycle are mostly unknown. In theory, they may be initiated in two ways:

(i) by a cascade of signalization triggered by the interaction between the bacteria and host cell receptors, at the plasma membrane before entry, or at the inclusion membrane later in infection;

(ii) by chlamydial proteins secreted in the host cell during the infection.

While there is no evidence for the first mechanism, the presence of a type III secretion system (TTSS) in chlamydiae come in strong support for the second one (Subtil *et al*., 2005). Based on sequence homology, chlamydiae carry an almost-complete repertoire of genes representing a TTSS (Hefty and Stephens, 2007). In contrast to most bacterial pathogens, the TTSS genes occur in clusters throughout the genome, rather than being concentrated on pathogenicity islands. Proteins that are secreted by TTSS are called effector proteins (Jamison and Hackstadt, 2008). Type III secretion system activity may be triggered extracellularly when the EB comes into contact with the host cell membrane and intracellularly when it comes into contact with the inclusion membrane into which effector proteins are secreted and may become either embedded or alternatively translocated into the host cell cytoplasm (Hsia *et al*., 1997).

Chlamydial TTSS is believed to translocate virulence effector proteins into the cytosol of their host cells. More than 20 *C. trachomatis* and *C. pneumoniae* proteins were detected within the cytoplasmic compartment of infected cells (Kleba and Stephens, 2008). Translocated actin recruiting phosphoprotein (TARP), is an effector protein translocated by TTSS, and is involved in the recruitment of actin to the *C. trachomatis* inclusion. Chlamydial outer protein N (CopN), another protein translocated by TTSS, is involved in TTSS down regulation as RBs start differentiating into EBs. While a number of cytosolic proteins are

shared, others are unique to each species, suggesting that variation among cytosolic chlamydial proteins contributes to the differences in the pathogenesis of the chlamydial species. The spectrum of chlamydial proteins exported differed concomitant with the progress of the developmental cycle (Peters *et al*., 2007; Kleba and Stephens, 2008; Betts *et al*., 2009; Wang *et al*., 2009).

The chlamydial TTSS also exports a family of proteins, the inclusion proteins (Incs), which localize on the membrane of the inclusion. Vesicles of the endo- and exocytic pathways and proteins involved in vesicle trafficking are recruited to the inclusion to facilitate effective chlamydial infection in infected cells. Incs are potentially involved in mediating such vesicular trafficking processes. Host cell components are capable of interacting and modifying segments of Incs which are exposed to the cytosolic face of the inclusion. Further, Incs have been reported to generate humoral immunity in infected humans and animals and cellular immunity by eliciting MHC class I-restricted CD8+ T cell responses (Gupta *et al*., 2009).

A research has identified 22 Incs localized in the inclusion membrane (Li *et al*., 2008). The cytosolic portion of IncA interacts with the IncA on other inclusion membranes facilitating homotypic vesicle fusion that when an epithelial cell is infected with multiple *C. trachomatis* EBs, they are internalized by endocytosis into individual phagosomal vacuoles that eventually fuse to form a single inclusion (Rockey *et al*., 2002). Non-fusing variants forming multiple non-fusogenic inclusions in infected cells have been identified, with the number of independent inclusions per cell varying directly with the multiplicity of infection. IncA was not detected in the inclusion membrane in each tested non-fusogenic strain (Suchland *et al*., 2000). Nonfusing strains of *C. trachomatis* more often produce subclinical infections than do normal fusing strains and have lower median inclusion forming unit counts (Geisler *et al*.,

2001). It is likely that, other Incs are responsible for a wide variety of pathogen/host cell interactions (Rockey *et al*., 2002; Li *et al*., 2008).

2.1.3 Structural Characteristics of *Chlamydia* Organisms

Chlamydia trachomatis EBs and RBs have a Gram-negative envelope with inner and outer membranes, but without detectable peptidoglycan (PG) although genomic analysis has revealed that both *C. trachomatis* and *C. pneumoniae* encode for proteins that form a nearly complete pathway for synthesis of PG, including penicillin-binding proteins. This finding may be the basis for the so-called ″chlamydial peptidoglycan paradox" because researchers have known for decades that chlamydial development is sensitive to β-lactam antibiotics (Hammerschlag, 2002).

In bacteria, PG serves primarily to protect the integrity of the organism under conditions of low external osmolarity. Instead of PG, the osmotic stability of EBs is presumably, due to a high degree of disulfide cross-linking of cysteine-rich cell-envelope proteins, which serve as a functional equivalent to PG. By contrast, this cross-linking is loose in the osmotically fragile RBs (Mpiga and Ravaoarinoro, 2006). When chlamydiae-infected cells are treated with β-lactam antibiotics that target cell-wall synthesis, chlamydial cell division is arrested and RBs become large and aberrant, which suggests that PG has a predominant role in RB development. This morphology is in contrast to other bacteria that lyse when treated with cell wall inhibitors, unless the antibiotic treatment occurs in an isotonic medium. Hence, it is probable that the inclusion provides an isotonic niche for chlamydiae and that PG has a minimal role in maintaining the osmotic integrity of the bacteria (Hammerschlag, 2002; McCoy *et al*., 2003; Skipp *et al*., 2005; McCoy *et al*., 2006).

The use of microarray and proteomic analysis methods has revealed that PG synthesis genes are expressed during early stages of the chlamydial life cycle, when EBs convert to RBs and when RBs begin to expand in size and divide. At these times, the pH of the inclusion is such that enzymes for PG synthesis are optimally active. Later in the developmental cycle, the environment of the inclusion potentially changes, which may render PG synthesis enzymes less active (McCoy *et al*., 2003; McCoy *et al.*, 2006).

The outer cell membrane of chlamydial cell envelope is made up of upto 50% proteins and 30% lipids as well as fatty acids and carbohydrates (Mardh, 2005).

Chlamydiae have a lipopolysaccharide (LPS) surface structure which is common to all species of the genus *Chlamydia*. The LPS has 2 antigenic domains; one is shared with other Gram-negative bacteria while the other is specific for chlamydiae organisms. Lipopolysaccharide contains a genus specific epitope which is highly antigenic. The pro-inflammatory cytokine response to *C. trachomatis* at the invasion phase is suggested to be mediated by LPS. Chlamydial LPS possesses significant lower endotoxic activity than enterobacterial LPS which has been attributed to the unusual long-chain fatty acids content of its lipid A **(**Heine *et al*., 2003**).**

The outer membrane proteins (OMPs) have a single predominant protein which is the MOMP of 40 kilodalton (KDa), comprising about 60% of Omps. This protein maintains the structural rigidity of the outer membrane and facilitates porin formation, permitting diffusion of solutes through the intracellular reticulate body membrane. It is believed to play a role in pathogenesis and possibly adhesion. Along with the lipopolysaccharide, the major outer membrane protein (MOMP) makes up the surface of the elementary body cell. Disulphide bond interactions within and between MOMP molecules and other components form high molecular weight oligomers (Anonymous, 2015). The MOMP of *C. trachomatis* contains

serovar, subspecies and species specific epitopes that can be defined by monoclonal antibodies. Variations in these epitopes explain the absence of strain-specific immunity and multiple re-infections by different serovars or by the same mutated serovar. The MOMP of *C. pneumoniae* is more homogenous and less immunogenic than that of other chlamydiae. Other Omps, such as Omp2 and the small Omp3, are present in smaller amounts (Millman *et al*., 2001; Karinen, 2006).

Several heat shock proteins (Hsps) have been found in chlamydial cell walls. The genes encoding Hsp10, Hsp60 and Hsp70 have been cloned and sequenced. All three Hsps can be found in the outer membrane complex of both EBs and RBs. Especially chlamydial Hsp 60, but also Hsp 70 and Hsp 10 have been implicated as important agents in the immunopathology of chlamydial infection (Karinen, 2006). Multiple *C. trachomatis* re-infections evoke Hsp60 accumulation. As *C. trachomatis* Hsp60 displays 48% identity to human Hsp60, chlamydial Hsp60 could break human tolerance to its own Hsp60, inducing auto-immunity. Thus, by allowing chlamydial Hsp60 accumulation in the host, recurrence due to persistence and re-infections would be at the origin of harsh chlamydial disease (Mpiga and Ravaoarinoro, 2006).

2.1.4 Genome of *C. trachomatis*

The genome is small, 6.6 - 9.5 x 10^8dalton. It compares in size to rickettsial DNA. It is half the size of the *Neisseria* DNA and one-fifth the size of DNA in *Escherichia coli* (Darougar *et al*., 1981). The entire genome of *C. trachomatis*, which is approximately 1000 kilobasepair (kbp) length, has been sequenced. The omp1 gene coding for MOMP, has been studied (Mardh, 2005).

In addition to the chromosome, almost all tested strains of *C. trachomatis* contain a cryptic 7.5-kbp plasmid of unknown function. The study of Carlson *et al*. (2008) supported a primary role for the plasmid in in-vivo infectivity and suggested that virulence is controlled, at least in part, by the plasmid's ability to regulate the expression of chromosomal genes.

2.2 Chlamydial Infection

2.2.1 History

Descriptions of a "chlamydia – like" disease of human eyes resembling the disease now known as "Trachoma" (meaning 'rough eye') have been found in ancient Chinese and Egyptian manuscripts (Collier, 1990). In fact, the disease has been recognized since antiquity, having been described in the Ebers papyrus, the oldest known medical writing in the Western World (Schachter *et al*., 1978; Jawetz *et al*., 1991). In 1907, Halberstaedter and Von Prowazek, working in Java, described the transmission of trachoma from man to orang-utans by inoculating their eyes with conjuntival scrapings (Halberstaedter, *et al*., 1907). In Giemsa stained conjunctival scrapings, they found intra-cytoplasmic vacuoles (chlamydial inclusions) containing numerous minute particles (small chlamydial elementary bodies (EBs) and larger chlamydial reticulate bodies (RBs)), which they correctly inferred represented the causal agent of trachoma. The newly discovered organisms were called the Chlamydozoa (from the Greek Khlamus, a mantle/cloak) because of the blue staining matrix in which the particles were apparently embedded (Collier, 1990). This was however, not correct as the Chlamydiae are not "mantled prorozoans", but the Greek-derived stem remains as a tribute to these outstanding workers.

Similar inclusions were subsequently described in the conjuntival cells of babies with non-gonococcal ophthalmia neonatorum in the uterine cervix from some of their mothers and the

urethral epithelium from male patients with non-gonococcal urethritis (Lindner, 1911). Thus, trachoma, inclusion conjunctivitis of the new born and infection of the adult genital tract were caused by similar infective agent (now *Chlamydia trachomatis*) all of which were capable of passing filters that otherwise generally retained bacteria. This property, coupled with inability of those agents to grow in artificial media, led to the erroneous belief that these agents were viruses.

In 1929-30, widespread out breaks occurred of an atypical and often severe pneumonia, acquired from psittacine birds (budgerigars, parrots and so forth), which was termed 'Psittacosis'. These outbreaks stimulated research and Levinthal Coles and Lillie independently described minute basophilic particles in Giemsa-stained blood and tissue from the infected birds and human patients. Bedson and co-workers soon proved the aetiological relationship of these particles with psittacosis and went on to describe the characteristic developmental cycle that now defines all members of the order Chlamydiales. Bedson referred to this agent (subsequently improperly referred to as Bedsoniae) as an "obligate intracellular parasite with bacterial affinities", a concept of great insight that was not accepted for another thirty years.

As early as 1934, Thygeson, an ophthalmologist, had drawn attention to the resemblances between the development and morphology of the inclusion conjunctivitis and of those found in psittacosis. The finding of a common complement-fixing antigen strenghthened the idea that these agents and those of Lymphogranuloma venerum (LGV) and that of mouse pneumonitis were related within thesame unique group. By 1935, the psittacosis agent had been grown in the chick embryo chorio-allantoic membrane while the LGV agent was propagated first, in monkey brain, in 1931 (Collier, 1990).

The trachoma agent was first isolated in the chick embryo yolk sac in China in 1957 by T'ang and collegues (Wang, 1999). Names for this bacterium included 'Bedsonia', 'Miyagwanella', 'Halprowia', Ornithosis, TRIC and PLT agents. The term 'Chlamydia' appeared in literature in 1945 and chlamydiae were proven not to be viruses in 1965 with the advent of tissue culture techniques and of electron microscopy, when evidence for bacterial – RNA, ribosomes and cell wall structures in Chlamydiae finally became over whelming. However, chlamydiae were still grouped with Rickettsia until the genus *Chlamydia* was validated in 1966.

DNA-DNA re-association studies and subsequently gene sequencing led to two new species in the late 90s; *Chlamydia pneumoniae* and *Chlamydia pecorum* (both now placed into the genus *Chlamydophila*) (Everett *et al*., 1999).

New molecular diagnostic methods based on nucleic acid amplification led to Chlamydiae being discovered in tissues and cells never before reported (joints, atherosclerotic plaques, brains, etc) and associated with diseases of previously unknown aetiology (arthritis, Alzheimer's disease, coronary artery diseases etc).

2.2.2 Clinical Spectrum

Typically, *C. trachomatis* serovars A through C of biovar trachoma infect the conjunctival epithelium and lead to ocular infections that can progress to trachoma, the leading cause of preventable blindness. In developing countries, these serovars remain endemic. It is estimated that there are about 162 million infected people worldwide, and 6 million of them are blind (Mabey and Fraser-Hurt, 2001; Mabey *et al*., 2003). Serovars D–K, Da, and Ia infect the genital epithelium and cause urogenital tract infections. These serovars remain the major

causes of STDs in developed as well as in developing countries, with about 92 million new infections each year (Bjartling *et al*., 2000; Toth *et al*., 2000). The LGV biovar has four invasive serovars (L1, L2, L2a and L3) that are able to infect not only the genital epithelium, but also monocytes and lead to a systemic disease known as Lymphogranuloma Venerum (LGV) (Mabey and Peeling, 2002).

The different serovars display well-documented and unique tissue tropisms. The trachoma serovars (A-C) are rarely isolated from the genital tract with the exception of serovar B variants, which have been associated with a very low incidence of urogenital disease. In contrast, genital serovars D–K are not associated with blinding trachoma; however, they can cause ocular infection when newborn infants acquire the organism during passage through the infected birth canal or when adults accidently inoculate the eye with infected genital secretions (Caldwell *et al*., 2003).

Despite this diversity in tissue tropism and disease manifestations, complete genomic sequencing of several Chlamydiaceae has shown that the gene order and content among the different species is remarkably conserved, with the exception of a region termed the plasticity zone (PZ). This observation led to the suggestion that host organ and cellular tropism may be attributed to the few genes localized in the PZ (Caldwell *et al*., 2003)**.**

Genital chlamydial infection:

Genital chlamydial infection is the most common among STIs worldwide (Kučinskienė *et al*., 2006). In the US in 2006, more than one million cases of chlamydial infection were reported to the Centers for Disease Control and prevention (CDC), corresponding to a rate of 347.8 cases/100000, with an increase of 5.6% as compared with the rate in 2005 (Be´be´ar and de Barbeyrac, 2009)**.** Most of these infections are asymptomatic and, if not treated, can lead to severe complications, mainly in young women. Risk factors for infection include young age,

high frequency of partner change, multiple partners, unprotected sex and being unmarried (Manavi, 2006). The majority of researchers think that higher prevalence of chlamydial infection among young-aged female adolescents may be due to insufficiently developed cervix, which is especially susceptible to STIs (Sedlecki *et al*., 2001).

Chlmydia trachomatis is a cause of cervicitis, non gonococcal urethritis (NGU) and pelvic inflammatory disease (PID) in women and NGU, epididymitis and proctitis in men. Infection of the urethra and lower genital tract may cause dysuria, whitish or clear urethral or mucopurulent vaginal discharge and post-coital bleeding. The bulk of infections is asymptomatic and therefore remains undetected (CDC, 2005).

If untreated, ascending infection may result. In women, ascending infection can cause endometritis, salpingitis, PID and perihepatitis. This happens in up to 40 % of women with untreated chlamydial infection. Manifestations of upper genital tract infection in women are irregular uterine bleeding, pelvic discomfort or abdominal pain; however some infections may still remain silent (Molano *et al*., 2003). Women infected with chlamydiae are up to 5 times more likely to become infected with HIV, if exposed (Joyee *et al*., 2005).

In pregnant women, there is little evidence and this is conflicting, to implicate *C. trachomatis* in chorioamnionitis and adverse pregnancy outcome. Babies who are born to infected mothers can get chlamydial infection during passage in the infected birth canal with the risk of developing pneumonia and conjunctivitis (Be´be´ar and de Barbeyrac, 2009).

Complications among men are rare. Infection sometimes spreads to the epididymis, causing pain, fever, and rarely, sterility (CDC, 2005).

Serious sequelae (blindness, tubal infertility, ectopic pregnancy, etc.) due to chlamydial diseases are observed only if they remain chronic (Mpiga and Ravaoarinoro, 2006). Re-

exposure or persistent infection is thought to drive an immunopathological inflammatory response resulting in tissue fibrosis and scarring that characterize all chlamydial diseases (Caldwell *et al*., 2003).

2.2.3 Immune Response

Chlamydia trachomatis is a strong immunogen, which stimulates both humoral and cell mediated immune responses. In addition to the immunogenic antigens, the outcome of chlamydial infection depends on interaction and balance of cytokines secreted by the activated lymphocytes. Interferon gamma (IFN-γ) has been described as a single most important factor in host defense against *Chlamydia*, while disease susceptibility has been linked with enhanced expression of Interleukin- 10 (IL-10) (Rank *et al.*, 1992). Immune system changes or disturbances induced by *C. trachomatis* may favour its own survival in the infected host, and induce persistent infections (Malhotra *et al.*, 2013).

Various studies have disclosed that the CD4+Th1 response is absolutely required to resolve primary infection, even when CD4+Th2 response is ineffective (Mpiga and Ravaoarinoro, 2006). The antichlamydial action of Th1 effectors is mediated principally via cytokines, specially the chlamydistatic IFN-γ. The antimicrobial mechanisms of these cytokines include depletion of intracellular tryptophan by activation of indoleamine 2,3-dioxygenase (IDO), induction of elevated nitric oxide (NO) through inducible NO synthase, deprivation of iron, via down-regulation of transferrin receptors and possibly the stimulation of phagolysosomal fusion or disruption of selective vesicular nutrient transport (He *et al*., 2007).

Depletion of tryptophan either results in chlamydiae cell death or causes chlamydiae to adopt a non-infectious, non-replicating form that retains viability (persistence). Strains of chlamydiae that possess a functional tryptophan synthase (i.e., genital but not ocular strains)

may use indole (perhaps produced by local microbial flora) as a substrate for tryptophan synthesis to counter the growth inhibitory effects of IFN-γ. In turn, tryptophan synthase gene, which is in the PZ is a virulence factor that differentiates *C. trachomatis* strains into genital and ocular disease pathotypes (Caldwell *et al*., 2003; Wood *et al*., 2003; Nelson *et al*., 2005; McClarty *et al*., 2007).

In the CD4+Th2 response, IFN-γ production is too low to exert chlamydistatic effects (Mpiga and Ravaoarinoro, 2006). In addition, it has been reported that even with the transfer of CD4+Th2 clones in nude mice with genital infection resulting in antibodies against multiple chlamydial antigens, the infection is only reduced but not resolved (Hawkins *et al*., 2002). Such a response is definitely regarded as ineffective because it does not dislodge RBs or persistent intracellular particles (Mpiga and Ravaoarinoro, 2006)**.** The predominant role of antibodies is in resistance to re-infection, by enhancing the uptake, processing and presentation of chlamydial antigens by antigen-presenting cells for rapid Th1 activation and clearance of infection (Longbottom, 2003).

Cytokines play an important role in induced immune response polarization. IFN-γ and IL-12 favour Th1 responses, but inhibit Th2 responses. In contrast, IL-4 and IL-10 stimulate Th2 responses, but suppress Th1 responses. For this reason, initial steps during infection are crucial in the induction of an appropriate response. Several authors have noticed that IFN-γ and IL-12 required to polarize the immune response towards the Th1 profile could come from natural killer cells and dendritic cells, respectively (Tseng and Rank, 1998; Matyszak *et al*., 2002). However, the early source of IL-4 and IL-10 allowing the induction of Th2 responses is not known (Mpiga and Ravaoarinoro, 2006).

