# *CHLAMYDIA TRACHOMATIS* PREVALENCE IN THE GREATER ACCRA REGION AND THE WESTERN REGION OF GHANA

By

Helena Dela

(PG 7955712)

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Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology

## DECLARATION

I hereby do declare that with the exception of references to other people's work which I have duly acknowledged and cited, all experimental work described in this thesis was carried out by me. I do further declare that this thesis has not been presented either in part or whole elsewhere for another degree

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| _____ | _____ |
| Helena Dela | Date |
| (Student) | |
| _____ | _____ |
| Prof. Kwasi Addo | Date |
| (Co-Supervisor) | |
| _____ | _____ |
| Prof. E. H. Frimpong | Date |
| (Supervisor) | |
| _____ | _____ |
| Dr. T. B. Kwofie | Date |
| (Head of Department) | |

# DEDICATION

This work is dedicated to my lovely husband Gabriel and two sons, Daniel and David. You have always been my inspiration.

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# ABSTRACT

*Chlamydia trachomatis* is one of the most prevalent Sexually Transmitted infections (STIs) worldwide due to its frequent asymptomatic nature and is normally tested alongside *Niesseria gonorrhoeae* which is also a bacterial pathogen. It is known to cause infections which can lead to ectopic pregnancy and neonatal conjunctivitis among pregnant women and Pelvic Inflammatory diseases (PID) in women when left untreated. *C. trachomatis* can also lead to infertility among males. Five sites in two regions were selected for the study. These included the 37 Military hospital and Adabraka STI clinic, Accra (Greater Accra region) and the three clinics at Sekondi/Takoradi (2 Medical Reception Station (2MRS), the Naval Health Center and the Air Force Medical Center) in the Western region. Patients who met the eligibility criteria provided consent, filled out a questionnaire and were made to provide urine samples which were transported to the Noguchi Memorial Institute for Medical Research (NMIMR) at 4˚C for Nucleic Acid Amplification Testing (NAAT).A total of 35 *C. trachomatis* positives out of 340 (151 males and 189 females) patients were detected during the study, giving a prevalence of 10.3%. The prevalence of chlamydia was higher (19.8%, 14/73) among the Takoradi clinics than the Greater Accra clinics (7.9%, 21/267). The males showed higher percentages in all the risk factors analyzed than the females. The risk factors included burning during urination, discharge and bleeding from penis/vagina, foul smell from urine, pain in penis/vagina, pain when having sex, ulcers and warts on genital parts. Burning during urination showed a relationship of a patient testing positive for the disease in both males and females. Most of the symptoms presented did not have any association in testing positive for *C. trachomatis*. It is recommended that more innovative ways be found to test sexually active young people, since the disease remain subclinical in many people. Health authorities and Clinicians should be encouraged to use NAATs as a diagnostic tool and in the absence of laboratory testing, syndromic approach could be used for diagnosis.

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## LIST OF ABBREVIATIONS

CDC Center for Diseases Control

CP Crossing Point

DFA Direct Fluorescent assay

EB Elementary Body

EIA Enzyme immunosorbent Assay

FSW Female Sexual Workers

HIV Human Immunodeficiency Virus

IC Internal Control

NAAT Nucleic Acid Amplification Test

NAMRU-3 Naval Medical Research Unit 3

NMIMR Noguchi Memorial Institute for Medical Research

NSU Non Specific Urethritis

PB Persisting body

PCR Polymerase Chain Reaction

PI Principal Investigator

PID Pelvic Inflammatory Disease

RB Reticulate Body

RM Reaction Mix

STD Sexually Transmitted Diseases

WHO World Health Organization

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background

Sexually transmitted infections (STIs) rank among the top five reasons why adults seek medical treatment in developing countries (World Health Organisation, 1999). STIs are infections that are spread primarily through person-to-person sexual contact. There are more than 30 different sexually transmissible bacteria, viruses and parasites. Some of the common bacterial infections include *Neisseria gonorrhoeae* which causes gonorrhoea or gonococcal infection, *Treponema pallidum* known to cause syphilis, and *Chlamydia trachomatis* which causes chlamydial infections (WHO, 1999).

*Chlamydia trachomatis* is one of the most prevalent sexually transmitted pathogens. This is because more of the STI cases are caused by *Chlamydia trachomatis* than any other bacterial pathogen (CDC, 2010). This is due to its asymptomatic nature which leads to a more rapid, widespread transmission leading to critical implications for reproductive, maternal and newborn health. STIs are the main preventable cause of infertility, particularly in women (CDC, 2010). Also, patients infected with Chlamydia are up to five times more likely to become infected with HIV if exposed (CDC, 2010).

About 72%-75% of women and 25% of men with Chlamydia are symptom-free (WHO, 1999). In women, symptoms include increased vaginal discharge, burning during urination, or bleeding after sexual intercourse whilst complications could lead to pelvis sepsis leading to abscess formation, chronic and recurrent pelvic inflammatory disease, ectopic pregnancy, infertility and chronic pelvic pain. Non-gonococcal urethritis, burning and frequency of

urination with complications such as chronic genital tract infection, possibly resulting in infertility occur in men.