2.2.4 Risk and Demographic Factors for *Chlamydia trachomatis* Infection

The most common demographic correlate of infection with chlamydial infection in women is young age (<20 yr) (Ward, 1999). This could be explained by the anatomic differences in the cervix of the younger women, wherein the squamo-columnar junction, a primary host target for *C. trachomatis*, is everted and thus more exposed. Other factors associated with chlamydial infection include unmarried status, nulliparity, black race and poor socio-economic condition (Novak and Novak, 2013). A large number of sexual partners, a new sexual partner, lack of use of barrier contraceptive devices and concurrent gonococcal infection are also known to be associated with chlamydial infection (Stamm and Batteiger, 2010). Cervical chlamydial infections are also found to be associated with the use of oral contraceptives (Stamm and Batteiger, 2010).

2.2.5 Diagnosis of Genital Chlamydial Infections

i. Clinical Diagnosis

Clinical picture of the patients suffering from chlamydial infection could be misleading as up to 70-80 per cent of the infected women and 50 per cent of the infected men are asymptomatic. Typically, a female with uncomplicated chlamydial infection will present with odourless, mucoid vaginal discharge without pruritis. Dysuria without frequency or urgency will be complained of if urethra is involved. Further, in PID, history of severe abdominal pain with high fever, dyspareunia, prolonged menstrual cycles and intermenstural bleeding can be elicited. On examination, cervicitis with a yellow, cloudy, mucoid discharge can be seen from the os. The cervix tends to bleed easily when scraped with spatula or brush. Urinalysis will reveal the presence of >5 WBC/HPF (high power field), which is suggestive of urethritis (CDC, 2010). Chlamydial infections cannot be distinguished from other urethral infections clinically. Amine test (*i.e.*, significant odour release on addition of KOH to the vaginal

secretion) can help differentiate chlamydial infections from other lower genital tract infections but has a low specificity.

Chlamydial infection in males manifests as urethritis in 15-55 per cent of the affected less than or equal to 35 yr, occasionally epididymitis may be seen (Paavonen and Eggert-Kruse, 1999). Mild to moderate clear to white urethral discharge is seen in the morning before the patient voids. In epididymitis, history of unilateral testicular pain with scrotal erythema, tenderness or swelling over the epididymis may be elicited. The diagnosis can be established by the presence of mucopurulent discharge from penis which on Gram staining shows >5 WBC/HPF and absence of intracellular Gram negative diplococci. Reiter's syndrome may be a rare complication of untreated chlamydial infection. A reactive arthritis that includes triad of urethritis/cervicitis in females, conjuntivitis and painless mucopurulent eruption on palms and soles of feet is seen in Reiter's syndrome (Stamm and Batteiger, 2010). Females are more commonly affected than males. There is asymmetrical multiple joint involvements with predilection for lower extremities.

ii. Laboratory Diagnosis

1. Specimens:

Proficiency in specimen collection and transport is paramount to accuracy in diagnostic testing. Both the sensitivity and specificity of diagnostic tests for *Chlamydia trachomatis* have been shown to be directly related to the adequacy of the specimen. The host cell that harbours the organism should be included in the specimen collection as chlamydiae are obligate intracellular pathogens, especially in techniques involving direct visualization of the organism (Malhotra *et al.,* 2013).

The choice of sampling sites can influence the likelihood of recovering the pathogen. A 10-20 per cent increase in the recovery of *Chlamydial trachomatis* from genital tract has been

observed if both cervical and urethral specimens are taken in comparison to cervical sampling only (Black, 1997).

The type of specimen depends on the clinical picture, the diagnosis conditions and the laboratory technique used for detection, with the conditions of transport and storage being adapted to the particular technique (Essig, 2007).

Invasive specimens include urethral swabs in men, and endocervical or urethral swabs and specimens taken from the upper genital tract, in women (liquid from Douglas's pouch, endometrium and tubal specimens) (Be´be´ar and de Barbeyrac, 2009). Non-invasive self-collected specimens include first-void urine (FVU), vulvovaginal swabs, anal swabs and penile swabs. FVU specimens (first 10-30mL of urine) should be obtained at least 2 hours after the last micturition (Forbes *et al*., 2007). The bacterial load of these specimens is a major aspect of their suitability for the diagnosis, which can be made only by using NAATs (Michel *et al*., 2007). Self-collected vaginal swabs have a lower bacterial load than endocervical swabs, but a higher load than FVU and are very well adapted to screening programs (Schachter *et al*., 2005b). FVU is a suitable sample type for men (Gaydos *et al*., 2008).

2. Microscopy

In earlier days, Giemsa was used to stain EBs, which are eosinophilic in contrast to the basophilic RBs. Also dark-field microscopy can be used to detect chlamydiae organisms where EBs appear as yellow bodies due to their natural auto fluorescence. However, this method is insensitive compared to culture or other methods of diagnosis (Singh *et al*., 2002; Forbes *et al*., 2007).

3. Culture

The gold standard for diagnosing chlamydial infection is culture performed as described by Mardh *et al*. (1977). Chlamydial infection diagnosed by 2 non-culture tests, is now known as the expanded gold standard (Stary *et al*., 1996; Watson *et al*., 2002).

The most widely applied culture method is application of cycloheximide-treated McCoy cells (Ripa and Mardh, 1977). Cycloheximide inhibits the protein metabolism of the host cell, thereby favouring growth and multiplication of chlamydiae organisms, as being energy-parasites stealing ATP from the host cell. Inoculation of cells with chlamydiae requires attachment of the organism to the cell membrane. Different techniques can be employed to facilitate this primary interaction, but centrifugation at 2500 ×g for 1 hour is the most commonly used method (Wang *et al*., 2005).

After 48–72 hours of growth, detection of chlamydial inclusions in the cell cultures can be highly specific, if specific anti *C. trachomatis*-MOMP antibody staining is used, because stained *C. trachomatis* inclusions have a unique appearance with intense apple green fluorescence. Counterstain with rhodamine B gives the cells a red colour which makes the detection of chlamydiae-positive cases easier and the picture is attractive to the eye (Mardh, 2005). Less specific inclusion-detection methods using Enzyme-Linked Immunosorbent Assay (ELISA), iodine and Giemsa are not recommended (CDC, 2002).

To establish the diagnosis, at least 10 EBs have been recommended to be used as the cut-off level. A lower cut-off can be accepted, but must be related to the experience. However, a higher cut-off will mean that chlamydiae-infected individuals may be falsely classified as negative (Mardh, 2005).

The use of both urethral and cervical cultures has been recommended for detection of genital chlamydial infection, increasing the positive rate by approximately 5%. The current sampling recommendations are to obtain the specimen for *C. trachomatis* culture after all other specimens (i.e. those for Gram-stained smear, *N. gonorrhoea*e culture or Pap smear) (Mardh, 2005; Forbes *et al*., 2007). The type of sampling swab used is essential, a Dacron or rayon-tipped swab can be used, while both cotton and wood sticks can be highly toxic to chlamydiae organisms (Mardh and Zeeberg, 1981; Essig, 2007). Use of urine for culture is inadequate, as the positive rate is only 30% as compared to cervical swabs from the same individuals (Mardh, 2005). Several types of media can be used to transport specimens and include 2-sucrose phosphate, sucrose– glutamate phosphate, or other commercial media (Hammerschlag, 2003). Specimens should be refrigerated upon receipt, and if they cannot be processed for culture within 24 hours, they should be frozen at -70°C (Forbes *et al*., 2007).
Cell culture has near 100% specificity, but estimates of sensitivity are as low as 50% (Black, 1997; Crotchfelt *et al*., 1998**;** Shrier *et al*., 2004). Other drawbacks of chlamydial culture are that it requires a cold chain during transport and that results are not available for 3 to 7 days. For these reasons, culture should not be used routinely to screen patients for *C. trachomatis*. Culture is still an important tool to diagnose chlamydial infections in cases of suspected abuse and for evaluating possible oropharyngeal, conjunctival and rectal infections (Olshen and Shrier, 2005)

Cultures may serve the purpose of allowing rapid test of microbial cure after antibiotic therapy, that is during the second to third weeks after finishing treatment. DNA tests may then still be positive due to the presence of dead, but not yet shed EBs. The use of cultures also offers the possibility of performing antibiotic susceptibility tests of isolated strains and detecting non-plasmid containing strains (Mardh, 2005).

4. Non-amplification, Non-culture Tests

The first generation of nonculture tests to diagnose chlamydial infections include direct fluorescent antibody staining (DFA), ELISA, and DNA probe tests. These tests use direct visualization techniques to detect *C. trachomatis*. Although these tests largely have been replaced by NAATs, they are still used in some clinical settings (Olshen and Shrier, 2005).

a) *Direct Fluorescent Antibody Staining (DFA)*

DFA method use fluorescein-isothiocyanate (FITC) conjugated monoclonal antibodies that are directed at a *C. trachomatis*-specific epitope of the MOMP. DFAs are based on detecting EBs in smears, although staining of inclusions can also succeed if intact infected host cells are collected (Essig, 2007).

Viable organisms are not required for the test. The test provides a rapid turnaround time (1-2 hours) for results, making DFA tests useful especially for laboratories that test only a limited number of specimens. In addition, checking for the presence of columnar or metaplastic epithelial cells allows assessment of the adequacy of the sample (Steece, 2007). However, the DFA is not suitable for large volume testing. Proper specimen collection and transport is critical. It is less (50-70%) sensitive compared to NAATs (95-98%). The sensitivity of DFA tests of genital secretion is problematic in more longstanding infections where the number of EBs has often become low. The use of DFA tests of urine specimens is also a problem, as there are many artifacts in the sample that can mimic EBs. In addition, the specificity is less than with cell culture (95-98%). Unfortunately, DFA tests have been used by inexperienced staff in many countries, which have resulted in a large number of false-positive diagnoses (Mardh, 2005; Steece, 2007).

b) *Enzyme-Linked Immunosorbent Assay (ELISA)*

ELISA tests were widely used to diagnose genital chlamydial infections. Such tests are still used in economically less developed countries, mainly because of the price being lower than that for DNA-based tests. ELISA uses an enzyme-linked monoclonal or polyclonal antibody directed at the *C. trachomatis* LPS which is more soluble than MOMP. These tests are not species-specific for *C. trachomatis* and may cross-react with *Staphylococcus aureus*, or LPS of other Gram-negative bacterial species e.g., *E. coli*. This problem is accentuated when rectal; urine and pharyngeal samples are to be tested. Because of the low specificity, it has been recommended to use confirmatory tests in case of a positive ELISA (Essig, 2007; Forbes *et al*., 2007). ELISA tests can be automated. They are more reproducible than DFA, and the sensitivity of the best ELISA is comparable to that of culture and lower than that of NAATs (Be´be´ar and de Barbeyrac, 2009).

c) *Point-of-Care Testing*

Rapid or 'point-of-care' (POC) tests are proposed for patients who are unlikely to return for test results. POC tests for chlamydial infection, such as Clearview® and Surecell®, use ELISA technology (Mardh, 2005). They are subject to the same potential for false-positive results of ELISA, not suitable for non-invasive specimens, have moderate sensitivity (50-80 %, compared with culture), and are not recommended for laboratory settings (CDC, 2002).

A rapid test for diagnosis of chlamydial infection, recently developed by the Wellcome Trust, is based on a second-generation signal amplification ELISA for chlamydial LPS in a dip-stick type format. The initial results were promising (Michel *et al*., 2007; Mahilum-Tapay *et al*., 2007).

d) *DNA Probes*

The DNA probe tests are the most sensitive and most specific non-culture, non-amplification tests available to diagnose chlamydial infections. These tests use DNA probes that hybridize

to species- specific sequence of *C. trachomatis* rRNA. Hybridization then causes a chemiluminescent response that can be detected with a luminometer. Available data suggest that DNA probe tests are about as sensitive as the best antigen detection and cell culture method and relatively specific. These tests can be used for endocervical, urethral and conjunctival specimens, but they are not recommended for use on urine, rectal, oropharyngeal or vaginal specimens. They are recommended for detecting *C. trachomatis* in endocervical and urethral specimens in settings where NAATs are not available, or for testing conjunctival specimens when culture is not available (Olshen and Shrier, 2005; Schachter *et al*., 2005b).

5. *Nucleic Acid Amplification Tests (NAATs)*

NAATs are the most sensitive tests for screening and diagnosis of chlamydial infection of the genital tract (Martin *et al*., 2000; Black *et al*., 2002; Gaydos *et al*., 2004). They are Food and Drug Administration (FDA) approved for use with a large range of sample types, including vulvovaginal swabs and FVU (Be´be´ar and de Barbeyrac, 2009). For these reasons, commercially available NAATs, such as PCR, ligase chain reaction (LCR), strand displacement amplification (SDA) and transcription-mediated amplification (TMA) are the tests of choice for the diagnosis of *C. trachomatis* genital infections. PCR, LCR and SDA methods amplify bacterial DNA. These tests target the cryptic plasmid or the MOMP. The plasmid-based PCR used may have a higher sensitivity than the MOMP-based PCR because of the presence of multiple copies of the plasmid in every chlamydia organism (Palmer and Falkow, 1986; Ostergaard and Moller, 1995; Marions *et al*., 2008). TMA amplifies bacterial rRNA (Boyadzhyan *et al*., 2004; Marions *et al*., 2008).

As more screening for genital infections has been done by using NAATs, concerns have been raised about their specificity, particularly in screening low-prevalence populations. In some studies, positive NAAT results could not be reproduced (Gronowski *et al*., 2000; Castriciano

et al., 2002; Culler *et al*., 2003; Verkooyen *et al*., 2003; Koenig *et al*., 2004). These concerns led the CDC to recommend confirmatory testing of positive test results when the PPV is ~ 90% (CDC, 2002). However, the necessity of confirmatory testing of positive specimens is controversial (Schachter *et al*., 2005a; Schachter *et al*., 2006**;** Be´be´ar and de Barbeyrac, 2009). In the CDC guidelines, it was pointed out that less sensitive diagnostic tests, such as culture and ELISA, should not be used to confirm positive results of the more sensitive NAATs for *C. trachomatis*. This guideline exists because 30% or more of specimens positive by NAATs will be negative by culture or ELISA (Schachter *et al*., 2005a). The study of Schachter *et al*. (2005a) and Schachter *et al*. (2006) showed that the same principle applies when only NAATs are being used. They showed that confirmatory testing complicates the handling of a positive NAAT result, as the initial failure to confirm a positive result is wrong more often than it is right and adds cost to an already expensive screening test.

Their primary disadvantage is the cost, which could be reduced by pooling specimens (Be´be´ar and de Barbeyrac, 2009). The sensitivity of the NAATs can also be lowered by the presence of inhibitors in clinical specimens. Human chorionic gonadotropin, crystals, nitrites and hemoglobin all have been shown to inhibit NAAT performance. The sensitivity of NAATs on urine specimens also can be reduced if larger than recommended urine volumes are tested. NAATs cannot distinguish between living and dead organisms and, therefore, should not be used in patients treated for chlamydial infection in the previous 3 weeks. NAATs require skilled laboratory personnel to process specimens to reduce cross-contamination of specimens. Despite these limitations, NAATs remain the most sensitive type of test available for diagnosing chlamydial infections (Boyadzhyan *et al*., 2004).

A *C. trachomatis* new variant (nvCT), harboring a 377 bp cryptic plasmid deletion, was recently described, first in Sweden, and was named accordingly, the Swedish variant (Ripa

and Nilsson, 2006; Marions *et al*., 2008). The deletion in this nvCT includes the region targeted by current commercially available diagnostic PCR tests, e.g., Cobas Amplicor, Cobas TaqMan48, and Abbott m2000, which will give false negative results (Raherison *et al*., 2009). Some commercial kits are able to detect this variant. The BD ProbeTec™ ET System can detect the nvCT, as the amplification region is within an unaffected part of the cryptic plasmid (Marions *et al*., 2008). The LightMix 480HT and The COBAS® TaqMan® CT Test, v2.0 can also detect the nvCT as the former targets the MOMP gene1, while the latter has a dual-target strategy (cryptic plasmid DNA and MOMP gene genomic DNA) (Unemo *et al*., 2007; Marions *et al*., 2008; Hadad *et al*., 2008; Be´be´ar and de Barbeyrac, 2009). At this time, the spread of nvCT seems to be limited to some European countries like Sweden, Denmark, Norway, Ireland and France (de Barbeyrac *et al*. 2007; Hoffmann and Jensen, 2007; Lynagh *et al*., 2007; Moghaddam and Reinton 2007).

Recently, a technological innovation that came from PCR, named real-time PCR, has become more common for clinical diagnostics and in research laboratories, because of its ability to generate quantitative results. The main advantages are the possibility of quantification, along with greater sensitivity, precision and accuracy, as well as faster analysis without any post-amplification procedures. The time needed to produce a result is approximately 2 hours; the other techniques need more time for processing the material. In addition, specific primer design combined with melting curve analysis allows a reliable and sensitive identification of the organism. These advantages make this technique superior to the other methods (Eickhoff *et al*., 2003; Oliveira *et al*., 2008).

6. Serological Diagnosis

Serology is useful only in some cases of *C. trachomatis* infection and in sero-epidemiological studies. It suffers from several drawbacks, including the serological cross-reactivity between

C. trachomatis and *C. pneumoniae* species and the persistence of antibodies, which prevents a distinction being made between past and present infection. Although it is not recommended for the diagnosis of lower genital tract infections, or for screening in asymptomatic patients, serological testing may be useful for diagnosing LGV, neonatal pneumonia and upper genital tract infections, and for the evaluation of tubal-factor of infertility (Persson, 2002; Be´be´ar and de Barbeyrac, 2009).

The serological methods available are complement fixation, MIF and ELISA. The latter two allow the distinction among IgG, IgA and IgM. The MIF method, which is species and serovar-specific and which is considered to be the reference method, is laborious, and reading of the assay is subjective and therefore it is not suitable for a daily routine. ELISAs provide objective reading and allow the handling of more samples at the same time (Bax *et al*., 2003). Several ELISA have been developed. Promising results were obtained with these tests; using an antigen purified chlamydial EBs devoided of the genus specific LPS. However, serum cross reactivity persisted due to the presence of other genus specific epitopes as well as residual contaminants. The use of chemically or recombinant antigens seems to be attractive to improve chlamydial serodiagnosis. This approach requires the identification of the most immunodominant antigens in human *C. trachomatis* infections. Proteomic and immunoblot analyses demonstrated that the most immunogenic chlamydial components comprise the genus specific LPS, the MOMP and the omp2. ELISAs, which can make use of synthetic peptides from the variable domains of the MOMP or recombinant LPS, can be automated (Frikha-Gargouri *et al*., 2008; Frikha-Gargouri *et al*., 2009).

2.2.6 Screening

Chlamydial infection causes a sexually transmitted disease that can be treated easily and its sequelae are prevented by a short course of an inexpensive antibiotic. Screening programs for

chlamydial infection must be cost-effective and must be made acceptable to patients by using non-invasive procedures that allow sample collection at the patient's home. There are 2 main approaches to the design of screening programs: proactive, consisting of screening the entire target population; and opportunistic, targeting individuals who attend a healthcare setting (Jones and Boag, 2007). The opportunistic approach, targeting sexually active individuals less than 25 years of age or older women with risk factors (e.g., those who have a new sex partner or multiple sex partners) within a variety of healthcare settings, is used in most chlamydial screening programs in the US, the UK and France. Research studies are needed to establish the benefits and the disadvantages of chlamydial screening programs (Low, 2007).

2.2.7 Treatment

The treatment of chlamydial infection depends on the site of infection, the age of the patient, and whether the infection is complicated or not. Treatment also differs during pregnancy. (Malhotra *et al.*, 2013)

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

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

be re-tested after completing the treatment, unless the symptoms persist or re-infection is suspected.

Treatment of PID: Recurrent chlamydial infection increases the risk for developing ectopic pregnancy and PID. PID can be treated on an outpatient basis unless indicated (accompanied by severe illness, nausea, vomiting, high-grade fever, tubo-ovarian abcess or intolerance or unresponsiveness to oral therapy). The CDC has recommended ofloxacin 400 mg orally (bd) or levofloxacin 500 mg orally once a day (OD) with or without metronidazole 500 mg orally (bd) for two weeks. In case of intolerance to the above mentioned regimen, ceftriaxone 250 mg intramuscular (IM) or cefoxitin 2 g (IM) as a single dose with concurrent probenicid 1 g orally in single dose plus doxycycline 100 mg orally (bd) with or without metronidazole 500 mg orally (bd) for two weeks (Sexually Transmitted Diseases Treatment Guidelines, 2010).

Treatment during pregnancy: Levofloxacin, ofloxacin and doxycycline are contraindicated during pregnancy. Therefore, azithromycin 1 g orally in a single dose or amoxycillin 500 mg orally thrice a day (tds) is recommended. Amoxycillin is reported to be more effective and with fewer side effects than erythromycin in treating antenatal chlamydial infection. Alternatively, erythromycin base 500 mg orally (qid) is a safe and effective alternative (Sexually transmitted diseases treatment guidelines 2010). Testing for cure is indicated in patients who are pregnant and should be performed three weeks after completion of treatment. If the risk of re-exposure is high, screening should be repeated throughout pregnancy.

Antimicrobial susceptibility

Antibiotics play a major role in treating chlamydial infections. Azithromycin and doxycycline are considered as first line drugs by the centres for disease control and prevention (CDC) (Workowski and Berman, 2010). The efficacy of these drugs for treatment of chlamydia is

high, however many researches have reported the problem of recurrent infections and treatment failures (Wang *et al*., 2005). Recent studies have indicated the re-emergence of antibiotic resistance in chlamydia which is feared to create severe problems in the treatment of the disease (Bhengraj *et al*., 2012).

Evaluation of in vitro susceptibility of chlamydiae is not currently performed, because a standardized method is lacking and minimum inhibitory concentration (MIC) results are not consistently reproducible and does not correlate with the patient's clinical outcome (Suchland *et al*., 2003). In vitro, the most active drug is rifampin, with the lowest MIC, followed by tetracyclines, macrolides and some fluoroquinolones (ofloxacin and the newer compounds) (Forbes *et al*., 2007)**.**

Acquired antimicrobial resistance of *C. trachomatis* seems to be exceptional (Be´be´ar and de Barbeyrac, 2009). The potential of *C. trachomatis* to develop antimicrobial resistance has not been well studied, although some case reports, suggest resistance as a cause of treatment failure (Mourad *et al*., 1980; Lefevre and Lepargneur, 1998; Somani *et al*., 2000). However, mutants resistant to fluoroquinolones and to rifampin have been produced in vitro (Dessus-Babus *et al*., 1998; Dreses –Werringloer *et al*., 2003), and 4 clinical isolates, that demonstrated in vitro resistance to macrolides, were shown to carry mutations in a 23S rRNA gene (Misyurina *et al*., 2004).

There have been no reports of clinical *C. trachomatis* isolates displaying in vitro homotypic resistance to antimicrobials. Heterotypic resistance of *C. trachomatis*, which refers to the replication of a heterogeneous population of resistant and susceptible bacteria from a subculture of a single resistant organism propagated on antimicrobial-containing medium,

can be observed when cells are inoculated with a large number of organisms (Wang *et al*., 2005).

2.2.8 Management of Genital Chlamydial Infection

Both patients and their sexual partners must be treated. For treatment of uncomplicated lower genital tract infections in adults, major progress has been made in the use of single dose therapy with azithromycin (1g) (CDC, 2006). A 7-day course of doxycycline, gives comparable results, but with lower rates of compliance (Lau and Qureshi, 2002).

Guidelines have been proposed in different countries for the treatment of upper genital tract infections. These require a longer treatment period (14–21 days), and the combination of several antibiotics to control other bacteria involved. The possibility of persistence of the infection after treatment may justify the use of a test of cure (5 weeks post-treatment) in cases of presumptive treatment failure or during pregnancy (Walker and Wiesenfeld, 2007).

2.2.9 **Prevention**

The control of STD is a public health priority and the importance of these infections has increased in salience over the past decade, with the growing evidence of co- transmission of HIV. The CDC guidelines (Johnson *et al.*, 2002) for the prevention and control of STDs are based on five major concepts: *(i)* Education and counselling on safer sexual behaviour in persons at risk. *(ii)* Identification of asymptomatic infected persons and of symptomatic persons unlikely to seek diagnostic and treatment services. *(iii)* Effective diagnosis and treatment of infected persons. *(iv)* Evaluation, treatment and counselling of sex partners of persons infected with a STD. *(v)* Pre-exposure immunization for vaccine preventable diseases.

The CDC strongly recommends that all sexually active women (≤25 yr) and women at increased risk of infection should be routinely screened for *Chlamydia*. However, screening for chlamydial infection is not recommended for men, including those who have sex with other men (Nelson and Helfand, 2001). Prevention of *C. trachomatis* infection can be done at primary, secondary and tertiary levels. Primary prevention involves preventing both exposure to and acquisition of chlamydial infection through lifestyle counselling and health education. Clinicians play an important role by enquiring about the risk taking sexual behaviour, by encouraging screening tests for those at risk, by ensuring that partners are evaluated and treated and by counselling about safe sex practices. Effective school based health programmes should be implemented among adolescents. Unfortunately, primary prevention has not gained popularity especially in the developing world (Horner, 2006). Secondary prevention means early detection of asymptomatic disease by screening in order to prevent the drastic sequelae of chlamydial infection. Chlamydial infection fills the general pre-requisite for disease prevention by screening, since these are highly prevalent, are associated with significant morbidity, can be diagnosed, and are treatable. Recent advances like testing non-invasive specimen, utilization of nucleic acid amplification tests and single dose therapy using azithromycin further enhance the efforts to prevent chlamydial infection. Tertiary prevention of acute and chronic chlamydial infection of the upper genital tract has largely failed because by the time patient becomes symptomatic substantial tubal damage already occurs.

Vaccines

Vaccination could be substantially more effective than other biomedical interventions in controlling epidemics of *Chlamydia* infection. Currently, the best public health intervention available is increasing the rate of screening and treating infected individuals. Administrating a protective vaccine to adolescents before their first sexual experience could induce a

significant reduction in prevalence which could not be obtained by screening teenagers, even with coverage of 100 per cent (Gray *et al.,* 2009). Unfortunately, no protective vaccines, either fully or partially, are available although there have been many attempts to develop one. The immunological characteristics of the genital tract and the tropism of *Chlamydia* for mucosal epithelial cells emphasize that a *C. trachomatis* vaccine must induce both mucosal and systemic protective responses (Mestecky *et al.,* 2005). The research goal for an efficacious human chlamydial vaccine has faced key challenges to define the elements of protective immunity to facilitate vaccine evaluation, the judicious selection of appropriate vaccine candidates that possess stable antigenic and immunologic properties and the development of effective delivery vehicles and adjuvants to boost immune effectors to achieve long term protective immunity. Progress in the functional immunobiology of *Chlamydia* has established the essential immunologic paradigms for vaccine selection and evaluation, including the obligatory requirement for a vaccine to induce T- helper type 1 immune response that controls *Chlamydia*. Major inroads are however, required in the construction and development of novel and effective delivery systems, such as vectors and adjuvants.

2.3 Chlamydia and Pregnancy

The prevalence of *C. trachomatis* infection in pregnant women ranges from 2-35 per cent (Black, 1997). Pregnant women with chlamydial infection are at increased risk for adverse outcomes of pregnancy and post-partum PID. Sequelae like still birth, low birth weight, neonatal death, decrease gestational periods, preterm delivery and premature rupture of membranes (PROM) have been reported (Ward, 1999). Nine per cent of the women with chlamydial infection who develop PID have tubal pregnancy (Johnson *et al.,* 2002). Early

pregnancy loss or recurrent pregnancy loss may be induced by asymptomatic chlamydial infection through the operation of immune mechanism.

2.4 Chlamydia and Pelvic Inflammatory Disease

Pelvic inflammatory disease is a general term that refers to infection and inflammation of the upper genital tract in women. It can affect the uterus (womb), fallopian tubes, ovaries and other organs related to reproduction (Moss, 2001). Twenty per cent of the women with chlamydial lower genital tract infection will develop PID (Price *et al.*, 2013) and 4 per cent will develop chronic pelvic pain (Paavonen and Eggert-Kruse, 1999). The clinical spectrum of chlamydial PID ranges from subclinical endometritis to frank salpingitis, tubo-ovarian masses, pelvic peritonitis, periappendicitis and perihepatitis. However, symptomatic chlamydial infections represent only the tip of the iceberg of all chlamydial infections as majority of genital chlamydial infections are asymptomatic.

2.5 Chlamydia and Infertility

Tubal infertility occurs when the infection spreads from the cervix, its point of initiation to the fallopian tubes keeping the eggs from being fertilized (Gerbese *et al*., 1998). A major cause of infertility in sub-Saharan Africa is Pelvic Inflammatory disease (PID) (Bello, 2004).

In PID cases due to *Chlamydia trachomatis*, high level of persistent circulating IgG antibody is produced (Joyner *et al*., 2002). *Chlamydia trachomatis* antibody testing could provide a clinically useful screening test for predicting or confirming tubal factor infertility in women and is therefore a desirable way to avoid laparoscopy (Thomas *et al*., 2000; Akande *et al.*, 2003).

Chlamydial PID is the single most important preventable cause of infertility. Approximately, 3 per cent of women with chlamydial genital tract infection develop infertility. After a single

episode of PID, the risk of tubal factor infertility is approximately 10 per cent, each repeat episode doubles the risk (Ray, 2006). Although the majority of patients are asymptomatic but re-infection/persistent infection with *C. trachomatis* leads to more severe tubal damage than other agents.

The role of *C. trachomatis* in the development of urethritis, epididymitis and orchitis in men is widely accepted. Though the role of this organism in prostatitis is controversial, but up to 35- 50 per cent incidence has been reported in patients with prostatitis (Cunningham and Beagly, 2008). Infection of the testes and the prostrate is implicated in the deterioration of sperm (decrease sperm motility, increase proportion of sperm abnormalities, significant reduction in sperm density, sperm morphology and viability and increased likelihood of leucocytospermia) affecting fertility. Chlamydial infection may also affect the male fertility by directly damaging the sperm as sperm parameters, proportion of DNA fragmentation and acrosome reaction capacity are impaired. However, the role of *C. trachomatis* in male infertility is not yet proven.

2.6 Chlamydia and Ectopic Pregnancy

Ectopic pregnancy is the condition which results when chlamydial infection ascends to the fallopian tubes causing scarring of the tubes which may interfere with the passage of fertilized egg to uterus and when this happens, the egg may attach itself to the fallopian tube and start to develop there. Ectopic pregnancy is an important cause of maternal deaths in Nigeria and in other developing countries. In Lagos, Nigeria, it was responsible for 8.6% of maternal deaths, and had a case fatality rate of 3.7%. The incidence was 23.1/1000 (1:43) deliveries and was responsible for 48.5% of gynecologic emergencies (Anorlu *et al*., 2005). Musa *et al.* (2009) reported 168 cases of ectopics out of 9,638 deliveries (1 in 57) in Jos, Nigeria, while Aboyeji *et al*. (2002) and Airede *et al*. (2005) reported 1.4% (1 in 69

deliveries) and 1.8 (18.1 per 1000 deliveries) incidence rates of ectopic pregnancy in Ilorin and Sokoto, respectively. In other parts of the world, the condition has also been reported to result in miscarriage and even cause the death of the mother (Russel *et al*., 1992). In England it was shown to result in 10% of deaths that occurred as a complication of pregnancy, child birth or the puerperium (Moss, 2001) and 48% of cases of ectopic pregnancy may be due to chlamydia, either recognized or unrecognized (Washington *et al*., 1993). Studies have also shown a strong correlation between ectopic pregnancy rates and chlamydial infection (Johnson *et al*., 1994). In 1987, more than 88,000 ectopic pregnancies occurred in the United States affecting nearly 17 in 1000 pregnant women between the ages of 15-44 (MMWR, 1990). The rate of ectopic pregnancies per 1000 live births has increased nearly five-fold since 1970 and 10% of women with symptomatic PID due to *Chlamydia trachomatis* may have ectopic pregnancy (Hay *et al*., 1997).

2.7 Chlamydia and HIV

Chlamydial infection of the genital tract facilitates the transmission of HIV. This is confirmed by various studies (Paavonen, 1996; Flemming and Wasserheit, 1999; Miller *et al.*, 2006). The combined epidemiology of these infections may partly be due to the fact that STDs including *C. trachomatis* and HIV have common sexual/behavioural risk factors. But, *C. trachomatis* and HIV have inter-relationship independent of the sexually transmissible risk factors (Joyee *et al.*, 2003). The possible inter-relationship between HIV infection and *C. trachomatis* includes: *(i)* the invasive intracellular pathogenesis of *C. trachomatis* can cause substantial damage to the genital epithelial layer that may facilitate HIV infection, and *(ii)* the immunological changes due to HIV infection may favour chlamydial infection.

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

2.8 Chlamydia and Co-infection with Other Sexually Transmitted or Reproductive Tract infections

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the two most common bacterial causes of lower genital tract infection. Clinical findings need to be corroborated with the laboratory investigations as the signs and symptoms of both are indistinguishable. Therefore, in the syndromic approach used in resource-limited settings, urethral discharge (UD) is simultaneously treated for both. *C. trachomatis* is recovered more often from women who acquire gonorrhoea than from similarly exposed women who do not acquire gonorrhoea. In individuals with gonorrhoea, there exists a 15-40 per cent higher risk of acquiring Chlamydia. Further, individuals infected with both *C. trachomatis* and *N. gonorrhoeae* shed larger number of *C. trachomatis* than those infected with *C. trachomatis* alone. These data suggest that acquisition of a gonococcal infection either reactivates a persistent chlamydial infection or increases the susceptibility of the host to *Chlamydia*. Post-gonococcal urethritis is often due to *C. trachomatis* infection which is not cured by conventional therapy against gonorrhoea. Co-infection of *C. trachomatis* with *N. gonorrheae* has been reported to range between 1.1 to 67 per cent (Tapsall *et al.*, 1996; Divekar, 2000; Lyss, 2003; Donati, 2009; Bala *et al.*, 2011)

Dibua *et al.* (2013) reported 61% prevalence rate of Chlamydia and HIV co-infection as well as 13.5% rate of Chlamydia/gonorrhoea co-infection among gynaecologic patients in South-Eastern Nigeria. In a study in STD patients in New Delhi, 19.9 per cent prevalence of *C. trachomatis* was observed (Mittal, 1992). The co-infection of *C. trachomatis* with bacterial

vaginosis was found to be 12.7 per cent, candidiasis in 10.9 per cent cases, syphillis in 3.6 per cent cases and chancroid in 1.8 per cent cases. However, co-infection with *N. gonorrheae* was not found. Two cases with multiple infections were also reported (*i.e.* one with *C. trachomatis, Candida albicans*, HIV and syphilis and the other with *C. trachomatis*, *C. albicans*, HIV and bacterial vaginosis). In another study, the prevalence of *C. trachomatis* in STD patients was found to be 30.8 per cent (Joyee, *et al.,* 2003). Thirty per cent of the *Chlamydia* infected cases had HIV infection, while the analysis revealed that 50 per cent of the HIV positive cases happened to be proven *C. trachomatis* positive cases.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at some Tertiary Health Care Centers in the North Central Zone, who gave ethical approval. They were the National Hospital, Abuja, in the Federal Capital Territory, the Federal Medical Centers, Lokoja and Makurdi in Kogi and Benue States respectively and the General Hospital, Minna in Niger State. The North-Central region of Nigeria consists of Benue State, Kogi, Kwara, Nassarawa, Niger and Plateau States as well as the Federal Capital Territory. The region, also referred to as Middle Belt, has a population of 20,246,257 which is 14.46% of the total population of Nigerians (>140,003,542) according to the 2006 census (NPC, 2006). The Federal Capital Territory is the home of Abuja, the capital of Nigeria. It has a total population of 1,405,201 and population density of 190/km^2. Kogi state has a population of 3,258,487, made up of 1,691,737 males and 1,566,750 females. It has an area of 29,833 km^2 and a population density of 70/km^2. Niger state has a population of 3,950,249 people (18th of 36) and a population density of 52/km^2 (130/sq mi). It has an area of 76,363 km^2 (29,484 sq mi) ranking as 1st of 36. Benue is a state located at 7°20′N 8°45′E in the MidEast region of Nigeria with a population of about 4,219,244(NPC, 2006). Makurdi is the State Capital.

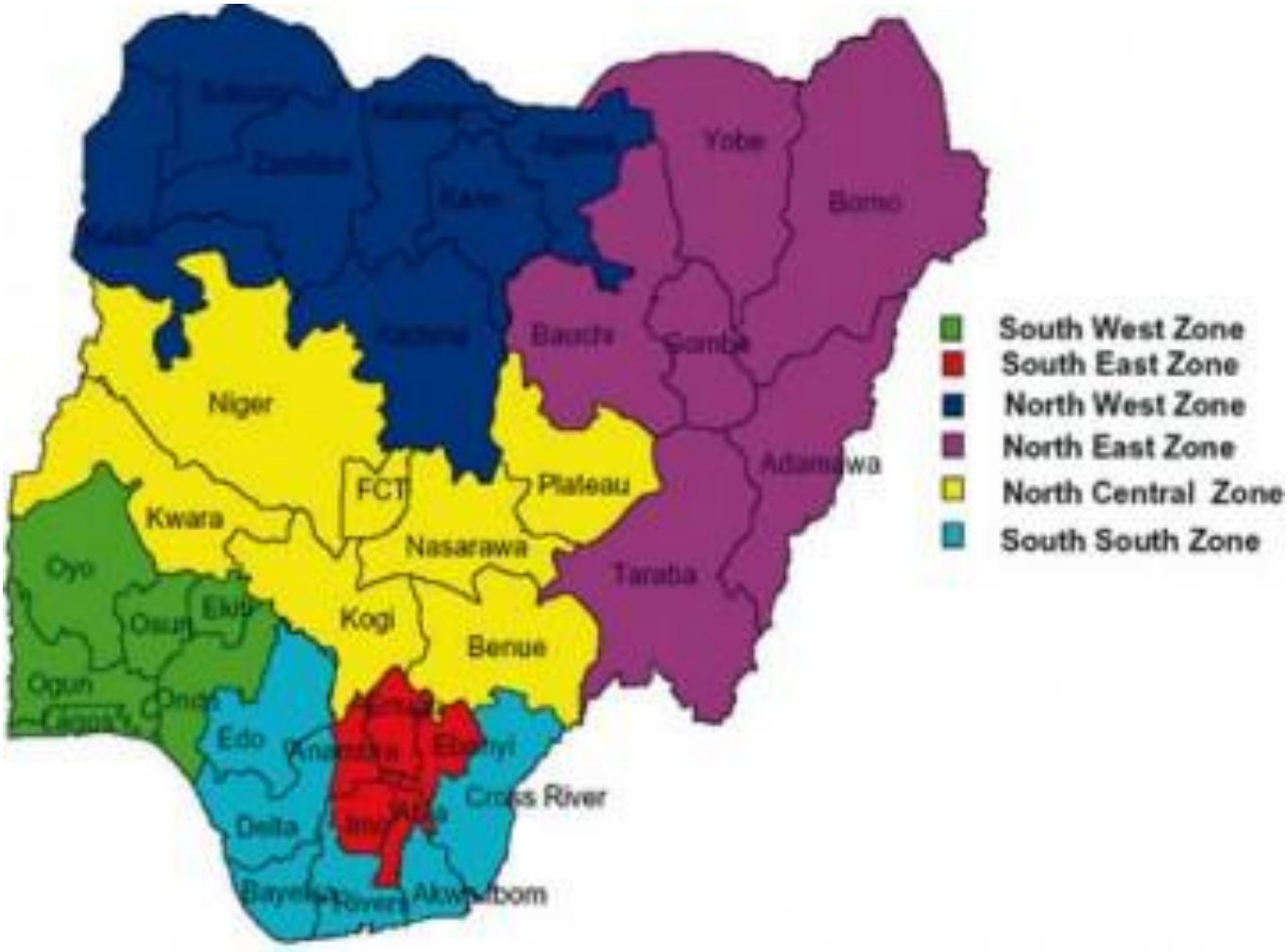


Figure 3: Map of Nigeria Showing the Study Area Coloured in Yellow

3.2 Study Population

Study population was drawn from antenatal clinic attendees and gynaecological patients presenting with pelvic inflammatory disease, infertility and ectopic pregnancy.