Owing to varied characteristics of the study population and different methods used for *C. trachomatis* detection, there is a wide variation in prevalence of Chlamydia infection worldwide (Buve *et al*., 2001). Approximately 4 million cases of *Chlamydia trachomatis* infection are reported per year in the US, with an overall prevalence of 5 %( CDC, 2002). In Ethiopia, the prevalence of Chlamydia infection of the cervix was 5.9% (Buve *et al*., 2001). A study that was conducted among 162 gynaecolgical patients (women) who were admitted to a postpartum ward of Korle-Bu Teaching Hospital, Accra, Ghana identified that 4.9% were infected with *C. trachomatis* (Bensti *et al.*, 1985*).*The common complaint (10%) presented by these women was lower abdominal pain.

A surveillance that was carried out among 377 female sex workers (FSW) in Accra presenting cases of cervical infections also showed results of *C. trachomatis,* 10.1% in August 2000. The method used was by the examination of gram-stained smears from the participants (Deceuninck *et al.,* 2000). .

In a study conducted by Ikeme *et al.*, 2011, incorporating the Enzyme Immunosorbent Assay (EIA), detected an overall 29.4% prevalence of *C. trachomatis* which involved a population comprising 136 female undergraduate students and 150 non-student women in a population-based prospective study comprising female residents of Enugu, South Eastern Nigeria (Ikeme *et al.*,2011).

Also in a Cameroonian study conducted by Ngandjio *et al.*, 2003, the prevalence of *Chlamydia trachomatis* infection was 3.78% out of 1,277 volunteer students screened by

direct fluorescence assay and Cobas Amplicor PCR. The infection was associated with the non-use or inconsistent use of condoms in women and a previous sexually transmitted infection in men (Ngandjio *et al*., 2003).

The prevalence that has been mentioned in other studies varies because of the different methods of tests that were used which could lead to the results being either too high or too low. The performance of nucleic acid amplification tests (NAATs) with respect to overall sensitivity, specificity, and ease of specimen transport is better than that of any of the other tests available for the diagnosis of chlamydial and gonococcal infections (CDC, 2002). In the United States, NAATs that have been cleared by the Food and Drug Administration (FDA) for the detection of *C. trachomatis* and *N. gonorrhoeae* infections are recommended as screening or diagnostic tests because they have been evaluated in patients with and without symptoms (CDC, 2002).

Due to the increasing prevalence of *C. trachomatis* worldwide, investigating its prevalence is necessary to be able to estimate the disease burden currently in certain parts of the country. Three main sites were enrolled which included the three military clinics of the Ghana Armed Forces attached to 2 Garrison in the port city of Sekondi/Takoradi, the 37 Military Hospital, and Adabraka STI clinic in Accra, Ghana. After consenting to participate in the study, first-void urine samples were collected from patients. Testing was carried out using one type of Nucleic Acid Amplification Test (NAAT), Polymerase Chain Reaction (PCR) applying the use of the LightCycler 480 II systems from Roche Diagnostics, Germany.

## 1.2 Problem statement

There is a gradual increase of reproductive health issues, which has led to infertility and a decrease in the mortality rate in Ghana. This can be attributed to different STIs which include HIV, Syphilis and gonorrhea. Unfortunately, *C. trachomatis* is not popularly known but is prevalent in Ghana. Different studies have been conducted to investigate the prevalence of *C. trachomatis* in Ghana (Opoku *et al*., 2010). Results that researchers have come across vary owing to the different methods that are used in testing for the disease. NAATs have been endorsed by the FDA in the U.S, as a reliable method in testing for Chlamydia (CDC, 2002).

## 1.2 Justification of the Study

It is important to know the prevalence of *C. trachomatis* in all the regions in Ghana, but due to logistics constraint, I was limited to two regions of Ghana, the Western and Greater Accra regions in order to supervise properly according to the standard operating procedures of the study. This study enlightens us not just on the prevalence of the study using NAAT as the diagnostic tool, but also to understand the demographic information in addition to the symptoms that were presented by the patients at the various health facilities.

## 1.3 General Objective

This study was to determine the prevalence of *C. trachomatis* and to estimate the disease burden in patients at the three military clinics of the Ghana Armed Forces attached to 2 Garrison in the port city of Sekondi/Takoradi, the 37 Military Hospital, and Adabraka STI clinic in Accra, Ghana.

## 1.4 Specific Objectives

To determine the prevalence of *C. trachomatis* infections in males and females.

To examine *C. trachomatis* positive cases in relation to patients' demographic information and their symptom presentation.

# CHAPTER TWO

## LITERATURE REVIEW

### 2.1 *Chlamydia trachomatis* — The Bacterium

*Chlamydia trachomatis* is a gram negative, obligate intracellular bacterium, which was first regarded as a virus (Rours *et al*., 2011). Chlamydiae are highly complex organisms with genus, species and serovar specificity, and the most easily detected antigen is the group antigen, shared by all members of the genus. The major genus specific antigen has been identified as lipopolysaccaride (LPS), and is expressed on the surface of Chlamydia organisms (Everett *et al*., 1999).

Chlamydia requires an eukaryotic host cell to fuel its own growth and replication. The bacterium belongs to the family *Chlamydiaceae*, consisting of two genuses; *Chlamydia* and *Chlamydiophila*. They in turn are divided into numerous species, of which three are responsible for human disease; *C. pneumoniae*, *C. psittaci* and *C. trachomatis.* The distinguishing characteristics between the three species concern the host range, clinical expression, and antibiotic susceptibility (due to folate biosynthesis), the staining characteristics (due to glycogen inclusions), inclusion morphology, shape of the elementary body, and limited DNA sequence homology (Rours *et al*., 2011).