3.2.1 Inclusion Criteria:

1. Females in the reproductive age
2. Consenting patients

3.2.2 Exclusion Criteria:

1. Females outside the reproductive age
2. Patients that have taken antibiotics during the previous 2 weeks.
3. Patients that have applied local vaginal antiseptics during the last 2 weeks.
4. Non consenting patients

3.3 Sample Size

The minimum sample size to be collected shall be 378 using a prevalence of 56.1 in the zone (Jos) by Mawak *et al.* (2011) as determined by the formula:

$$n = \frac{Z^2 Pq}{L^2}$$

Where: n= number of samples

Z=standard normal deviate at 95% CI (1.96)

P= prevalence of the disease =56.1% (0.561) (Mawak *et al.*, 2011)

q= 1-p = 1- 0.561 = 0.439

L = allowable error of 0.05

n= 378

However, a total of 600 samples were collected for more representation, with the following breakdown: 50 samples from gynaecology cases and 100 from antenatal clinic attendees, making a total of 150 from each of the four study sites (150 X 4 =600).

3.4 Ethical Approval

The study was granted ethical approval by the ethical committees of the various Hospitals chosen for the study viz; Federal Medical Centers Lokoja and Makurdi, The National Hopital Abuja and the General Hospital, Minna. Consent of the study participants was also sought before they were included in the study. Photocopies of the approval letters and the consent form are included in the list of appendices.

3.5 Questionnaire Administration

A structured interview and information from the patients' record files were used to obtain data on socio-demography, previous history of PID and any STD, treatment regimen etc.

3.6 Materials

Chlamydia IgG ELISA test kits, Determine kits for the test of HIV1/2 manufactured by Alere Medical Co. Japan, HIV 1/2 STAT-PAK DIPSTICK kit manufactured by Chembio Diagnostic Systems, INC, USA and *Chlamydia trachomatis* PCR kit.

3.7.1 Specimen Collection

i. Blood sample collection

Five (5) ml of blood sample was collected from each consenting participant by venepuncture and stored in venoject vacutaneers and allowed to clot. The sera were separated by spinning the blood in a centrifuge at 3000g and stored at -20^{o} till use.

ii. Endocervical swab specimen collection

Patients were put in the lithotomy position. A sterile single-use plastic Cusco's speculum was used for exposure of the external os of the cervix (no antiseptic solution was applied to the speculum). The mucous was removed from the exocervix using a cotton swab (Black, 1997; Essig, 2007). A swab was then taken from each patient using Dacron swabs.

3.7 Methods

3.7.1 Test for *Chlamydia* IgG Antibody by ELISA

Chlamydia IgG ELISA test kit by DIAGNOSTIC AUTOMATION, INC, Calabasas was used. It employs the LGV type 2 broadly reacting antigen of *Chlamydia trachomatis*. It detects *Chlamydia trachomatis*, *Chlamydia psittaci* and *Chlamydia pneumonia* (TWAR) antibodies.

Principle of the test

Purified *Chlamydia trachomatis* antigen is coated on the surface of microwells. Diluted patient serum is added to wells and the *Chlamydia trachomatis* IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and the TMB chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader in a parallel manner with calibrator and controls.

Materials provided

1. Microwell strips: *Chlamydia trachomatis* antigen coated wells (12x8 wells)
2. Sample diluents: Blue color solution (1 vial (22ml)
3. Calibrator: Factor value (f) stated on label. Red Cap (1 vial (150ul)

4. Negative control: Range stated on label. Natural Cap (1 vial (150ul)
5. Positive control: range stated on label. Green Cap (1 vial (150ul)
6. Washing concentrate 10X: White Cap (1 bottle (100ml)
7. Enzyme Conjugate: Red color solution (1 vial (12ml)
8. TMB Chromogenic Substrate: Amber bottle (1 vial (12ml)
9. Stop solution (1 vial (12ml)

Preparation for assay

One (1x) Washing buffer was prepared by adding distilled water to 10x wash concentrate to a final volume of 1 litre.

Assay procedure

One in forty dilutions were prepared by adding five µl of the test samples, negative control, positive control and calibrator to 200ul of sample diluents and mixing well, then 100ul of the diluted sera, calibrator and controls were dispensed into the appropriate wells. For the reagent blank, 100ul of sample diluent was dispensed into 1A well position. The holder was tapped to remove air bubbles from the liquid and to mix well. The samples were then incubated for 30 minutes at room temperature. After 30 minutes, liquid was removed from all wells and they were washed three times with washing buffer. Later, 100µl of enzyme conjugate was then dispensed into each well and wells were incubated at room temperature for another 30 minutes. Excess enzyme conjugate was then removed from all wells and they were washed three times with washing buffer. One hundred (100) µl of TMB chromogenic substrate was dispensed into each well and the wells were incubated for another 30 minutes at room temperature.

At the end of 30 minutes, 100ul of 2N HCl was added to stop the reaction. After addition of stop solution, the wells developed yellow coloration with different intensities. The colour intensity of each well is proportional to the antibody concentration in it. After making sure

that there were no air bubbles in each well, the holder was taken to the microwell reader and the optical density (OD) read at 450nm. The result was printed out by a printer built in the ELISA machine.

Calculation of results

To obtain cut off OD value, the OD of the calibrator was multiplied by factor (f) printed on the label calibrator. The Chlamydia IgG index of each determination was then calculated by dividing the OD value of each sample by the obtained OD value of cut-off.

a. Calibrator absorbance = 4.00
b. Factor label value = 0.35 (As provided by the manufacturer)
c. Cut-off absorbance = 4.00 X factor label value

4.00 X 0.35 = 1.4

Interpretation of results

IgG index of 0.9 or less is sero-negative for IgG antibody, IgG index of 1.0 or greater is sero-positive for IgG antibody while IgG index of 0.91 – 0.99 is equivocal.

3.7.2 Test for HIV

Determine kits for the test of HIV1/2 manufactured by Alere Medical Co. Japan were used to screen the blood samples. It is an in vitro, visually read, qualitative immunoassay for the detection of antibodies to HIV-1/2 in human serum, plasma or whole blood.

Principle of the test Procedure

Alere Determine kit for the test of HIV1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV-1/2. Sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium

colloid –antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site.

If antibodies to HIV-1and/or 2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site.

If antibodies to HIV-1and/or 2 are absent in the sample, the antigen- selenium colloid flows past the patient window and no red line is formed at the patient window site.

Materials Provided

Determine kits for the test of HIV1/2 Test card, 10 cards (10 tests/card) HIV-1/2 recombinant antigen and synthetic peptide coated and 1 Bottle (2.5ml) Chase buffer (list No. 7D2243) prepared in phosphate buffer.

Test Procedure

The protective foil cover was first removed from each test and 50ul of sample was added to the sample pad and the result was read in a minimum of fifteen minutes.

Results Interpretation

Tests were considered **positive** when two red bars appeared in both the control window and the patient window of the strip, they were considered **negative** when one red bar appeared in the control window of the strip and no red bar appeared in the patient window of the strip and finally **Invalid when n**o red bar appeared in both the control window of the strip and patient window of the strip.

The Determine positive samples were further tested using HIV 1/2 STAT-PAK DIPSTICK kit manufactured by Chembio Diagnostic Systems, INC, USA.

HIV 1/2 STAT-PAK DIPSTICK kit

The Chembio HIV 1/2 STAT-PAK DIPSTICK assay is a single use, immunochromatographic screening test which uses a cocktail of antigens to detect antibodies to HIV 1 and 2 in serum, plasma or whole blood. Reactive results are supportive evidence of exposure to HIV1/2 and can be used to support a clinical diagnosis of HIV 1 or HIV 2. Non reactive results, however, should not be used to exclude infection with HIV 1 or 2.

Principle of Test

The Chembio HIV 1/2 STAT-PAK DIPSTICK assay employs a combination of antibody binding protein, which is conjugated to colloidal gold dye particles, and antigens to HIV 1/2, which are bound to the membrane solid phase. The sample being tested and running buffer are applied to the sample pad. The running buffer facilitates the lateral flow of the specimen through the membrane and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye- conjugated immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST area producing a pink/purple line. In the absence of HIV 1/2 antibodies, the sample continues to migrate along the membrane and produces a pink/purple line in the control area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimens and reagents have been applied properly and have migrated through the device.

Materials Provided

Each kit contains 30 HIV 1/2 STAT-PAK DIPSTICK test strips, 1 Running Buffer bottle (8ml), 30 Disposable sample loops (5µl), 30 Backing Cards, 1 Product Insert to perform 30 tests.

Test procedure

The backing cards were first removed and one segment was torn off for each test to be run. Specimen identity was entered in the space marked ID on the card. The HIV 1/2 STAT-PAK DIPSTICK test strip was removed from the vial and the red liner was as well removed from the adhesive strip on the back of the test strip then discarded. The test strip was placed unto the backing card in the space marked with green tape facing up and the arrows on the tape facing in the same direction as the arrows on the backing card. The backing card and dipstick assembly were placed on a clean flat surface. The five µl sample loop provided was used to touch the sample to fill the circular opening of the loop with sample. Holding the sample loop vertically, it was touched to the sample pad in the centre of the SAMPLE (S) area of the dipstick to dispense five µl of sample on the sample pad. The running buffer bottle was inverted and held vertically over the sample area to add three drops of buffer onto the sample. The test result was read between 15 and 20 minutes after the addition of the running buffer.

Interpretation of result

Two pink lines, one in the TEST area and one in the CONTROL area indicated a reactive result and one pink coloured line in the CONTROL area with no coloured line in the TEST area indicate non-reactive result. When no distinct line was visible in the CONTROL area that indicated the test was invalid.

3.7.3 Chlamydia Testing by PCR

Ten endocervical swab Samples corresponding to Chlamydia ELISA positive sera were selected for each of the four study sites to make a total of forty (40) and were subjected to PCR assay.

Preparation of samples for PCR

*DNA extraction***:** This was done using DNA extraction kit ZR Genomic DNA^{TM} –Tissue MiniPrep D3050 manufactured by ZYMO RESEARCH CORP. The kit contained: Proteinase K, and storage buffer (2x5mg), 2X digestion buffer (5ml), Genomic lysis buffer (50ml), DNA Pre-wash Buffer (15ml), g-DNA Wash buffer (50ml), DNA Elution Buffer (10ml), Zymo-Spin II Columns and collection Tubes (100 tubes).

Procedure

The swab extract in phosphate buffered saline was adjusted to 100µl with water in a microcentrifuge tube and then 95µl of 2X Digestion Buffer and five µl of Proteinase K were added. The mixture was swirled and the tube incubated at 55^{o}C for 20 minutes, then 700µl of Genomic Lysis Buffer was added to the tube and mixed thoroughly by vortexing, and the mixture was transferred to Zymo-spin II Column in a collection tube and centrifuged at 10000xg for one minute. An aliquot of 200µl of DNA Pre-Wash Buffer was added to the spin column in a new collection tube and centrifuged at 10000 x g for one minute before 400µl of g-DNA Wash Buffer was added to the spin column and centrifuged at 10,000 x g for one minute. The spin column was transferred to a clean microcentrifuge tube followed by addition of 50µl of DNA Elution Buffer was added to the microcentrifuge tube and the tube incubated for five (5) minutes at room temperature. Thereafter the tube was centrifuged at 12000 x g for 30 seconds to elute the DNA. The eluted DNA was used immediately for the PCR assay.

End point PCR

Primers: The following pairs of oligonucleotide primers specific for the omp1 *C. trachomatis* gene coding for the major outer membrane protein (MOMP) were selected. The sequences of these oligonucleotide primers were as follows:

Primer 1

Sense: TGACTTTGTTTTCGACCGTGTT (198-221)
Antisense: ACATTCCCAC/GAA/GAGCTGC (621-604) (Brunham *et al.,* 1994)
Amplicon size: 345bp

Primer 2

Sense: CT1 (5_-GCC GCT TTG AGT TCT GCT TCC TC-3_) and
Anti sense: CT5 (5_-ATT TAC GTG AGC AGC TCT CTC AT-3_) (Ngandjio *et al.,* 2003)
Amplicon size: 245bp.

PCR Mixture and DNA Amplification

For PCR, Gene Amp(R) PCR reagents from Perkin Elmer, U.S.A. with AmpliTaq(R) DNA polymerase was used. A master mixture of these reagents was made for the samples. The final reaction mixture of 50µI for each sample contained 0.5µM each primer; 100µM each of dATP, dCTP, dGTP and dTTP; 50 mM KCI; 10 mM Tris-HCI, pH 8.3; 1.5 mM MgCI2; 0.01% gelatin and 1.25 units of DNA polymerase (*Thermus aquaticus*) enzyme and 9ul of sample DNA. Each microfuge tube containing the PCR mix of 50µI was mixed and subjected to 40 cycles of amplification. Each cycle composed of sequential incubations of 94°C for 1 minute for DNA denaturation, 52°C for 1 minute for annealing primer to these templates, and 72°C for 2 minutes for DNA chain extension. At the end of 40 cycles, samples were kept for another 7 minutes at 72°C for completion of extension of DNA chain. The PCR product samples were immediately frozen for further analysis.

Amplified Product Detection

Visualisation of amplified product was carried out by agarose gel electrophoresis. A 10µI of post-PCR mixture was subjected to electrophoresis on 1% agarose gel in presence of ethidium bromide. A DNA ladder was also run simultaneously to confirm the size of the amplified product.

1% Agarose gel was prepared by mixing 1g of agarose into 2ml of Tris-Acetate EDTA (TAE) and 98ml of distilled water and heating the mixture at $55^{o}C$ for two minutes in a microwave. Ten microlitre (10µl) of Ethidium Bromide was added to the heated mixture and poured into a gel tray fitted in a gel tank. Combs were inserted into the molten gel and it was allowed to solidify at room temperature. After solidification of the agarose gel, 300 ml of TAE Buffer (6ml TAE + 294 ml distilled water) was poured into the gel tank and the combs were gently removed. Ten microlitre (10µl) of DNA ladder was loaded into the first well of the gel while 10µl of each PCR product was loaded into the remaining wells accordingly. The electrophoresis machine was set at 60V for 45 minutes. The Gel was then viewed under the UV light of the gel documentation and documented.

3.7.5 Statistical Analysis of the Results

A univariate analysis of association with chlamydial infection was performed on all potential risk factors by using the chi-square test. Data analysis was performed with GraphPad Software Inc.

CHAPTER FOUR

4.0 RESULTS

4.1 Seroprevalence of *Chlamydia* IgG Antibody among Pregnant Women and Gynaecologic Patients Attending some Tertiary Hospitals in the North-Central Nigeria

Altogether, six hundred (600) blood samples were collected and tested, of which four hundred (400) were from pregnant women and two hundred (200) from gynaecologic patients. Of the two hundred (200) gynaecologic patients, 113 (56.5%) were pelvic inflammatory disease (PID) patients, 76 (38.0%) were infertility patients while 11(5.5%) were ectopic pregnancy patients. The distribution of the study participants is shown in Figure 4. Of the total six hundred (600) blood samples analysed, three hundred and fifty four (354) were positive for *Chlamydia* IgG antibody, indicating a prevalence of 59.0%. This result is shown in Figure 5.

4.2 Seroprevalence of *Chlamydia* IgG Antibody among Pregnant Women and Gynaecologic Patients Attending some Tertiary Hospitals in the North-Central Nigeria Based on the Study Groups

The study population was made up of two groups, pregnant women and gynaecologic patients. Of the 400 pregnant women tested, 230 were positive for *Chlamydia* IgG with a prevalence rate of 57.5% while 124 out of the 200 gynaecologic patients tested positive giving a prevalence rate of 62.0%. Chi square analysis of the result showed a statistically significant difference ($\chi 2$ =111.0, P = 0.0001), between the prevalence rates among the pregnant women and the gynaecologic patients. The results are as shown in Figure 6.

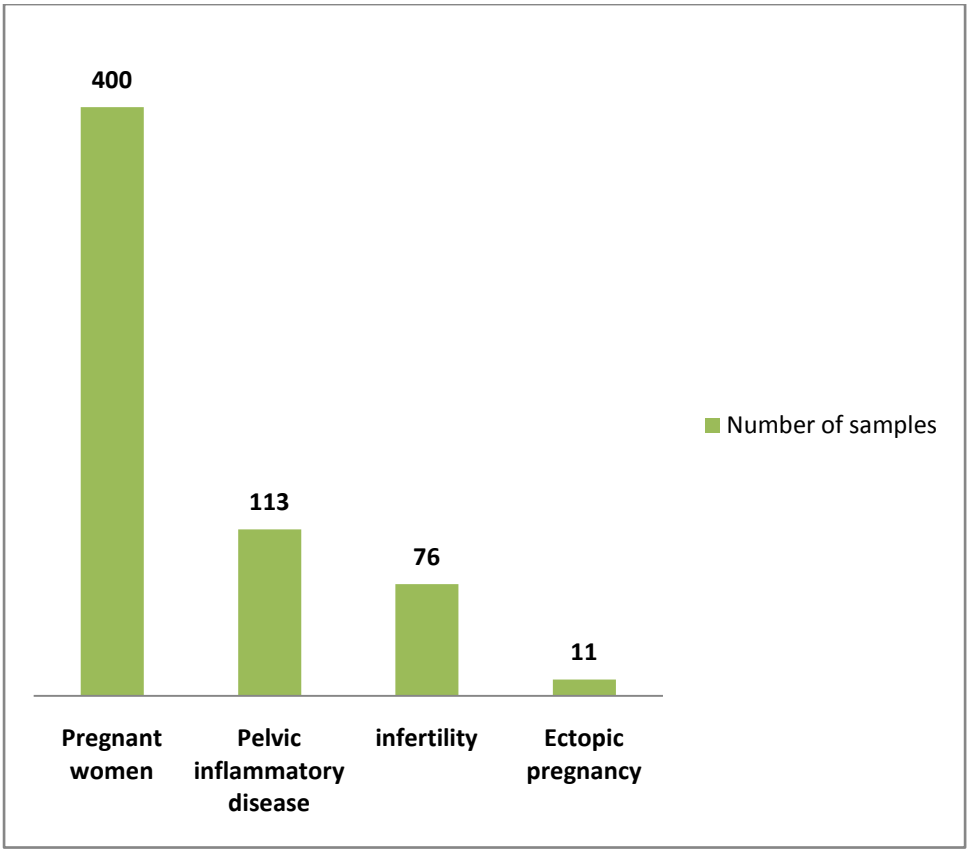


Figure 4: Distribution of the Study Population

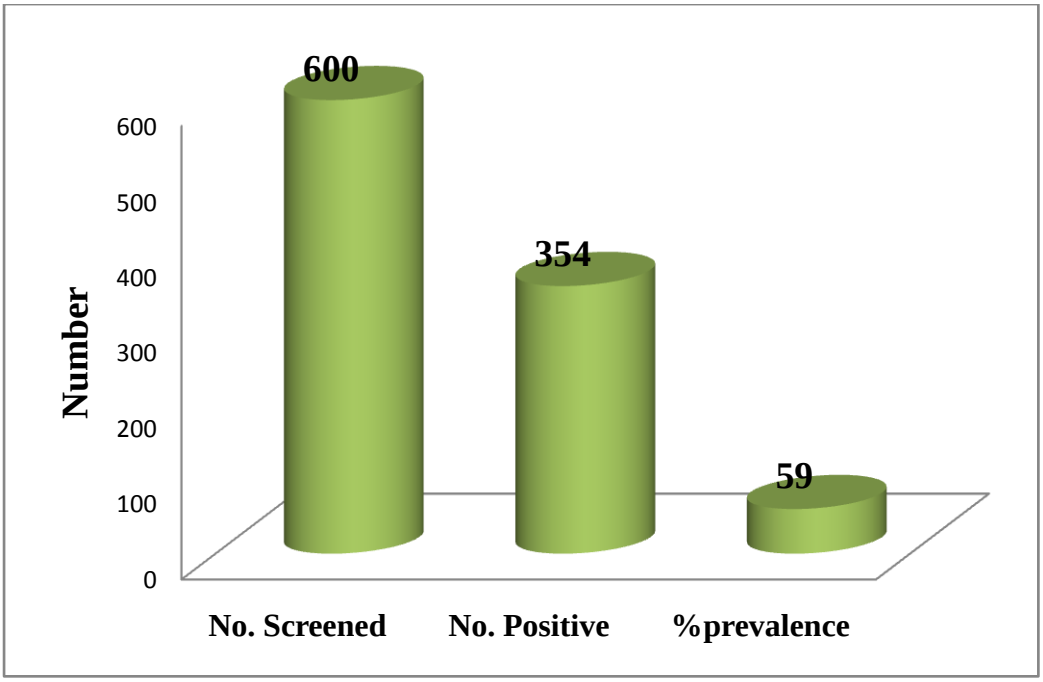
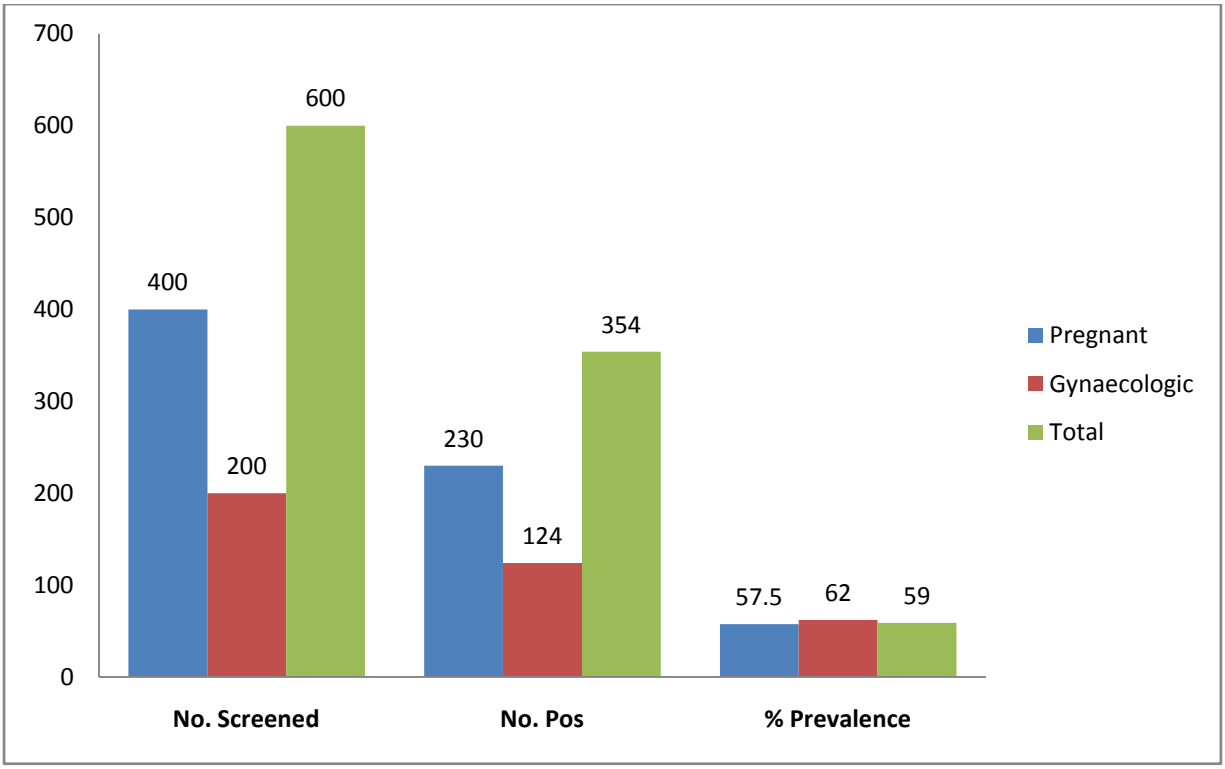


Figure 5: Seroprevalence of *Chlamydia* IgG Antibody among Pregnant Women and Gynaecologic Patients Attending some Tertiary Hospitals in the North-Central Nigeria



$\chi 2 = 111.0$, $P = 0.0001$

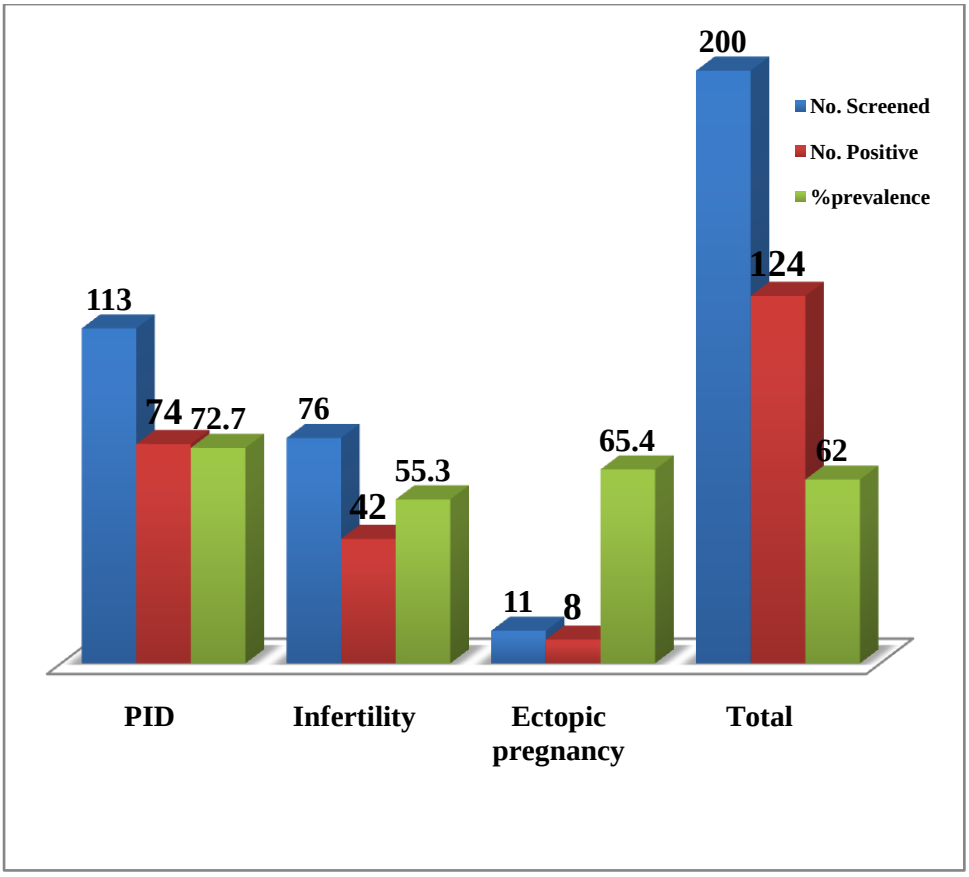
Figure 6: Seroprevalence of *Chlamydia* IgG Antibody among Pregnant Women and Gynaecologic Patients Attending some Tertiary Hospitals in the North-Central Nigeria Based on the Study Groups

4.3 Percentage Distribution of *Chlamydia* IgG Antibody among Gynaecologic Patients Attending some Tertiary Hospitals in the North-Central Nigeria

The gynaecologic group was made up of patients of pelvic inflammatory disease (PID), infertility and ectopic pregnancy. Of the 113 patients of PID tested, 74 were positive giving a prevalence rate of 65.5% and out of the 76 infertility patients tested, 42 were positive giving a prevalence rate of 55.3% while 8 of the 11 ectopic pregnancy patients tested positive with a prevalence rate of 72.7%. The results are as shown in Figure 7. Chlamydia was not found to be statistically associated with the Chlamydia complications in the study participants; PID (OR: 1.4, CI: 0.91 – 2.15), Infertility (OR: 0.84, CI: 0.52 – 1.36) and Ectopic pregnancy (OR: 1.87, CI: 0.49 – 7.13) even though the prevalence rates were high.

4.4 Seroprevalence of *Chlamydia* IgG Antibodies in the North-Central Nigeria Based On the Sampling Sites

The six hundred blood samples constituted one hundred and fifty (150) from each of the Hospitals viz; The National Hospital, Abuja, the Federal Medical Centre, Makurdi, Federal Medical Centre Lokoja, and finally, General Hospital, Minna. Of the 150 samples from each site, 100 were from regnant women and 50 from gynaecologic patients. Of the 150 samples assayed from each of the sites, 127, 107, 77 and 43 samples were found positive for the National Hospital, Abuja, the Federal Medical Centre, Makurdi, Federal Medical Centre Lokoja, and the General Hospital, Minna, respectively giving prevalence rates of 84.7%, 71.3% 51.3% and 28.7% respectively. The sero-prevalence of *Chlamydia* IgG antibodies in the North-Central Nigeria based on the sampling sites is shown in Figure 8.



$\chi 2$= 2.55 P=0.279

Figure 7: Percentage Distribution of *Chlamydia* IgG Antibody among Gynaecologic Patients Attending some Tertiary Hospitals in the North-Central Nigeria

Key:-

PID: Pelvic Inflammatory Disease

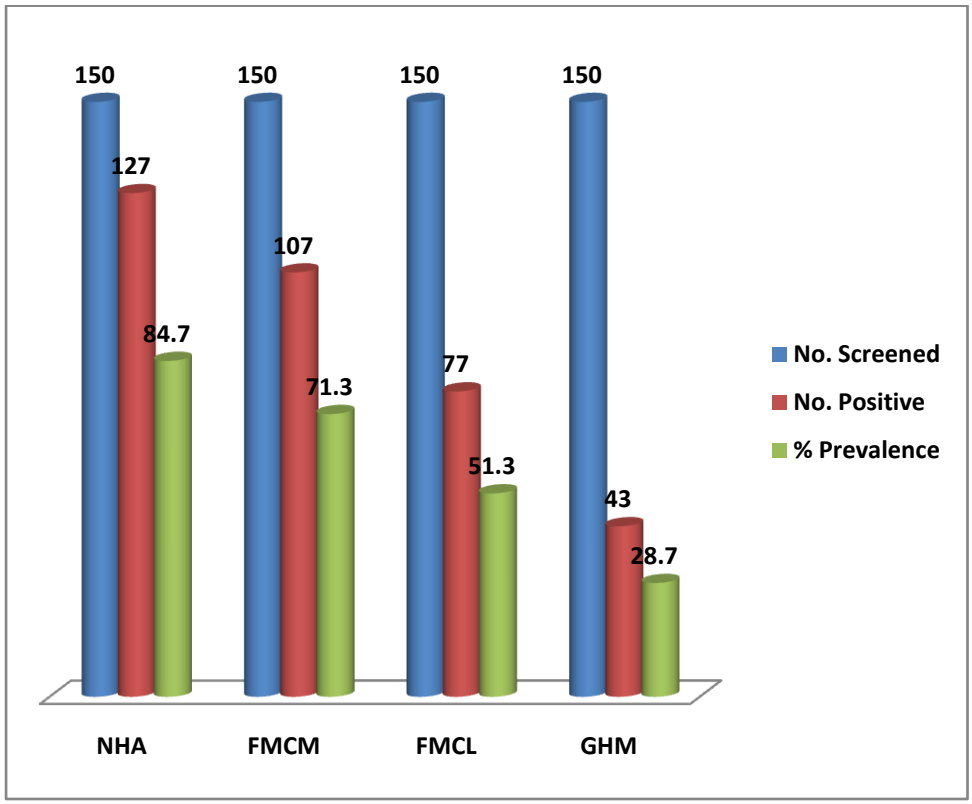


Figure 8: Seroprevalence of *Chlamydia* IgG Antibodies in some Tertiary Health Care Centers in the North-Central Nigeria based on the sampling sites

Key:

NHA: National Hospital, Abuja FMCM: Federal Medical Center, Makurdi

FMCL: Federal Medical Center, Lokoja GHM: General Hospital, Minna

4.5 Prevalence of HIV 1/2 among the Study Participants in Some Tertiary Hospitals of the North-Central

All the participants in the study groups were also screened for HIV to determine the relationship between Chlamydia and HIV among them. Of the 600 samples tested for HIV, 87 were positive giving a prevalence rate of 14.5%. Abuja had the highest prevalence rate of 24.6% (37 in 150), followed by Benue, 16.7%, (25 in 150) then Kogi, 12.0% (18 in 150) and finally, Niger, 4.7% (07 in 150). The difference between the various parts was statistically significant. The results of HIV prevalence in the North Central are shown in Figure 9.

4.6 Relationship between Chlamydia and HIV in Some Tertiary Hospitals of the North Central

As for relationship between Chlamydia and HIV, 61 subjects tested positive for both Chlamydia and HIV, 293 were positive for Chlamydia only while 18 had only HIV. Results are shown in Figure 10.

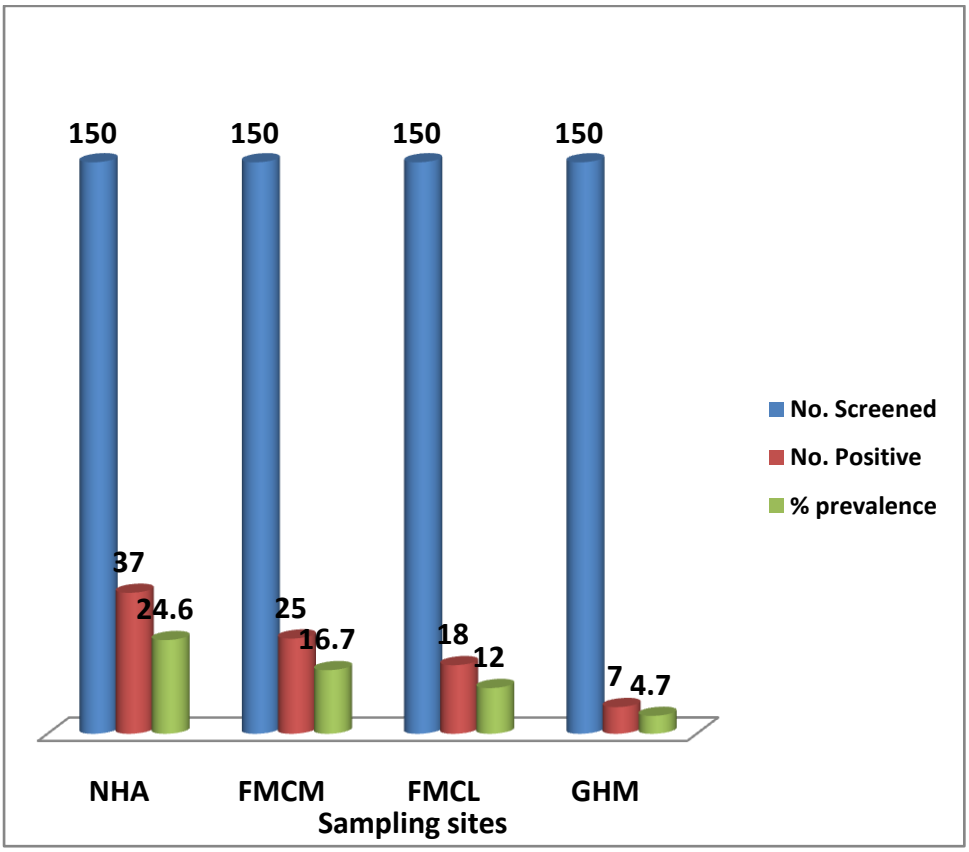
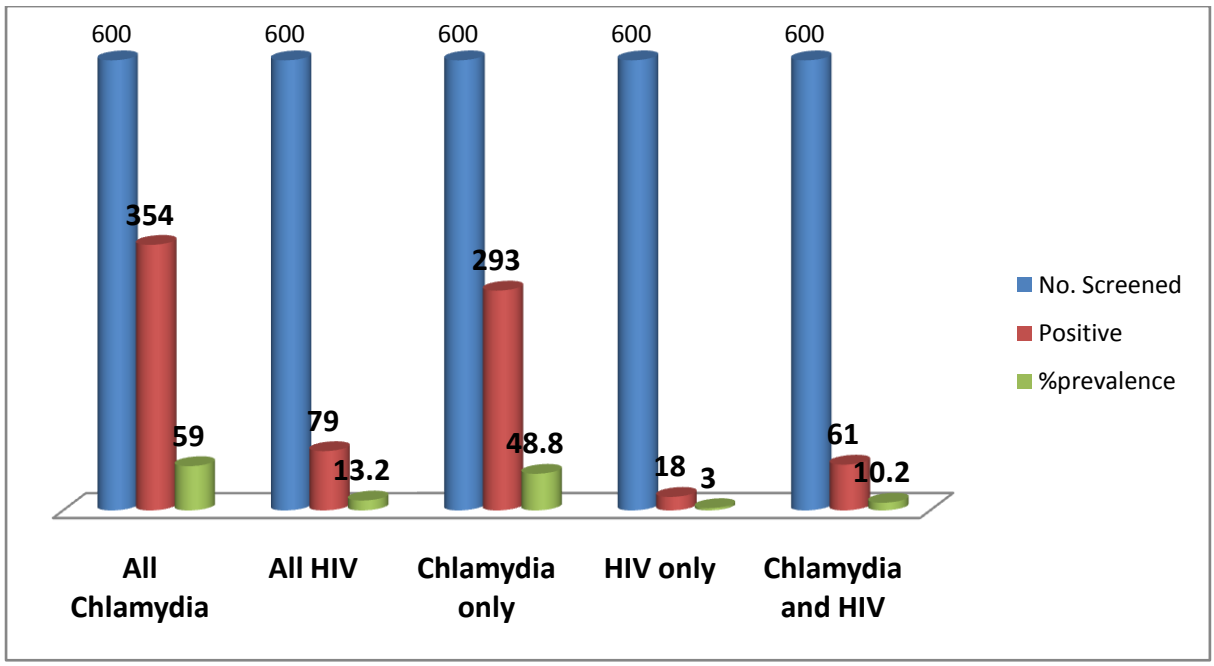


Figure 9: Prevalence of HIV 1/2 among the Study participants in some Tertiary Health Care Centers in the North-central, Nigeria

Key:

NHA: National Hospital, Abuja FMCM: Federal Medical Center, Makurdi

FMCL: Federal Medical Center, Lokoja GHM: General Hospital, Minna



$\chi 2 = 779.7$ $p < 0.0001$

Figure 10: Relationship between Chlamydia and HIV in Some Tertiary Hospitals of the North Central, Nigeria

4.7 Seroprevalence of *Chlamydia* IgG Antibodies in the National Hospital, Abuja Based On the Study Groups

The group of gynaecologic cases had a higher prevalence rate of 92.0% while the group of pregnant women had a lower rate of 81%. The two prevalence rates were high but there was no statistically significant difference between the two groups as shown in Table 1.

4.8 Seroprevalence of *Chlamydia* IgG Antibodies among Gynaecologic Patients Attending the National Hospital, Abuja

In the National Hospital, Abuja, of the three goups of gynaecologic cases considered, pelvic inflammatory disease patients had the highest prevalence rate of 72.7% while infertility patients had the lowest; of 55.3%. The seroprevalence of Chlamydia IgG antibodies in the Hospital based on the various gynaecologic cases is shown in Table 2. There was no statistically significant difference between the prevalence rates in the various cases.