*Chlamydia pneumoniae* formally known as TWAR (after the laboratory designations of the first two isolates, TW-183 and AR-39) was first obtained in 1969's in chick embryo yolk sac culture (Kuo et *al.*, 1995). Chlamydia has been recognized as an important acute respiratory pathogen in man, especially very common among children between the ages of 5 and 14 years. In children, this infection is usually mild or asymptomatic, but it may be more severe

in adults. It causes upper and lower respiratory tract infection which includes pharyngitis, sinusitis, bronchitis and otitis media (Del Piano *et al*., 1994).

*Chlamydia psittaci*, a common pathogen of avian and lower mammals is relatively uncommon is humans, causes psittacosis in birds but could be transmitted to those keeping psittacine birds (true parrots, parakeets, and macaws) and those handling and slaughtering poultry (Okoror *et a*l., 2007).

*C. trachomatis* was the first Chlamydia species to be discovered and has been divided into subgroups based on antigenic variation in the major outer membrane proteins (MOMP) (serovars) and on clinical expression (biovars) (Rours *et al*., 2011). The serovars include A, B, C, D, E, F, G, H, I, J, K and L. Seventy percent of the non-lymphogranuloma venereum (LGV) STIs are due to serovars D, E and F, which are also responsible for neonatal disease. Serovars D through K deal with urogenital and neonatal chlamydial infections (Rours *et al*., 2011).

The life-cycle of Chlamydia is unique with an intracellular growth or replicate form, the reticulate body (RB), and an extracellular metabolically inert, ineffective form, the elementary body (EB), Figure 1. (Beatty *et al*., 1994; Byrne *et al*, 2004).

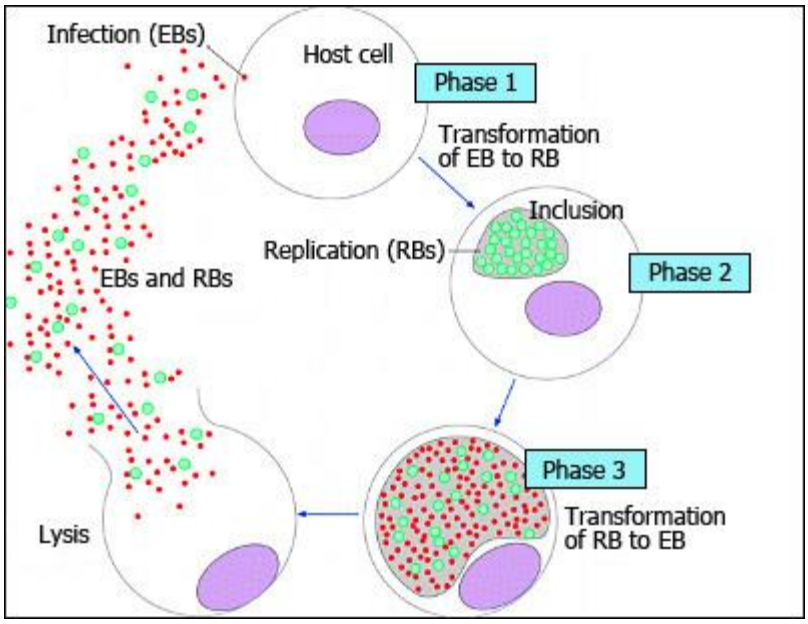


Figure 1: Life Cycle of *Chlamydia trachomatis* (www.cytologystuff.com)

The EB is metabolically inactive but is initially involved in the attachment to the host cells. When the EB adheres to the eukaryotic cell, it enters the cell by endocytosis and stays in the intracellular vacuole, called an inclusion body, through its entire lifecycle. This EB changes to a metabolically active and dividing form called the reticulate body (RB), which is adapted for intracellular multiplication. The RBs (diameter, 0.5 to 1 mm) are able to synthesize their own RNA, DNA, and protein inside the inclusion body with the help of the ATP produced by the host cell (Schachter *et al*., 1999). This entire cycle takes about 18 to 24 hours, and as the RBs are not stable outside the host cell, some of them re-organize into EBs that are infectious. After about 72 hours, the host cells rupture and there is a release of the infectious EBs by exocytosis after which infection of other cells may take place (Schachter *et al*., 1999).

The infected cells produce and secrete inflammatory mediators, stimulate infiltration of polymorphonuclear cells and lymphocytes, and secrete growth factors, which lead to the

formation of lymphoid follicles, chronic inflammation and even fibrotic changes. After an incubation period of 10 days (varying between 7 and 21 days) patients may present with a variety of clinical manifestations that result from the host inflammatory response and cell destruction. In addition to this replicate cycle associated with acute infection, recticulate bodies can cease to divide into elementary body. They rather form Persisting bodies (PBs) which cause persistent infections (Schachter *et al*., 1999).

**2.2 Disease History**

Human diseases caused by *C. trachomatis* were described as long ago as in Egyptian papyri (the eye disease trachoma) but were first visualized in 1907 by Halberstaedter and von Prowazek in Berlin. The genus part of the name, Chlamydia, comes from the Greek word "chlamys", which means cloak or mantle which refers to the appearance of the intracytoplasmic inclusions in infected cells, which lie like a mantle around the host cell's nucleus. The species part of the name, trachomatis is also Greek and means rough or harsh. This name is perfectly associated with the actions of this disease. Halberstaedter and von Prowazek were able to see the typical intracytoplasmic inclusions in stained conjunctival scrapings from orangutans that had been inoculated with human trachomatous material. Shortly thereafter, similar inclusions were identified in conjunctival scrapings from infants with trachoma. At the same time, the same types of inclusions were found in the genital tracts of mothers of the affected infants and also in the urethras of the fathers. These inclusions were associated with non-gonococcal urethritis. A tissue culture method was developed in 1965 (Gordon and Quan, 1965), and it was shown in several studies that nearly half the cases of non-gonococcal urethritis in adults were chlamydial infections (Richmond *et al*., 1972).