4.9 Seroprevalence of *Chlamydia* IgG antibodies in the Federal Medical Center, Makurdi

In Federal Medical Center, Makurdi, of the two study groups considered, the sero-prevalence of *Chlamydia* IgG though high in both groups, it was higher among the gynaecologic cases (76.0%) than in the pregnant women (69.0%). There was also no statistically significant difference between the two groups. The result is as shown below in Table 3.

Table 1: Seroprevalence of *Chlamydia* IgG Antibodies in the National Hospital, Abuja Based On the Study Groups

Group	No. Screened	No. Positive	%prevalence
Pregnant	100	81	81.0
Gynaecologic	50	46	92.0
Total	150	127	84.7
$\chi 2$= 3.15	P=0.076		

Table 2: Seroprevalence of *Chlamydia* IgG Antibodies among Gynaecologic Patients Attending the National Hospital, Abuja

Cases	No. Screened	No. Positive	%prevalence
PID	24	23	72.7
Infertility	23	21	55.3
Ectopic pregnancy	03	02	65.4
Total	50	46	92.0

$\chi 2 = 2.13$ P = 0.34

Table 3: Seroprevalence of *Chlamydia* IgG antibodies in the Federal Medical Center, Makurdi

Group	No. Screened	No. Positive	%prevalence
Pregnant	100	69	69.0
Gynaecologic	50	38	76.0
Total	150	107	71.3
$\chi 2 = 1.07$	P= 0.301		

4.10 Seroprevalence of *Chlamydia* IgG Antibodies among Gynaecologic Patients Attending the Federal Medical Center, Makurdi

Looking at the seroprevalence among the three groups of the gynaecologic cases considered in the Federal Medical, Center, Makurdi, Ectopic pregnancy group recorded the highest rate of 100%, followed by the pelvic inflammatory disease and the infertility groups which had almost thesame rates of 75% and 73% respectively. There was also no statistically significant difference between the groups. The results are as shown below in Table 4.

4.11 Seroprevalence of *Chlamydia* IgG Antibodies in Federal Medical Center, Lokoja Based on the Study Groups

The seroprevalence of *Chlamydia* IgG antibodies in Kogi based on the study groups showed that pregnant women had higher prevalence rate of 56.0% than the gynaecologic cases group (42.0%). However, there was no statistically significant difference between the two groups. The results are as shown below in Table 5.

4.12 Seroprevalence of *Chlamydia* IgG Antibodies among Gynaecologic Patients Attending the Federal Medical Center, Lokoja

The seroprevalence of *Chlamydia* IgG antibodies in Kogi based on the gynaecologic cases showed that the ectopic pregnancy group had the highest prevalence of 66.7% followed by the infertiliy group (54.5%) and finally the PID group (22.7%). There was a statistically significant difference between the various groups as shown in Table 6.

Table 4: Seroprevalence of *Chlamydia* IgG Antibodies among Gynaecologic Patients Attending the Federal Medical Center, Makurdi

Cases	No. Screened	No. Positive	%prevalence
PID	33	25	75.0
Infertility	15	11	73.0
Ectopic pregnancy	02	02	100.0
Total	50	38	76.0

$\chi 2 = 1.07$ P = 0.16

Key:-

PID: Pelvic Inflammatory Disease

Table 5: Seroprevalence of Chlamydia IgG antibodies in Kogi based on the study groups

Group	No. Screened	No. Positive	%Prevalence
Pregnant	100	56	56.0
Gynaecologic	50	21	42.0
Total	150	77	51.3
$\chi 2 = 2.65$	P = 0.104		

Table 6: Seroprevalence of Chlamydia IgG antibodies in Kogi based on the gynaecologic cases

Cases	No. Screened	No. Positive	%Prevalence
PID	22	12	22.7
Infertility	22	12	54.5
Ectopic pregnancy	06	04	66.7
Total	50	21	42.0

$\chi 2 = 6.08$ P = 0.048

Key:-

PID: Pelvic Inflammatory Disease

4.13 Seroprevalence of *Chlamydia* IgG antibodies in General Hospital, Minna Based on the Study Groups

The seroprevalence of *Chlamydia* IgG antibodies in Niger based on the study groups indicated that the gynaecologic cases had higher prevalence (38.0%) than the pregnant women (24.0%). The difference in prevalence was however not statistically significant. The result is as presented in Table 7.

4.14 Seroprevalence of *Chlamydia* IgG Antibodies among Gynaecologic Patients Attending the General Hospital, Minna

The seroprevalence of *Chlamydia* IgG antibodies in Niger based on the three types gynaecologic cases considered indicated that the PID cases group recorded the highest prevalence (44.0%) followed by the infertility group (25.0%) and there was no case of ectopic pregnancy throughout the period of sample collection at this site. The difference in prevalence was also not statistically significant, and the result is as presented in Table 8.

Table 7: Seroprevalence of *Chlamydia* IgG Antibodies in General Hospital, Minna Based on the Study Groups

Group	No. Screened	No. Positive	%Prevalence
Pregnant	100	24	24.0
Gynaecologic	50	19	38.0
Total	150	43	28.7
$\chi 2 = 3.24$	P = 0.072		

Table 8: Seroprevalence of *Chlamydia* IgG Antibodies among Gyanecologic Cases Attending the General Hospital, Minna

Group	No. Screened	No. Positive	%prevalence
PID	34	15	44.0
Infertility	16	04	25.0
Ectopic pregnancy	-	-	-
Total	50	19	38.0

$\chi 2 = 1.72$ P = 0.423

Key:-

PID: Pelvic Inflammatory Disease

4.15 Chlamydia and HIV Co-infection in the National Hospital, Abuja

The relationship between chlamydia and HIV was considered at the various sites of the study. In the National Hospital, Abuja an association between chlamydia and HIV was recorded with $\chi 2$ of 11.39 and P-value of 0.0007 as shown below in Table 9.

4.16 Chlamydia and HIV Co-infection in the Federal Medical Center, Makurdi

In the Federal Medical Center, Makurdi, there was no statistically significant association between chlamydia and HIV as shown below in Table 10.

4.17 Chlamydia the Federal Medical Center, Lokoja

In the Federal Medical Center, Lokoja, there was also no statistically significant association between chlamydia and HIV as shown below in Table 11.

4.18 Chlamydia and HIV co-infection inthe General Hospital, Minna

In the General Hospital, Minna, a statistically significant association between chlamydia and HIV was recorded with χ^2 of 4.65 and P-value of 0.031 as shown below in Table 12.

Table 9: Chlamydia and HIV Co-infection in the National Hospital, Abuja

		Chlamydia	
		Positive	Negative
HIV	Positive	21	09
	Negative	106	12
$\chi 2 = 11.39$	P = 0.0007		

Table 10: Chlamydia and HIV Co-infection in the Federal Medical Center, Makurdi

		Chlamydia	
		Positive	Negative
HIV	Positive	12	06
	Negative	95	37
$\chi 2 = 0.19$	P = 0.603		

Table 11: Chlamydia and HIV Co-infection in the Federal Medical Center, Lokoja

		Chlamydia	
		Positive	Negative
HIV	Positive	06	03
	Negative	71	70
$\chi 2 = 0.93$	P = 0.335		

Table 12: Chlamydia and HIV Co-infection in the General Hospital, Minna

		Chlamydia	
		Positive	Negative
HIV	Positive	03	01
	Negative	40	106
$\chi 2 = 4.65$	P = 0.031		

4.19. Agarose-gel profiles of the Polymerase Chain Reaction (PCR) products for primer 1

Lanes M2 is the 100bp ladder with standard molecular weights. Lane 6 is a sample positive for *Chlamydia trachomatis* showing specific amplification of the 345 bp outer membrane protein 1 (omp1) gene of *Chalamydia trachomatis.* Figure 11 shows the documented gel image of the result.

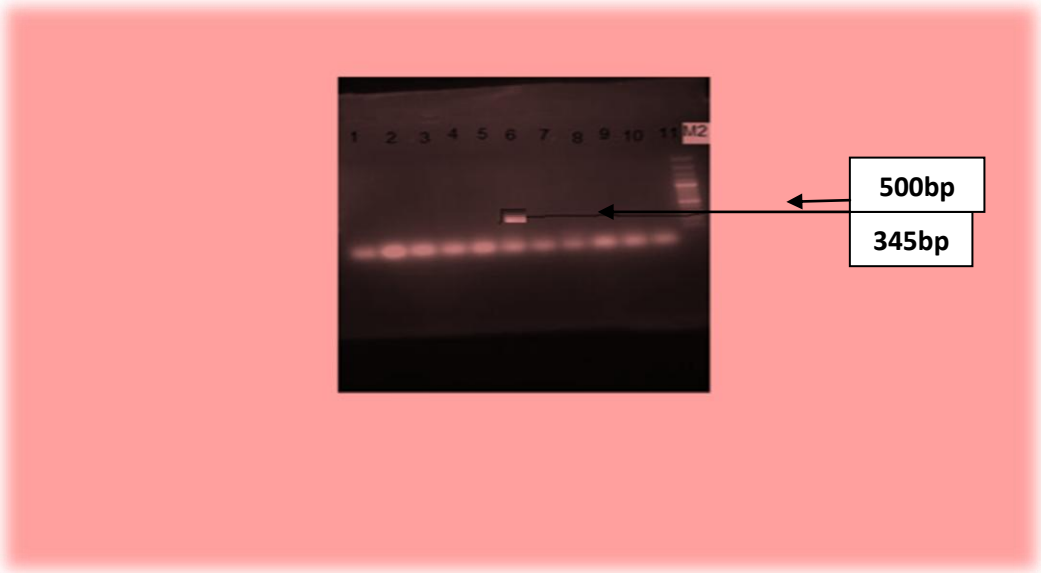


Plate 1: Agarose – gel Image of the PCR for *Chlamydia trachomatis omp*1 Gene Using Primer 1 for Samples 1-11

M2 is a 100bp ladder, Lanes 1- 11 are the samples with only Lane 6 showing an amplicon

4.20. Agarose-gel profiles of the Polymerase Chain Reaction (PCR) products for primer 2

On the upper row, Lane 1 (labelled M1) is the 100 base pair ladder with standard molecular weights. Lanes 2 to 13 are the samples. There was no amplicon in this row indicating negative result. On the lower row, lane 1 (labelled) M2 is a 100 base pair ladder with standard molecular weight, while lanes 2 to 5 are the samples. Lane 2 is a sample positive for *Chlamydia trachomatis* showing specific amplification of the 245 bp outer membrane protein 1 (omp1) gene of *Chlamydia trachomatis.*

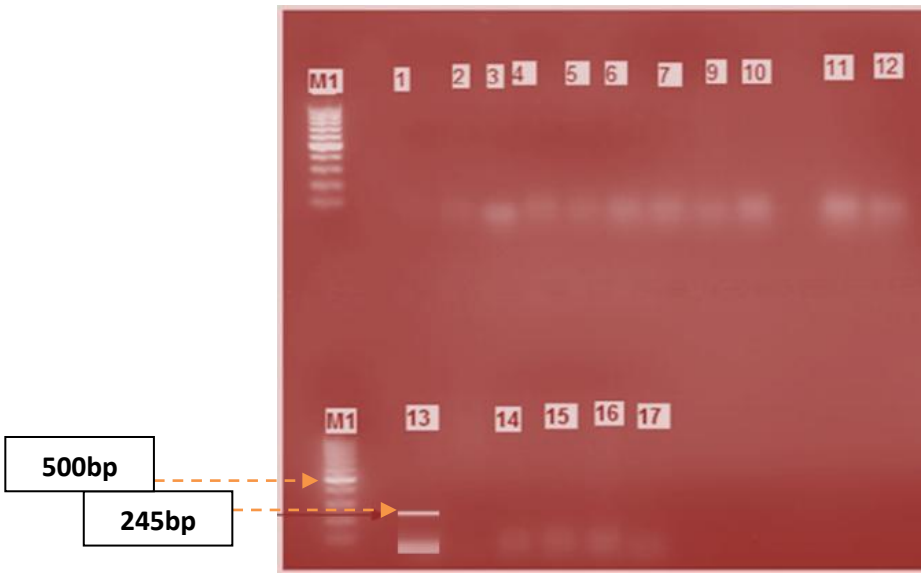


Plate 2: Agarose – gel Image of the PCR for *Chlamydia trachomatis omp*1 Gene Using Primer 2 for Samples 1-17.

Upper row; Lane 1 (M1) is the 100bp ladders with standard molecular weight. Lanes 2 to 13 are the samples with all showing no amplicon.

Lower row; Lane 1 (M1) is the 100bp ladder with standard molecular weights. Lanes 2 to 5 are the samples with only sample 13 (Lane 2) showing an amplicon indicating presence of the amplified 245 bp portion of the *omp*1 gene of *Chlamydia trachomatis*.

4.23 Seroprevalence of *Chlamydia* IgG Antibodies in some Tertiary Health Care Centers in the North-Central Nigeria Based on Age Groups of the Study Participants

As for age groups, the results showed that the age group of 15-19 had the highest prevalence in both the pregnant women and the gynaecologic cases while the age group of 40-44 recorded the lowest rate in both. The difference between the various age groups in the pregnant women was statistically significant but insignificant for the gynaecologic cases.This can be seen as presented in Table 13.

4.24 Seroprevalence of *Chlamydia* IgG Antibodies in some Tertiary Health Care Centers in the North-Central Nigeria based on the Age at Marriage of the Study Participants

When the age at marriage of the study participants was considered, those married at teenage had the highest prevalence of 69.3% and 67.0% for the pregnant women and the gynaecologic patients respectively. There was a statistically significant difference between the different age groups of marriage for the pregnant women but none for the gynaecologic cases. The results are as shown in Table 14.

4.25 Seroprevalence of *Chlamydia* IgG Antibodies in some Tertiary Health Care Centers in the North-Central Nigeria Based on the Age at First Intercourse of the Study Participants

The result of seroprevalence of *Chlamydia* IgG antibodies in parts of the North-Central Nigeria based on the age at first intercourse of the study participants showed that among the gynaecologic cases, the age group of 15 – 19 had the highest prevalence of 78.0% while those of age group 30 – 34 had the lowest of 20.0%. There was statistically significant difference between the age groups. Of the 400 pregnant women recruited for the study, only 236 of them responded to this question out of which 76 were positive for Chlamydia. The difference between the various age groups was however not significant statistically. The results are as presented in Table 15.

Table 13: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Age Groups of the Study Participants

Pregnant women				Gynaecologic cases			
Age group	**No. Screened**	**No.pos**	**%prev**	**Age group**	**No. screened**	**No. Pos**	**%prev**
15-19	34	26	76.5	15-19	15	11	73.3
20-24	97	63	64.9	20-24	30	20	66.7
25-29	125	85	68.0	25-29	65	45	69.0
30-34	91	42	46.2	30-34	45	28	62.2
35-39	29	09	31.0	35-39	30	14	46.7
40-44	24	05	20.8	40-44	15	06	40.0
Total	400	230	57.5	Total	200	124	62.0
$\chi 2 = 42.83$		P<0.0001		$\chi 2 = 8.61$		P = 0.125	

Table 14: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Age at Marriage of the Study Participants

Pregnant women				Gynaecologic cases			
Age at marriage	**No. Screened**	**No. pos**	**%prev.**		**No. screened**	**No. pos**	**%prev.**
15-19	137	95	69.3	15-19	58	39	67.0
20-24	153	81	52.9	20-24	49	29	59.0
25-29	78	46	58.9	25-29	33	19	57.0
30-34	18	05	27.8	30-34	17	09	52.0
35-39	14	03	21.4	35-39	43	28	65.0
Total	400	230	57.5	40-44	200	124	62.0
$\chi 2 = 23.48$	P=<0.0001				$\chi 2 = 2.01$	P = 0.734	

Table 15: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Age at First Intercourse of the Study Participants

Pregnant women				Gynaecologic cases			
Age at first sex	**No. Screened**	**No. pos**	**%prev.**	**Age at first sex**	**No. screened**	**No. pos**	**%prev.**
15-19	63	27	42.9	15-19	96	75	78.0
20-24	79	23	29.1	20-24	54	33	61.0
25-29	56	13	23.2	25-29	30	12	40.0
30-34	38	08	21.1	30-34	20	04	20.0
Total	236	71	28.4	Total	200	124	62.0
	$\chi 2 = 7.65$	P= 0.054			$\chi^2 = 19.48$		P = 0.0002

4.26 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Educational Level of the Study Participants

The seroprevalence of Chlamydia IgG antibodies in parts of the North-Central Nigeria based on the Educational level of the study participants is shown in Table 16. From the results, starting with the group of pregnant women, the tertiary level of education group had the highest prevalence (67.4%), followed by the secondary level (62.1%) and the informal education group had the lowest (30.8%). In contrast, for the gynaecologic cases, the tertiary level of education group had the lowest prevalence (30.9%) while the secondary level had the highest (85.0%). The results were statistically significant in both study groups.

4.27 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Occupation of the Study Participants

The distribution of chlamydia infection among the study groups based on occupation showed that among the pregnant women, the group of unskilled occupation had the highest prevalence (70.8%) followed by the skilled group (53.9%) while the none group (housewives) had the lowest prevalence (36.5%). Among the gynaecologic cases however, the semi-skilled group had the highest prevalence (83.1%) followed by the unskilled group (53.8%) while the skilled group had the lowest prevalence (40.9%). The results are as shown in Table 17.

4.28 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Marital Status of the Study Participants

The distribution of chlamydia infection among the study groups based on marital status showed that among the pregnant women, the group of married had a slightly higher prevalence (57.8%) than the single group (54.3%). The difference was not statistically significant as can be seen in Table 23. In contrast, among the gynaecologic cases, the single group had a much higher prevalence (86.0%) than the married group (55.4%). The result was statistically significant and is presented in Table 18.

Table 16: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Educational Level of the Study Participants

Pregnant women				Gynaecologic cases			
Educational level	**No. screened**	**No. pos**	**%prev.**	**Educational level**	**No. screened**	**No. pos**	**%prev.**
Informal	26	08	30.8	Informal	23	11	47.8
Primary	73	28	38.4	Primary	42	27	64.3
Secondary	169	105	62.1	Secondary	80	68	85.0
Tertiary	132	89	67.4	Tertiary	55	17	30.9
Total	400	230	57.5	Total	200	124	62.0
$\chi^2 = 22.4$	P = <0.0001			$\chi^2 = 40.32$	P = 0.0001		

Table 17: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Occupations of the Study Participants

Pregnant women				Gynaecologic cases			
Occupation	**No. screened**	**No. Pos**	**%prev.**	**Occupation**	**No. screened**	**No. pos**	**%prev.**
Skilled	128	69	53.9	Skilled	44	18	40.9
Semi skilled	52	23	44.2	Semi skilled	83	69	83.1
Unskilled	168	119	70.8	Unskilled	26	14	53.8
None	52	19	36.5	None	47	23	48.9
Total	400	230	57.5	Total	200	124	62.0
$\chi^2 = 25.97$		P= <0.0001		$\chi^2 = 28.33$		P = 0.0001	

Table 18: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Marital Status of the Study Participants

Pregnant women				Gynaecologic cases			
Marital status	**No. screened**	**No. Pos**	**%prev.**	**Marital status**	**No. Screened**	**No. Pos**	**%prev.**
Married	365	211	57.8	Married	157	87	55.4
Single	35	19	54.3	Single	43	37	86.0
Total	400	230	57.5	Total	200	124	62.0
$\chi 2 = 0.155$		P = 0.694		$\chi 2 = 13.45$		P = 0.0002	

4.29 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on the Family Type of the Study Participants

The distribution of chlamydia infection among the study groups based on family type showed that among the pregnant women, the group of monogamous had the highest prevalence (60.4%) followed by the not applicable (unmarried) group (54.2%) while the polygamous group had the lowest prevalence (42.3%). The difference between the groups was statistically significant and the result is shown in Table 19. Same is the case for the gynaecologic patients, where the group of monogamous had the highest prevalence (70.7%) followed by the not applicable (unmarried) group (46.7%) while the polygamous group had the lowest prevalence (30.4%). The difference between the groups was also statistically significant and the result is shown in Table 19.