## 2.3 Clinical Manifestations

The initial damage that chlamydia causes often goes unnoticed. However, chlamydial infections can lead to serious health problems with both short- and long-term consequences. *Chlamydia trachomatis* causes trachoma, a chronic inflammatory disease of the eye eventually leading to blindness (Rours *et al*., 2011). Trachoma mainly thrives in isolated rural communities where people live with limited access to water and health care. In some communities, the disease is so common that blindness from trachoma is simply accepted as a fact of life. The disease spreads easily, primarily from child to child, and child to caregiver. Trachoma affects the eyelids and conjunctiva (outside covering) of the eye, usually with very little discomfort until later in the disease. When infected, the conjunctival covering of the eye becomes red and irritated (inflamed). The disease begins in childhood, and initially the infection clears up on its own. Repeated infections by the bacteria are common and, unless treated, can result in scarring of the conjunctival surface of the lids. The lids become scarred and the lid margins may turn in, causing eye irritation and pain followed by scarring of the cornea by the inward-turned lashes (trichiasis), which scrape the cornea. Corneal scarring results in decreased or total loss of vision. In order to see properly, it is necessary for the cornea (front window of the eye) to remain clear. Chlamydia can also cause adult inclusion conjunctivitis, often preceded by genital tract infection, and newborn conjunctivitis (ophthalmic neonatorum) or pneumonia. This happens when the baby is infected while passing the birth-canal (Mecansy *et al*., 2003). Chlamydia can also cause pre-term delivery when left untreated. In a study conducted in the Netherlands to investigate the effect of Chlamydial infection in pregnant women, of all deliveries before 32 weeks and 35 weeks gestation showed 14.9% and 7.4% respectively. *C. trachomatis* infection

contributes significantly to early premature delivery and should be considered a public health problem, especially in young women and others at increased risk of *C. trachomatis* infection.

Also, untreated chlamydia in women can spread into the uterus or fallopian tubes and cause pelvic inflammatory disease (PID). Symptomatic PID occurs in about 10 to 15 percent of women with untreated chlamydia (Oakshott *et al*., 2010). However, chlamydia can also cause subclinical inflammation of the upper genital tract ("subclinical PID"). Both acute and subclinical PID can cause permanent damage to the fallopian tubes, uterus, and surrounding tissues. The damage can lead to chronic pelvic pain, tubal factor infertility, and potentially fatal ectopic pregnancy. Some patients with chlamydial PID develop perihepatitis, or "Fitz-Hugh-Curtis Syndrome", an inflammation of the liver capsule and surrounding peritoneum, which is associated with right upper quadrant pain (Cates *et al.*, 1991). Ectopic pregnancy occurs when scarred fallopian tubes may allow the passage of sperm to fertilize the egg but the fertilized egg may be too large to pass through the scarred tubes into the uterus for implantation. Instead the fertilized egg travels backward and gets implanted into the fallopian tube or in the abdomen. This is associated with mortality of the foetus and mother. Ectopic pregnancy is the main cause of maternal mortality in the first trimester of pregnancy in developing countries (Paavonen and Eggert-Kruse, 1999).

Some of the major complications that occur in men include urethritis, prostatis, epididymitis, proctitis, reactive arthritis, and decreased fertility. Urethritis is inflammation of the urethra (urine tube) that runs along the underside of the penis. Symptoms include: a white cloudy discharge from the tip of the penis, pain or a burning sensation when the patient urinates, the urge to urinate often, irritation and soreness around the tip of the penis. There are many

causes of urethritis but chlamydia infection is the most common (Scheibel *et al*., 1983). When a person has urethritis and the cause is not known then it can be referred to as Non-specific urethritis (NSU) which is also treated with the same antibiotics as Chlamydia. Prostatis is a condition that occurs when urethritis is left untreated (Scheibel *et al*., 1983). Epididymitis is a condition that causes swelling and tenderness in the epididymis. The epididymis is part of a man's reproductive system that carries sperms from the testicles, so if the testicles are affected, it is called epidymo-orchitis (NHS-UK, 2013). If left untreated, epididymitis can sometimes lead to infertility. Chlamydia can cause a reactive arthritis (inflammation of the joints). In some people the arthritis develops as part of a syndrome and they also develop inflammation of the urethra (urethritis) and the eyes (conjunctivitis). Reactive arthritis is more likely to occur in men than women (NHS-UK, 2013).

Studies have shown that *C. trachomatis* facilitates an HIV infection because infected and sore mucous membranes are rich in blood-vessels and lymphocytes, a predisposition to be infected with HIV. Therefore, a step in decreasing the spread of HIV is to decrease the spread of *C. trachomatis* (Jonsson *et al*, 1997).