4.30 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Order of Marriage of the Study Participants

The distribution of chlamydia infection among the study groups based on order of marriage showed that among the pregnant women, the group of those in their first marriage had the highest prevalence (58.2%) followed by the group of 'not applicable' (unmarried) (54.3%).

As for the gynaecologic cases, those cases in their third marriage had the highest prevalence (66.7%) closely followed by the 'not applicable' (ie unmarried) (65.1%) and those in their second marriage (64.3%) groups, while the group of 'first' had the lowest prevalence of 58.2%. The differences were however not statistically significant and the results are shown in Table 20.

Table 19: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Family Type of the Study Participants

Pregnant women					**Gynaecologic cases**		
Family type	**No. Screened**	**No. Pos**	**%prev.**	**Family type**	**No. screened**	**No. Pos**	**%prev**
Monogamous	313	189	60.4	Monogamous	147	104	70.7
Polygamous	52	22	42.3	Polygamous	23	07	30.4
Not applicable	35	19	54.2	Not applicable	30	14	46.7
Total	400	230	57.5	Total	200	124	62.0
$\chi 2 = 6.15$	P = 0.046			$\chi 2 = 15.06$	P = 0.0005		

Table 20: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Order of Marriage of the Study Participants

Pregnant women				Gynaecologic cases			
Order of marriage	**No. Screened**	**No. Pos**	**%prev.**	**Order of marriage**	**No. screened**	**No. Pos**	**%prev.**
First	340	198	58.2	First	140	85	60.7
Second	25	13	52	Second	14	09	64.3
Third	-	-	-	Third	03	02	66.7
Not applicable	35	19	54.3	Not applicable	43	28	65.1
Total	400	230	57.5	Total	200	124	62.0
$\chi 2$ = 0.54		P= 0.99			$\chi 2$ = 2.44		P = 0.486

4.31 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Number of Sexual Partners of the Study Participants

The distribution of chlamydia infection among the study groups based on number of lifetime sexual partners showed that among the pregnant women, the group of those who have had more than one sex partner had the highest prevalence (88.2%) while the group of those who have maintained a single sex partner had the lower prevalence (50.3%) and the difference was significant statistically. As for the gynaecologic cases, such was the case where the group of those who have maintained a single sex partner had the lower prevalence (56.0%) compared to those who have had more than one partner (75.0%). The difference was statistically significant as can be seen in Table 21.

4.32 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Nature of Presentation of the Disease in the Study Participants

The distribution of chlamydia infection among the study groups based on nature of disease presentation, showed that among the pregnant women, the group of those who did not present with any symptoms of urogenital infection had higher prevalence (71.2%) while the group of those with such symptoms had lower (32.9%). The difference was found to be statistically significant. As for the gynaecologic cases, thesame trend was observed where the asymptomatic group had a higher prevalence of 67.6% compared to the symptomatic group (25.9%). The difference was also statistically significant as can be seen in Table 22.

Table 21: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Number of Sexual Partners of the Study Participants

Pregnant women				Gynaecologic cases			
No. Of sexual partners	**No. screened**	**No. Pos**	**%prev.**	**No. Of sexual partners**	**No. screened**	**No. Pos**	**%prev.**
1	324	163	50.3	1	143	81	56.6
>1	76	67	88.2	>1	57	43	75.0
Total	400	230	57.5	Total	200	124	62.0
$\chi 2 =$ 36.0		P< 0.0001			$\chi 2 = 4.961$		P = 0.026

Table 22: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Nature of the Disease presentation in the Study Participants

Pregnant women				Gynaecologic cases			
Symptomatic status	**No. Screened**	**No. pos**	**%prev.**	**Symptomatic status**	**No. screened**	**No. Pos**	**%prev.**
Symptomatic	143	47	32.9	Symptomatic	27	07	25.9
Asymptomatic	257	183	71.2	Asymptomatic	173	117	67.6
Total	400	230	57.5	Total	200	124	62.0
$\chi 2 = 55.18$	P = <0.0001			$\chi 2 = 17.078$	P = <0.0007		

4.32 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on some urogentital samples of the Study Participants

The distribution of chlamydia infection among the study groups based on the type of symptoms of the uro-genital tract infection showed that among the pregnant women, the group of those who complained of burning pain on urination had the highest prevalence (26.7%) closely followed by the group of dyspareunia (pain during intercourse) (25.9%) while the group of those with lower abdominal pain had the lowest (4.7%). The difference was found to be statistically significant. As for the gynaecologic cases, the difference was statistically significant as can be seen in Table 23.

4.33 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Number of Life Births of the Study Participants

The distribution of chlamydia infection among the study groups based on number of life births showed that among the pregnant women, the group of none (those who have not had life birth including those having their first pregnancy ie primigravida) had the highest prevalence (64.2%) while the group of those who had 7-9 life births had the lowest (37.5%). The difference was found to be statistically significant. As for the gynaecologic cases, the results followed thesame trend as can be seen in Table 24.

Table 23: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on some Urogenital Tract Infection symptoms of the Study Participants

Pregnant women				Gynaecologic cases			
UTI symptoms	**No. Screened**	**No. Pos**	**%prev.**	**UTI symptoms**	**No. Screened**	**No. Pos**	**%prev.**
AVD	99	12	12.1	AVD	56	09	16.1
BOU	60	16	26.7	BOU	36	14	38.9
DYSP	54	14	25.9	DYSP	23	11	47.8
LAP	107	05	4.7	LAP	76	21	27.6

Key: AVD = abnormal vaginal discharge, BOU= burning on urination UTI = urinary tract infection, DYSP = dyspareunia, LAP = lower abdominal pain

Table 24: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Number of Life Births of the Study Participants

Pregnant women				Gynaecologic cases			
No. Of life births	**No. Screened**	**No. Pos**	**%prev.**	**No. Of life births**	**No. screened**	**No. Pos**	**%prev.**
1-3	90	51	56.7	1-3	79	48	60.8
4-6	81	47	58.0	4-6	38	21	55.3
7-9	40	15	37.5	7-9	13	05	38.5
>9	38	20	52.6	>9	03	01	33.3
None	151	97	64.2	None	67	49	73.1
Total	400	230	57.5	Total	200	124	62.0
$\chi 2 = 0.83$		P = 0.011		$\chi 2 = 8.41$		P = 0.078	

4.35 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Adverse Pregnancy Outcomes of the Study Participants

The distribution of chlamydia infection among the study groups based on adverse pregnancy outcome showed that among the pregnant women, the group of none (i.e. those who had not experienced any adverse pregnancy outcome) had the highest prevalence (62.7%) while the group of those who have experienced stillbirth had the lowest (35.1%). The difference was not found to be statistically significant. As for the gynaecologic cases, the group of those who have had miscarriage had the highest prevalence while the lowest was seen among the group of 'none'. The difference was also not statistically significant as can be seen in Table 25.

4.36 Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Other Risk Factors of Chlamydia

The distribution of chlamydia infection among the study groups based on other risk factors for chlamydia showed that among the pregnant women, the group of those who used intrauterine device (IUD) for contraception had the highest prevalence (71.9%) followed by those who have experienced abortion (60.0%) while the group of those who smoke had the lowest prevalence (50.0%). The difference was however, not found to be statistically significant. As for the gynaecologic cases, the result is similar where the group of those who used intrauterine device (IUD) for contraception had the highest prevalence (39.4%) followed by the group of those that had history of sexually transmitted diseases (33.7%). The lowest prevalence was recorded among those who did not experience any of the other risk factors considered here. The difference was as well not statistically significant as can be seen in Table 26.

Table 25: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Adverse Pregnancy Outcomes of the Study Participants

Pregnant women				Gynaecologic cases			
Adverse pregnancy outcome	**No. Screened**	**No. pos**	**%prev.**	**Adverse pregnancy outcome**	**No. Screened**	**No. Pos**	**%prev.**
Stillbirth	54	19	35.1	Stillbirth	27	18	66.7
Preterm	27	15	55.6	Preterm	13	07	58.4
Miscarriage	78	45	57.7	Misc	29	23	79.3
None	241	151	62.7	None	131	76	58.0
Total	400	230	57.5	Total	200	124	62.0
$\chi 2 = 13.67$	P= 0.057			$\chi 2 = 5.19$	P= 0.159		

Table 26: Seroprevalence of *Chlamydia* IgG Antibodies in Some Tertiaty Hospitals in the North-Central Nigeria Based on Other Risk Factors of Chlamydia

Pregnant women				Gynaecologic cases			
Risk factor	**No. screened**	**No. Pos**	**%prev.**	**Risk factor**	**No. Screened**	**No. Pos**	**%prev.**
Previous STD	56	32	57.1	Previous STD	83	28	33.7
Use of IUD	32	23	71.9	Use of IUD	94	37	39.4
None of the above	263	146	55.5	None of the above	37	09	24.3
$\chi 2 = 3.34$		P = 0.9493			$\chi 2 = 3.68$	P= 0.4506	

Key: STD= Sexually Transmitted Disease IUD= Intrauterine Device

CHAPTER FIVE

5.0 DISCUSSION

The seroprevalence of *Chlamydia* IgG antibody in some Tertiary Hospitals in the North Central, Nigeria was found to be 59.0% in this study. This high prevalence is in line with the report by (CDC) in 2005 that in the United States, chlamydial genital infection is the most frequently reported infectious disease, and the prevalence is highest in persons aged <25 years (CDC, 2005). It is also similar to the findings of Mawak *et al*. (2011) and Dibua *et al.* (2013) which was 56.1% among gynaecologic clinic attendees in Jos and Nsukka respectively. A 51.0% prevalence among pregnant and non-pregnant women and their spouses attending pre and antenatal clinic in the College of Medicine of the University of Lagos has been reported (Okoror *et al*., 2000). A slightly lower prevalence of 40.7% of *C. trachomatis* has also been reported from the South-Eastern part of Nigeria (Okoror *et al*., 2007). Other lower prevalences of 38.3% and 31.0% in Zaria (Tukur *et al*., 2006 and Koledade *et al.*, 2014) and 13.3% in Benin City (Isibor *et al*., 2005) have also been reported.

The high prevalence found in this part of the country in this study can be considered as an evidence of endemicity in the population and this may be due to the fact that Chlamydia infections usually present with no clear cut symptoms and are as a result left untreated or mistaken for other infections such as gonorrhoea. This often leads to serious sequelae such as pelvic inflammatory disease (PID), endometrirtis, salpingitis, ectopic pregnancy and infertility (Mabey *et al*., 1992). Other factors that could be attributable to the high prevalence and endemicity are sociocultural inhibition that prevents women from reporting sexual symptoms and non-avaialbility of facility to detect the causative agent (*Chlamydia trachomatis*) in many health care centres in this part of the world (Okonofua *et al*., 1995). The high prevalence could have also been caused by the high prevalence of HIV-AIDS in the

area. According to the Technical Report of the 2010 National HIV Sero-prevalence Sentinel Survey, the North-central geopolitical zone had the highest prevalence rate of AIDS and studies have shown the association between the two infections (chlamydia and HIV) such that the presence of one facilitates that of the other. Genital Chlamydia infection has been linked to an increasing risk for acquisition of HIV disease (Laga *et al*., 1993; Ho *et al*., 1995; Brunham, *et al*., 1996; Stamm, 1999; Joyee, *et al*., 2005) while on the other hand, immunosuppresion due to HIV may lead to more severe Chlamydia disease condition like PID in those who are infected with *Chlamydia trachomatis* (Thomas, *et al*., 2002). Thus early diagnosis and treatment of Chlamydia infections is important to prevent HIV risk and devastating clinical consequence. The two infections (Chlamydia and HIV infections) have also being reported to have common risk factors such as multiple sexual partners (Joyee, *et al*., 2005).

The possible relationships between HIV infection and *Chlamydia trachomatis* are that the invasive intracellular pathogenesis of *Chlamydia trachomatis* can cause substantial damage to the genital epithelial layer which may facilitate HIV infection (Hitchcock, 1999) and immunological changes due to HIV infection may favor *Chlamydia trachomatis* (Debattista *et al*., 2002). Chlamydia is one of the non – ulcerative sexually transmitted infections which elicit localised inflammations and immune responses characterised by the infiltration and accumulation of immune cells expressing CD4 surface proteins essential for the binding of HIV prior to entry, thus also facilitating the entry of HIV (Altes *et al*., 2002; Joyee *et al*., 2005; Wodarz *et al*., 2007).

The results of this study also showed that the prevalence of Chlamydia was slightly higher among gynaecologic cases (62.0%) than in the pregnant women (57.5%). This is similar to an earlier report in Delta State by Omo-Aghoja *et al.* (2007), that the prevalence of serum

Chlamydia antibody was significantly higher in cases (65.8%) (tubal infertility) compared with controls (pregnant women) (17.3%; $P < 0.01$). Although it is not recommended for the diagnosis of lower genital tract infections, or for screening in asymptomatic patients, serological testing has been shown to be useful for diagnosing LymphoGranulomaVenerum (LGV), neonatal pneumonia and upper genital tract infections, and for the evaluation of tubal-factor of infertility (Persson, 2002; Be´be´ar and de Barbeyrac, 2009).

On the PCR detection of *Chlamydia trachomatis*, this is one of the first studies to use a PCR assay to screen for genital chlamydia among the study group in the study are, to the best of our knowledge. Various measures to minimize the risk of contamination were implemented; a limited number of samples were manipulated together and DNA extraction and PCR amplification took place in separate rooms. Of the forty (40) samples selected from the IgG ELISA positive samples, only two (5.0%) were detected to be positive by the PCR assay. This disparity in result could be due to any or all of the following reasons; the *Chlamydia* IgG test used in the serological assay could detect both past and present or active infection since IgG is the latest circulating antibody in the body (Coico *et al.*, 2003) while the PCR assay only detects active infection. In view of this, most of our study participants could be in the inactive stage of the infection and hence not detected by the PCR assay. *Chlamydia trachomatis* serology has been used for both diagnostic purpose and large epidemiological studies (Morre *et al.*, 2002), however, its test employs either group (genus) specific lipopolysaccaride (LPS) or reticulate bodies as antigens and there is potential for false positive results (Orienston, 1998). These false positive results could be due to cross reactivity from *Chlamydia pneumoniae* which is a specie of the genus *Chlamydia* as reported by Forsey, (1987) or from other bacteria such as *Acinetobacter* (Brade and Brunner, 1979; Papp *et al.*, 2014). This is in contrast to the polymerase chain reaction assay, a nucleic acid amplification assay which has been reported to be highly sensitive and specific at the same

time (Papp *et al.,* 2014). Brade and Brunner, (1979) reported earlier that the false positive results due to cross reactivity could be from high sero-prevalence of *Chlamydia pneumonie* in populations which could be up to 60%. Okoror *et al.,* 2007 also reported that women harboured antibodies to *Chlamydia pneumoniae* in a study they conducted among patients attending gynaecological clinics in South-Eastern Nigeria. Another reason could be due to the endocervical specimen inadequacy which has been shown to affect the sensitivities of nucleic acid amplification tests (Kellog *et al.,* 1990; Kellog *et al.,* 1991; Welsh *et al.,* 1997; Beebe *et al.,* 1999). Chlamydiae are intracellular organisms which infect the columnar epithelial cells, hence to diagnose them using culture and non-culture tests which require cervical samples, there is need for cellular adequacy which is a significant problem as pointed out by Welsh *et al.* (1997) and Rogers *et al.* (2006). The lack of specimen adequacy remains a serious shortcoming in many screening programs and research studies. In some of the public health screening programs funded by the CDC for the prevention of infertility due to STDs, as many as 30% of specimens were inadequate in spite of extensive clinician training (Black, 1997). In fact this has made the Centers for Disease Control and Prevention to recommend monitoring of columnar cell content in endocervical swab specimens to assess specimen quality (CDC, 2002). Nucleic Acid Amplification Tests (NAATS) are theoretically capable of detecting as little as one organism in a sample (Schachter and Moncada, 2002). However, in practice, this sensitivity is rarely achieved because of sample inhibition, due to factors such as beta-human chorionic gonadotropin, crystals, nitrites, and hemoglobin, as well as loss of DNA during extraction (Castriciano *et al.,* 2002), which can hinder the amplification reaction and yield false- negative results (Chong *et al.,* 2003).

The low detection by PCR in endocervical swabs in this study could also be due to certain substances (blood/tissue products) in clinical samples such as cervical swabs which may inhibit DNA amplification assays as earlier reported by Bauwens *et al.* (1993). In particular,

Polymerases have been shown to be sensitive to these inhibitory substances (Bauwens *et al.,* 1993). The frequency of inhibition has been determined by analysing specimens which were negative by PCR but positive by one of the other methods being used in comparison such as culture (Bass *et al.,* 1993; Bauwens *et al.,* 1993; Mahony *et al.,* 1994 and Pasternack *et al.,* 1996). Another reason for this low PCR detection could be the study group (Pelvic inflammatory disease, ectopic pregnancy and infertility cases) who may have been exposed to Chlamydia. Specimens from women with a high probability of *Chlamydia trachomatis* infection (such as those included in this study), may be more likely to contain PCR inhibitors secondary to tissue inflammation as reported by Toye *et al.,* 1998. In their study, Toye *et al* reported that among *Chlamydia trachomatis* positive women, PCR inhibitors were significantly more common in cervical swabs than in other specimens and inhibition was detected almost three times more commonly in specimens from *Chlamydia trachomatis* positive women than from uninfected ones. In a meta –analysis of thirty studies by Watson *et al.* (2002), nucleic acid amplification tests used on non – invasive samples such as urine are more effective at detecting asymptomatic chlamydial infection than the invasive (such as cervical swab specimens). Also on the study group, studies have shown that recently acquired infections shed more infectious units than older infections (which may have led to the conditions of our cases) and are therefore more likely to have positive cultures which use the same samples (cervical and urethral swabs) as the nucleic acid amplification tests (Kellog, 1989; Thejls *et al.,* 1994). This variation in PCR and ELISA results is supported by the finding of Kellog *et al.,* 1991 that 6 of 22 ELISA positive samples were PCR negative, and they concluded that variations in specimen quality had a significant impact on the incidence of both true negative and false positive ELISA results and could significantly influence understanding of the prevalence of chlamydial infections in women. In a study by Hillis *et al.,* 1997, about 68% of PID cases that were not detected by PCR were serologically positive

by at least one of the markers (IgM/IgA/IgG). Lan *et al*. (1994) also made similar observation and this showed that chlamydial etiology was identified more often using serology in comparison with PCR in PID patients. Based on these findings, we can say that though serological tests cannot replace the sensitive and specific direct tests, they may be useful in identifying chlamydial etiology in ascending upper genital tract infections, where direct tests may often fail to detect the presence of organism. In clinical point of view, it has earlier been showed that non-invasive serological testing may be also helpful in reducing the risk of introducing infections to the upper genital tract by avoiding procedures that involve instrumentation such as laparoscopy and hysterosalphingography (HSG) (Theunissen *et al*., 1994). Even though the number of cases in the upper genital tract disease groups in the present series is small, our findings suggest the utility of chlamydial serology in the diagnosis and management of female upper genital tract infections.

Also only a subset of the ELISA positive samples (11.2%) was chosen for the PCR. This may not be representative enough. However, the use of this few samples' number was due to financial constraint. The samples were stored for a long period (over sixteen months) before analysis. This however, may not have affected the result. Dommelem *et al.,* 2013 showed that storage conditions and duration hardly affect *Chlamydia trachomatis* DNA detection by PCR in a negative manner in their study in which they kept samples for as long as two years. Otoikhian *et al.,* 2012 reported a 52.63% (30 in 57) prevalence among asymptomatic females in Delta State, Nigeria. This is extremely higher than our 5.0%. This may be because urine samples were used in their study, which have been reported to contain less PCR inhibitors than the cervical swab samples used in our own study (Watson *et al.,* 2002). In fact, studies have shown that urine can be used to reduce the inhibitors on cervical swabs. Wilcox *et al.,* (2000) reported that PCR inhibitors on cervical swabs appeared to be diluted to non – detectable levels when swabs were placed in urine. Our finding however agrees with that of

Ngandjio *et al.,* 2003 who screened 1,277 volunteer students in Yaounde, Cameroun by PCR using endocervical swab specimens and found 3.78%. Ortashi *et al.,* 2004 also reported a similar low detection rate of 7.3% among Sudanese women attending an Obstetrics and Gynaecology Clinic in Khartoum, Sudan.