### 2.4 Epidemiology

It is estimated that around 92 million chlamydia infections occurred worldwide in 1999, affecting more women (50 million) than men (42 million). Asia recorded the highest rate of infection rate (42.89%) followed by Sub-saharan Africa (15.89%) and 9.31% from Latin America and Caribbean (STD statistics worldwide-avert.org., 2014).

West Africa has recorded a low prevalence of both *Chlamydia trachomatis* and *Neisseria gonorrhoeae* when compared with East Africa and the Western world (Opoku et al., 2010).

Women presenting vaginal discharge in five countries in West Africa (Benin, Burkina Faso, Ghana, Guinea, and Mali), tested a 3.2% prevalence (Pepin *et al.,* 2004). However, there have been a series of STI related studies that have been carried out in Nigeria. In one of such studies, investigating the prevalence of *C. trachomatis* infection among female undergraduates of the university of Port Harcourt using the Strand displacement and amplification technique, 44/400 females, (11%) tested positive for the disease. In another study carried out in South west Nigeria using the Enzyme Linked Immunosorbent Assay (ELISA) of symptomatic patients attending clinics, 546/699 (91.2%) patients tested positive for Chlamydia out of which 221(40.5%) males and 325 (59.5%) females were involved in the outcome (Okoror *et al*., 2014)

Some populations in East and Southern Africa had a prevalence of 5-11% and 7-17% for Chlamydia and Gonorrhea respectively (Mayaud *et al*., 1998). In a study to review sentinel surveillance of sexually transmitted infections in South Africa between 1985 and 2003, in selected sentinel populations, chlamydia had higher prevalence levels (13%) than other STIs in women visiting the STI clinic (Johnson *et al*., 2005)

In a study that was conducted by Drescher *et al*, 1988, cases of the incidence of urogenital *C. trachomatis* infection presented 3.6% positive cases of asymptomatic pregnant patients out of a total of 110 women. This included females presenting with cases of infertility that resulted in 3.6% positive cases and out of a total of 15 males with cases of urethritis, 6 (40%) tested positive for Chlamydia. The presence of *Chlamydia trachomatis* was determined using the Direct Fluorescent Assay (DFA) incorporating the use of a fluorescein labeled monoclonal antibody (MicroTrak/ SYVA Corporation) (Drescher *et al*., 1988).

A recent study conducted by Yirenya-Tawiah and others to investigate the prevalence of *C. trachomatis* and *N. gonorrhoeae* in women of reproductive age living in urogenital schistosomiasis endemic areas in Ghana, showed 6.3% and 2.6% positivity of Chlamydia and Gonorrhea respectively. Samples were examined using PCR incorporating the use of Cobas Amplicor from Roche diagnostics. They concluded that women with a history of urogenital schistosomiasis were at a higher risk of contracting the diseases (Yirenya-Tawiah *et al*, 2014).

**2.5 Risk factors**

*C. trachomatis* has shown to be more prevalent in women than in men worldwide (STD statistics worldwide- avert.org, 2014). This has led to studies carried out being more focused in women than in men leading to insufficient information of the disease in males. Only a few studies have been carried out which focuses on both males and females.

A study conducted to investigate the prevalence of genital chlamydia and gonococcal infections in women at risk of acquiring STIs in Kumasi metropolis, Ghana showed 4.8% positivity for chlamydia infection. The investigators took into consideration the age, sexual behaviours, use of contraceptives, infertility and symptoms were significant determinants of the disease (Opoku *et al*., 2010).

The prevalence of HIV and *C. trachomatis* was also carried out in Tanzania amongst 15-19 year olds in rural Mwanza region and their ages, sex, sexual behaviours, symptoms and their educational backgrounds were taken into consideration. Out of 4,749 males and 4686 females enrolled, 1.0% males and 2.4% females tested positive for *C. trachomatis* (Obasi *et al*, 2001).

However, a research that was carried out by Nyarko *et al*, January 2014 to determine the prevalence of *Chlamydia trachomatis* infection in Tarkwa-Nsusem Municipality and explore the relationship between age, gender, symptoms and diagnosis enrolled a total of 186 individuals which revealed a total prevalence of 22.5% and 19.7% for men and women respectively. The study found a positive relationship between symptoms and age, and between symptoms and positive diagnoses of Chlamydia among young adults in that locality (Nyarko *et al*, 2014).

To address this important deficit in research, this study carefully examined the relative effects of gender, age, educational level, religion and symptoms in acquiring *C. trachomatis*.

### 2.6 Treatment and Prevention of *Chlamydia trachomatis*

The treatment of urogenital infection in both men and women depend on several factors which include; the site of the infection, the age of the patient, whether the infection is complicated or uncomplicated and whether the patient is pregnant or not (Miller, 2006). Azithromycin and doxycycline are the recommended antibiotic for the treatment of uncomplicated urogenital infections while Erythromycin base or amoxicillin are recommended for treatment in pregnant women (CDC, 2002). Ophthalmia neonatorum can be treated with erythromycin base or ethylsuccinate (CDC, 2002). The WHO estimates that 6 million people are blind due to trachoma, making it the leading cause of preventable blindness in the world. This is why WHO has put in place a public health awareness strategy approach known as SAFE which combines treatment (Surgery and Antibiotics) with prevention (Facial-cleanliness and environmental improvement) (Emerson *et al*, 2006).