The distribution across age groups, age at marriage and age at first sex showed that the age group of 15 – 19 had the highest prevalence. The high prevalence of chlamydial infection among teenagers is probably due to sexual activity which is a risk factor for chlamydial infection (Nugent *et al*., 1992). According to Eng *et al*. (1997) and Sedlecki *et al*. (2001), female adolescents are more susceptible to sexually transmitted disease than older women because their cervical anatomic development is incomplete and is especially sensitive to infection by certain sexually transmitted pathogens, and for other features that characterize sexual behaviour and health care behaviour of the young people. For instance in a study carried out by Sedlecki *et al*., (2001), poor use of contraceptives among adolescent girls who constituted the subjects of the study, indicated low levels of safe sexual practices in the majority of the girls who were nineteen years of age. Only 17.6% of the girls positive to Chlamydia test used condoms as contraceptives. They also found out poor health care behaviour in the girls. Deblina –Datta *et al*. (2007) also reported highest prevalence of Chlamydia among females aged 14-19 years. The least prevalence according to our study was among those aged 40-44. This is in line with the finding of Sexually Transmited Diseases Surveillance Report (CDC, 2004) that the age bracket of 14-39 years accounted for over 95% of chlamydial cases in the United States. Studies have also shown that the incidence of Chlamydial infection in women decreases substantially after 30 years of age, likely because the target cell for *Chlamydia trachomatis* (i.e., the columnar epithelial cell, which is present on the ectocervix of young women) is replaced by squamous epithelium through the process of squamous metaplasia that occurs with age (Jacobson *et al.,* 2000). This implies that

younger women are more susceptible to *Chlamydia trachomatis* than adults. Our finding is also in conformity with that of Okoror *et al*., 2007, who found no positive case among the women aged 41-45 in their sudy. Old age was also associated with low risk of chlamydial infection as observed by Radcliffe *et al*., 2001.

The study revealed statistically significant association between chlamydial infection and age at marriage. The highest prevalence was seen among those who got married at teen age. In Nigeria, there is no legal minimum age for marriage and early marriage is still the norm in some areas. Parents see it as a way of protecting young girls from the outside world and maintain their chastity. Many girls get married at the age 12 and 13 and there is usually a large gap between husband and wife. Young married girls are at risk of contracting sexually transmitted infections from their husbands who may have sexual partners outside marriage and who may have more than one wife (polygamy). Men have also been known to have large reservoir of chlamydia infection and could repeatedly re-infect their partners even without knowing (Sule *et al*., 1997). Because of their age, lack of education and low status, young married girls are not able to negotiate condom use to protect themselves against HIV and other sexually transmitted diseases (Child marriage briefing in Nigeria population council, 2004).

Chlamydia is common in women with a higher number of sexual partners (Black, 1997). This lends support to the finding in this study that women with more than one lifetime partners had higher prevalence than those who maintain only one. The recorded value for number of lifetime partnes may even be higher than that, in reality due to the fact that women do not usually disclose information that relates to previous sexual habits for fear of stigmatization and cultural inhibitions. This view is also shared by Oloyede *et al*., 2009.

The distribution of Chlamydia across occupation categorized into skilled, unskilled and housewife, showed that the category of unskilled occupation had the highest prevalence rate. This might be explained by the fact that women with unskilled occupation (mainly traders) have more tendency of having multiple partners since they have more opportunity of meeting several and different kinds of people than those of the none category and lack adequate knowledge of how to protect themselves from sexual health risks than those of the skilled category.

Our finding also showed that genital Chlamydia presented more asymptomatically. This is in line with the nature of the disease as has been pointed out by several studies (Farley *et al.,* 2003; Wiesenfeld *et* al., 2005; CDC, 2006; Geisler, 2010; Detels *et al*., 2011) and this has been the biggest challenge in the control of the disease as pointed out by Black, (1997).

CHAPTER SIX
6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Three hundred and fifty four (354) of the total six hundred (600) blood samples analysed were positive, indicating a prevalence of 59.0%. Prevalence rates of 57.5% and 62.0% were obtained for the pregnant women and the gynaecologic patients respectively. Of the 113 patients of PID tested, 74 were positive giving a prevalence rate of 65.5% and out of the 76 infertility patients tested, 42 were positive giving a prevalence rate of 55.3% while 8 of the 11 ectopic pregnancy patients tested positive with a prevalence rate of 72.7%. Across the sampling sites, prevalence rates of 84.7%, 71.3% 51.3% and 28.7% respectively were recorded for the Federal Capital Territory (Abuja), Benue, Kogi and Niger States respectively. A prevalence rate of 17.2% for HIV was recorded for the study area with Abuja having the highest prevalence rate of 24.6% and Niger having the lowest (4.7%). As for age groups, the results showed that the age group of 15-19 had the highest prevalence in both the pregnant women and the gynaecologic cases while the age group of 40-44 recorded the lowest rates in both. When the age at marriage of the study participants was considered, those married at teenage had the highest prevalence of 69.3% and 67.0% for the pregnant women and the gynaecologic patients respectively. Based on the age at first intercourse, the age group of 15 – 19 had the highest prevalence of 78.0% while those of age group 30 – 34 had the lowest of 20.0%. In the group of pregnant women, those that attained tertiary level of education had the highest prevalence (67.4%), while in the gynaecologic cases, the secondary level had the highest (85.0%). The distribution of chlamydia infection among the study groups based on occupation showed that the group of unskilled occupation had the highest prevalence (70.8%) among the pregnant women while that of the semi-skilled group had the highest prevalence (83.1%) among the gynaecologic cases. Based on family type the

distribution showed that among the pregnant women, the group of monogamous had the highest prevalence (60.4%) and same is the case for the gynaecologic patients (70.7%). The distribution of chlamydia infection among the study groups based on number of lifetime partners showed that the group of those who have had more than one sex partner had the higher prevalence among both the pregnant women (88.2%) gynaecologic cases (75.0%) groups. The distribution of chlamydia infection among the study groups based on nature of presentation showed that the group of those who did not present with any symptoms of urogenital infection had higher prevalence for both groups. Only two out of the forty (40) endocervical swabs from the ELISA positive samples were confirmed positive by PCR.

6.2 Conclusion

The sero-prevalence of chlamydia in the North-Central zone of Nigeria was found to be high (59.0%). Chlamydia was found to be associated with HIV in the study area. The PCR detection of genital Chlamydia was 5.0% in the subjects tested. The risk factors associated with chlamydial infection in the Zone included, teenage, unskilled occupation, single marital status, more than one sexual partners in life and secondary school level of education. It was also found to present asymptomatically than with symptoms. Though high prevalence rates were found among the gynaecologic cases considered in the study (PID, ectopic pregnancy and infertility which are chlamydial complications), they were not found to be statistically associated with Chlamydia. The mere detection of the presence of anti-chlamydia IgG is of limited value, however, an estimation of IgG titer may serve useful in certain clinical situations, as in the case of ascending upper genital tract infections with *C. trachomatis*.

6.3 Recommendations

1. For an infection that is largely asymptomatic but has devastating effects on populations, only a preventive approach would have beneficial effects in controlling the disease and its effects on women's health in the country.

2. To overcome the problem of specificity in Chlamydia serology, recently developed specific synthetic peptides based on the major outer membrane protein (MOMP) of *Chlamydia trachomatis* should be used.

3. Future studies are required to investigate the genotypes of chlamydia in circulation in the study population and to investigate the role of *Chalmydia trachomatis* genotypes in disease manifestations.

6.4 Limitations

The study is hospital based rather than community based. The problem of external validity, i.e. generalizing hospital based data to community level analysis, therefore arises.

The study was also conducted in tertiary level hospitals, hence it can be argued that the patients attending these hospitals are self selected and may not represent the true range of patients in the community.

A prospective follow up study will appear to be more accurate for determining the association between the Chlamydia sequaelae and the various socio-demographic and reproductive risk factors. This would be difficult to carry out in a country such as Nigeria where there is likelihood of a high drop-out rate.

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APPENDICES

Appendix I: Questionnaire for a Study to Determine the Prevalence of Chlamydial Infection among Pregnant Women and Gynaecologic Patients in some Tertiary Health Care Centers in the North Central, Nigeria

General information

Serial number---------- Hospital number---------- Date----------

Age ---------- Age at marriage----------

Ethnic group---------- Age at first intercourse----------

Educational level: Primary { } Secondary { } Tertiary { } Informal { }

Occupation: skilled { } semi-skilled { } unskilled { } none { }

Spouse's occupation skilled { } semi-skilled { } unskilled { }

Obstetrics history

No. Of pregnancies: 1 { } 2{ } 3 { } >3{ } none { }

No. Of live biths: 1 { } 2{ } 3 { } >3{ } none { }

No. Of stillbirths: 1 { } 2{ } 3 { } >3{ } none { }

No. Of premature biths: 1 { } 2{ } 3 { } >3{ } none { }

Miscarriage: yes { } no { }

If yes how many? 1 { } 2{ } 3 { } >3{ }

Risk factors

Marital status: single { } married { } divorced { }

Family type: monogamous { } polygamous { }

Order of marriage: first { } second { } third { }

Number of lifetime partners: 1 { } 2{ } 3 { } >3{ }

Do you smoke? Yes { } No { }

History of abortion: Yes { } No { }

if yes how many times? 1 { } 2{ } 3 { } >3{ }

History of sexually transmitted disease: Yes { } No { }

Do you use IUD for contraception? Yes { } No { }

Urogenital symptoms

Any history of:

Abnormal vaginal discharge: Yes { } No { }

Burning on urination: Yes { } No { }

Pain during intercourse: Yes { } No { }

Lower abdominal pain Yes { } No { }

Are you currently on medication for any of these symptoms? Yes { } No { }

Appendix II: Consent Form

My name is Muhammad Hassan Isa, a student of Microbiology Department, Ahmadu Bello University, Zaria. I am undertaking a study on the prevalence of Chlamydia among pregnant women and gynaecologic patients in the North central, Nigeria. This study has been reviewed and granted approval by the Ethics Committees of the Federal Madical Centers, Makurdi and Lokoja as well as the Natinal Hospital, Abuja and General Hospital, Minna, Niger state.

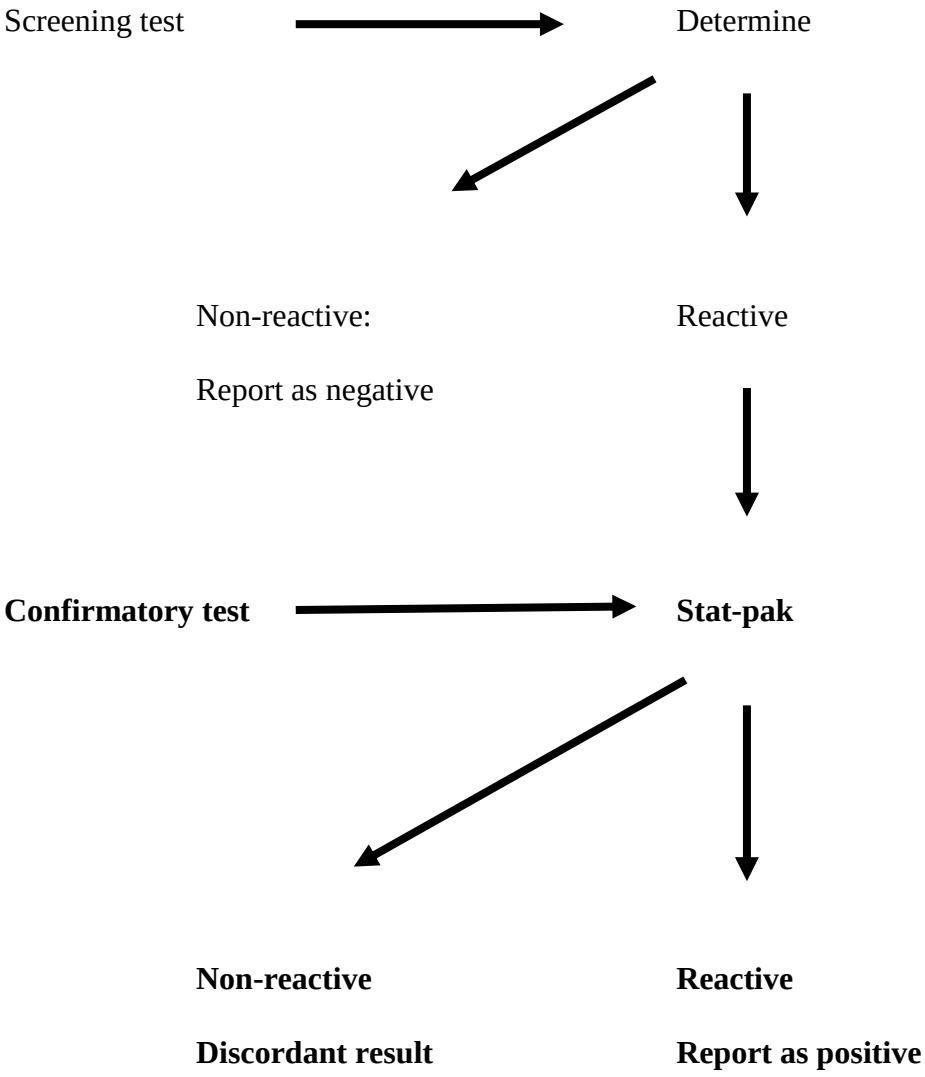
I would very much appreciate your participation in this study. This information will help the Government to plan health services and it will be strictly confidential and as well not shown to other persons. Should you have any queries, feel free to contact the Chairman of the Ethics Committee of this Hospital.

As part of the study, you will be asked some questions about your life style and 5ml of blood as well as endocervical swab sample shall be collected from you. All the answers you give shall be confidential. Participation in the study is completely voluntary. Should you come across any question you do not want to answer, you can leave it and go on tothe next or you can stop answering questions at any time. However, I hope you will participate in the study since your information is of paramount importance.

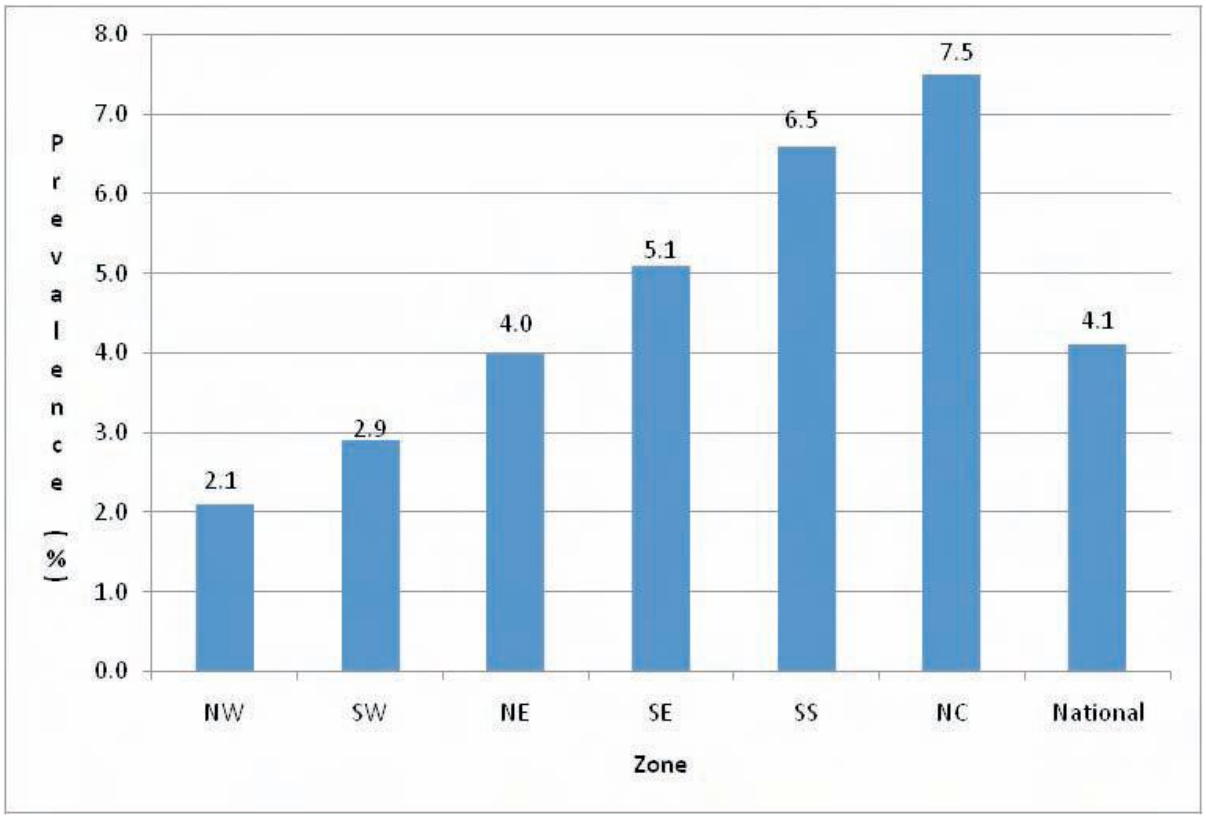
_____ _____

Signature of respondent Date

Appendix III: HIV Testing Algorithm



Appendix IV: Prevalence of HIV by Zones In Nigeria (HSS, 2010)



Appendix V: Univariate Analysis on Risk Factors for Chlamydial Infection among Pregnant Women and Gynaecologic Patients in some Tertiary Health Care Centers in Parts of the North Central, Nigeria

Factor	odds ratio	95% confidence interval	
		Lower	upper
Pregnant group			
Age group			
15-19	12.35	3.48	43.73
20-24	07.04	2.42	20.50
25-29	08.08	2.81	23.18
30-34	03.26	1.12	09.48
35-39	01.71	0.48	06.03
40-44	01.00	reference	
Age at marriage			
15-19	8.29	2.20	31.30
20-24	4.13	1.11	15.37
25-29	1.00	reference	
Age at first sex			
15-19	2.81	1.11	7.09
20-24	1.54	0.61	3.86
25-29	1.13	0.42	3.07
30-34	1.00	reference	
Educational level			
Informal	01.00	reference	
Primary	1.4	0.54	3.65
Secondary	3.69	1.57	8.98
Tertiary	4.66	1.88	11.56

Factor	odds ratio	95% confidence interval	
		Lower	upper
Occupation			
Skilled	2.03	1.05	3.94
Semi/skilled	1.38	0.63	3.02
Unskilled	4.22	2.19	8.12
None	1.00	reference	
Marital status			
Married	1.00	reference	
Single	0.84	0.42	1.69
No. Of life partners			
1	1.00	reference	
>1	7.35	3.55	15.21
Gynaecologic group			
Age			
15-19	4.13	0.88	19.27
20-24	3.00	0.83	0.83
25-29	3.38	1.06	10.76
30-34	2.47	0.75	8.17
35-39	1.31	0.37	4.62
40-44	1.00	reference	
Age at marriage			
15-19	1.09	0.48	2.53
20-24	0.78	0.33	1.81
25-29	0.73	0.29	1.85
30-34	0.60	0.19	1.89
35-39	1.00	reference	

Factor	odds ratio	95% confidence interval	
		Lower	upper
Age at first sex			
15-19	14.29	4.31	47.32
20-24	6.29	1.85	21.39
25-29	2.67	0.71	9.95
30-34	1.00	reference	
Educational level			
Informal	1.00	reference	
Primary	1.96	0.70	5.52
Secondary	6.18	2.22	17.19
Tertiary	0.49	0.18	1.32
Occupation			
Skilled	0.72	0.32	1.66
Semi/skilled	5.14	2.29	11.57
Unskilled	1.22	0.47	3.18
None	1.00	reference	
Marital status			
Married	reference	1.00	
Single	4.96	1.98	12.43
No. Of life partners			
1	1.00	reference	
>1	7.35	3.55	15.21