Prevention of *C. trachomatis* (applies to all STIs as well) involves two processes; Primary prevention which depends on the ability of changing sexual behaviors that contribute to the increases the risk of contracting STIs and the secondary prevention that involves the standardized detection and treatment of STIs (Sangani *et al*., 2004). The Centre for Disease Control outlines five major concepts for the prevention of STIs. This includes: Education and counseling individuals and communities at large on safer sexual behavior in persons at risk; identification of asymptomatic infected persons and symptomatic persons unlikely to seek diagnostic and treatment services; provision of effective diagnosis and treatment of infected persons; evaluation, treatment, and counseling of sex partners of persons infected with STI; and pre-exposure immunizations for vaccine-preventable STIs (CDC, 2002).

### 2.7 Scientific Methods of Testing for *Chlamydia trachomatis*

There are different methods of testing for the disease. One of the oldest methods is the cell culture method. Cell culture for *C. trachomatis* involves inoculating a confluent monolayer of susceptible cells with an appropriately collected and transported specimen. After 48 to 72 hours of growth, infected cells develop characteristic intracytoplasmic inclusions that contain substantial numbers of *C. trachomatis* elementary and reticulate bodies. These unique inclusions are detected by staining with a fluorescein-conjugated monoclonal antibody that is specific for the major outer membrane protein (MOMP) of *C. trachomatis.* Culture methods are time consuming and cost-effective due to their nature of propagation. They also require a special transport medium, are not automated, labour intensive, require expertise, subject to contamination and require storage of isolates at -70 ˚C if processing is delayed (Chemesky, 2005). Culture is less sensitive in testing for chlamydia in urine samples (Barnes *et al*., 1989). Unlike cell culture methods, Nucleic Acid Amplification

methods (NAAT) do not require viable organisms. They are designed to amplify nucleic acid sequences that are specific for the organism being detected and are highly sensitive due to their ability to produce a positive signal from as little as a single copy of target DNA or RNA. The ability of NAATs to detect *C. trachomatis* without pelvic examination or intraurethral swab specimen (for males); by the use of urine is a key advantage. Also, they are highly sensitive and specific with a quick turnaround time for results to be derived within less than a day. Some disadvantages of NAATs include the fact that specimens can contain amplification inhibitors that result in false-negative results. NAATs are also expensive and have a low performance in the presence of inhibitors (Crotchfelt *et al*., 1997). Another method of testing for *C. trachomatis* is the Enzyme immunosorbent assay (EIA) which incorporates the detection of chlamydial lipopolysaccharides (LPS) with a monoclonal or polyclonal antibody that has been labeled with an enzyme. This enzyme converts a colorless substrate into a colored product, which is detected by a spectrophotometer. This method is inexpensive, gives quick results, possesses the features that gives a rapid handling of large numbers of specimens and can be accepted for point-of – care tests. However, EIAs also gives false positives when it cross reacts with other chlamydiae and bacteria (Chemesky, 2005). DFA (Direct fluorescent immunosorbent assay) is another technique which uses fluorescein-conjugated monoclonal antibodies that bind specifically to bacterial antigen smears on slides and is suitable for all specimen types. It also has a rapid turnaround time but is labour intensive, time consuming, has low sensitivity for urine and requires a lot of expertise (Chemesky, 2005). Other tests include serological testing incorporating the use of Enzyme Linked Immunosorbent assays (ELISA) and Rapid test kits. The rapid test kit is an immunological antigen test, which provides an instant test

result without special instrumentation or requirement of a skilled lab technician. This test also provides a faster method of testing specimen to obtain quick results. Specimen collected should be tested on-site in order to get accurate results (Barnes *et al*., 1989).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Rationale for investigation

There has been little work that has been done in Ghana regarding the use of Nucleic Acid Methods (NAATs) to diagnose *C. trachomatis*. This may be due to the lack of adequate resources (funding), training and technical expertise, especially in the method of NAAT which is currently the most frequently used method in testing for *C. trachomatis* as well as *N. gonorrhoeae*. Another advantage of the NAAT is the nature of sample (urine or swab) that can be used in testing. The option of using urine as the preferred choice of sample made it less invasive during acquisition and therefore, easier in acquiring consent from patients who were eligible. NAATs are designed to amplify nucleic acid sequences that are specific for the organism being detected. They are able to produce a positive signal from as little as a single copy of the target DNA or RNA (CDC, 2002).

## 3.2 Criteria for study site selection

Five sites were chosen for the study. These were, 37 Military Hospital, the three clinics in Sekondi/Takoradi (2 Medical Reception Station (2MRS) which is an Army clinic, the Naval Sick bay and the Air Force medical center) and the Adabraka STI clinic. The 37 Military Hospital is the second largest hospital in Ghana which is also a major referral center, and serves as the premier medical facility for the Ghana Armed Forces. Approximately 75% of patients seen in the outpatient clinics at the hospital are civilians with no military affiliation which is estimated to see at least 500 STI cases annually. Takoradi on the other hand due to its recent find in oil has attracted majority of foreigners. The three clinics see to the day to

day health needs of both the military and civilian population in the town. Both U.S. and other foreign military service members stationed in Ghana, or arriving in the port cities in Accra and Takoradi may acquire short-leave and off-base privileges and may engage in illegal behavior with commercial sex workers. Adabraka STI clinic on the other hand which is a popular health facility in the Accra Metropolis sees to majority of civilians(commercial sex workers, homosexuals and lesbians) with all kinds of STI cases. The site also serves as a major site for people living with HIV for the collection of their antiretroviral drugs. However, the project was set up by the U.S Navy to benefit the U.S. military force health protection efforts through understanding the prevalence of Chlamydia and Gonorrhea in Ghana and also to enrich the epidemiologic data at the international level.

### 3.3 Sample Size Calculation

The prevalence rate for the study is 20.4% from the research conducted by Nyarko *et al*., 2014.

$$Ss = \frac{Z^2 * (p) * (1-p)}{c^2}$$

Where:

Z = Z value (e.g. 1.96 for 95% confidence level)

p = estimate prevalence of Chlamydia

c = margin of error at 5% (standard value of 0.05)

Ss= required sample size

$Ss= \{(1.96)^2 (0.204) (1-0.204)\}/ (0.05)^2$

$= 249.53$

$\approx 250$

In view of this calculation, total number of samples collected was three hundred and forty from June 2012 to June 2013. Analysis of samples was carried out using descriptive statistics in which graphs and tables were used. For the statistical test, chi-square was used. A total of 340 people were enrolled in the study with 151 males and 189 females. Adabraka STI clinic enrolled a total of 133 patients, 37 Military hospital enrolled 134 whiles the Takoradi sites (Airforce, Army and Navy) enrolled a total of 73 patients between January 2014 to the end of November 2014.

**3.4 Informed consent**

All patients who attended the selected clinics with the symptoms of the disease during the study period were invited to participate in the study. Upon arrival, the patients were given study forms which included consent forms, and in the case where the patient was below eighteen years of age, a child accent form with a Parental consent form for the parent or guardian accompanying the patient to provide consent and a questionnaire to capture the patient basic information on his/her health status.

The Physician, health care provider or designated research assistant carried out the consent procedures, and clearly inquired from the patient their willingness to participate in any/ all of the potential segments of the study. This specifically implied the patient clarifying consent to providing a urine sample and giving information for the questionnaire to be filled. Patients who consented were given urine collection containers to provide first void urine (the first catch when urinating). Patients who also consented to filling out the questionnaire were made to fill them out in a private area where a translator and a witness were provided

when necessary. Patients who opt to fill out the forms by themselves were made to fill out a Test of understanding of English form to be sure that the patient has the basic requirement literacy to be able to fill out the study forms. However, the use of study site numbers was incorporated to enhance confidentiality, preventing the use of names or any other form of identification.

**3.5 Criteria for Inclusion of Subjects**

**3.6 Inclusion Criteria: This included;**

1. Patients who showed chlamydia-like symptoms at the clinics.

   Chlamydia-like symptoms include: burning during urination, discharge from the penis/ vagina, bleeding from the penis/ vagina, foul smell from urine, pain in the penis/ vagina, and pain when having sex.

2. Patients who were 18 years and above were made to provide independent consent

3. Patients below the age of 18 years were made to participate only when they gave parental consent

4. Pregnant women were also included

**3.7 Exclusion Criteria**: This included;

1. Patients who were unwilling to consent

2. Patients who were willing to consent but were without chlamydia-like symptoms.

3. Patients < 12 years of age

4. The adolescent age group between 12 and <18 years without written assent (which can be in the form of initials or thumbprint) and written parental consent from a parent or guardian.

### 3.8 Sample Processing

Urine samples that were collected from the patients were kept refrigerated at 4˚C onsite when storage was for between 3-5 days and were kept frozen in case of long storage. These conditions were implemented for samples from all other sites. Samples were collected from the sites and transported in cold boxes with icepacks to obtain the optimum temperature of 4˚C during transportation to the Noguchi Memorial Institute for Medical Research, Legon, Accra. Urine was aliquoted into 2.5mls tubes after the required amount of urine (500µl) was taken through DNA (Deoxyribonucleic acid) extraction.

#### 3.8.1 Urine Extraction

1. The urine samples were vortexed in the collection bottle and 500µl of urine were aliquoted into a 1.5ml microcentrifuge tubes.
2. The urine was centrifuged at 5000×g(7500rpm) for 10mins to pellet any bacteria in the urine
3. The supernatant was removed and discarded.
4. Bacterial pellet was suspended in 180µl of buffer ATL.
5. A 20µl volume of proteinase K was added, mixed by vortexing and incubated at 56°C until the tissue was completely lysed. There was occasional vortexing during incubation to disperse the sample or placed in a shaking water bath or on rocking platform.
6. Brief centrifugation of the 1.5ml microcentrifuge tube was carried out to remove drops from the inside of the lid.

7. 200µl of Buffer AL was added to the sample, mixed by pulse-vortexing for 15s, and incubated at 70°C for 10min. Brief centrifugation of the tube was carried out to remove drops from inside the lid.

8. There was an addition of 200µl ethanol (96-100%) to the sample and mixed by pulse vortexing for 15secs and incubated at 70°C for 10mins. Brief centrifugation of the 1.5ml microcentrifuge tube was carried out to remove drops from inside the lid.

9. The mixture from step 8 was carefully applied (including the precipitate) to the QIA amp Mini spin column (in a 2ml collection tube) without wetting the rim.

   NB: This can be done best by dividing the mixture into two (400µl each)

   The cap was closed, and centrifuged at 6000xg (8000rpm) for 1 min. The QIAamp Mini spin column was placed in a clean 2ml collection tube (provided) and the tube containing the filtrate was discarded.

10. The QIAamp Mini spin column was carefully opened 500µl of Buffer AW1 was added without wetting the rim. The cap was closed and centrifuged at 6000×g (8000rpm) for 1 min.

11. The QIAamp mini spin column was carefully opened and 500µl of Buffer AW2 was added and centrifuged at full speed at 20,000×g(14,000rpm) for 3mins.

12. The QIAamp Mini spin column was placed in a new 2ml collection tube. Centrifugation was carried out at full speed at 20,000(14,000rpm) for 1min.

13. The QIAamp Mini spin column was finally placed in a clean 1.5ml microcentrifuge tube and the collection tube containing the filtrate was discarded. The QIAamp Mini spin column was carefully opened and 100µl Buffer AE or distilled water was added.

    Note that in getting more yield of DNA, it was advised to add 50µl, centrifuge and add another 50µl. Incubation at room temperature for 1 min before centrifugation at 6000×g (8000rpm) for 1min also helped in getting more yield.

### 3.8.2 NAAT Testing

DNA was stored at 4 ˚C (short term storage) and at -20 ˚C (long term storage) before NAAT testing was carried out. Primers for the procedure were purchased from TIBMOLBIOL which was the LightMix kit for *Chlamydia trachomatis*. The enzyme was purchased from Roche diagnostics which was made up of the enzyme, PCR high grade water and magnesium chloride.

The procedure for the NAAT testing was as follows:

### 3.8.3 Enzyme Reconstitution

1. Roche Master: 60ul of 1b (Colourless cap) of the Reaction Mix Hybprobe 10X concentration was pipetted into One Vial of 1a (Red cap) Fast stat enzyme.

2. Reaction Mix, RM (Green Cap): Reconstitute with 66ul of PCR grade water was added to the Reaction mix, vortexed and Span.

3. Internal Control, IC (White Cap): 66ul of PCR grade water was used to reconstitute this reagent, vortexed and Span.

However, RM & IC are stable for 5 days at 4°C after reconstitution.

In the LightMix kit was also a standard row which had a lyophilized concentration of *C. trachomatis* present which needed to be prepared before being used in the experiment as Standard controls to serve as a guide when the results, Crossing points (CPs) were being read and interpreted. The Standard controls (Sts) CPs ranged between 18 to 35 and in each NAAT testing carried out, these Standard controls were expected to be included to ensure accuracy and precision in the results.

### 3.8.4 Preparation of Standard Row

Each vial with 40ul PCR grade water from $10^1$ to $10^6$ concentrations were reconstituted and pipetted about ten times for thorough mixing. (This was done in Template addition workstation).

The Master mix was carried out using the table below. This table below was derived after troubleshooting to optimize the reagents to derive the right volumes suitable for the experiment to be carried out.

| Components | X1 | X10 |
|---|---|---|
| **PCR-Grade Water** | 6.6 | 66 |
| **$Mg^{2+}$** | 2.4 | 24 |
| **Reagent Mix** | 2.0 | 20 |
| **Internal Control** | 2.0 | 20 |
| **Roche Master (Enzyme)** | 2.0 | 20 |
| **Total** | 15ul | 150ul |

**Table 1: Master mix calculation**

Ideally, a plate map was designed to serve as a guide during template addition. Below is an example of the template layout with NTC= Negative Test Control, S= Sample and St= Standard

**PLATE MAP**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | NTC | S 1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | St1 |
| **B** | | | | | | | | | | | | St2 |
| **C** | | | | | | | | | | | | St3 |
| **D** | | | | | | | | | | | | St4 |
| **E** | | | | | | | | | | | | St5 |
| **F** | | | | | | | | | | | | St6 |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

**Table 2:** Plate map showing sample arrangement

Finally, there was an addition of 5ul of sample (DNA Extract) to make up the total volume to 20ul for amplification.

Amplification of the sample included the four basic steps in PCR (Denaturation, cycling, melting, and cooling) with specific ramp rates for all the cycles.

| Program Step | Denaturation | Cycling | | | Melting | | | Cooling |
|---|---|---|---|---|---|---|---|---|
| **Parameter** | | | | | | | | |
| **Analysis Mode** | None | Quantification Mode | | | Melting Curves Mode | | | None |
| **Cycles** | 1 | 50 | | | 1 | | | 1 |
| **Target** | 95 | 95 | 62 | 72 | 95 | 40 | 85 | 40 |
| **Hold/HH:MM:SS** | 00:10:00 | 00:00:05 | 00:00:05 | 00:00:15 | 00:00:30 | 00:02:00 | 00:00:00 | 00:00:00 |
| **Ramp Rate ($^{o}$C) 96** | 4.4 | 4.4 | 2.2 | 4.4 | 4.4 | 1.5 | - | 1.5 |
| **Ramp Rate ($^{o}$C) 384** | 4.6 | 4.6 | 2.4 | 4.6 | 4.6 | 2.0 | - | 2.0 |
| **Sec Target** | - | - | 55 | - | - | - | - | - |
| **Step size** | - | - | 0.5 | - | - | - | - | - |
| **Step Delay (Cycles)** | - | - | 1 | - | - | - | - | - |
| **Acquisitions Mode** | None | None | Single | None | None | None | Continuous | None |
| **Acquisitions (per $^{o}$ C)** | - | - | - | - | _ | _ | 1 | - |

**Table 3:** Table showing cycling conditions

Crossing Points (CPs) were generated upon completion of every run. A CP value that ranged between 18 and 35 was generated with a sigmoid curve illustrating the time, cycle and rate of the peak. CP values for samples below 18 and above 35 were considered negative.

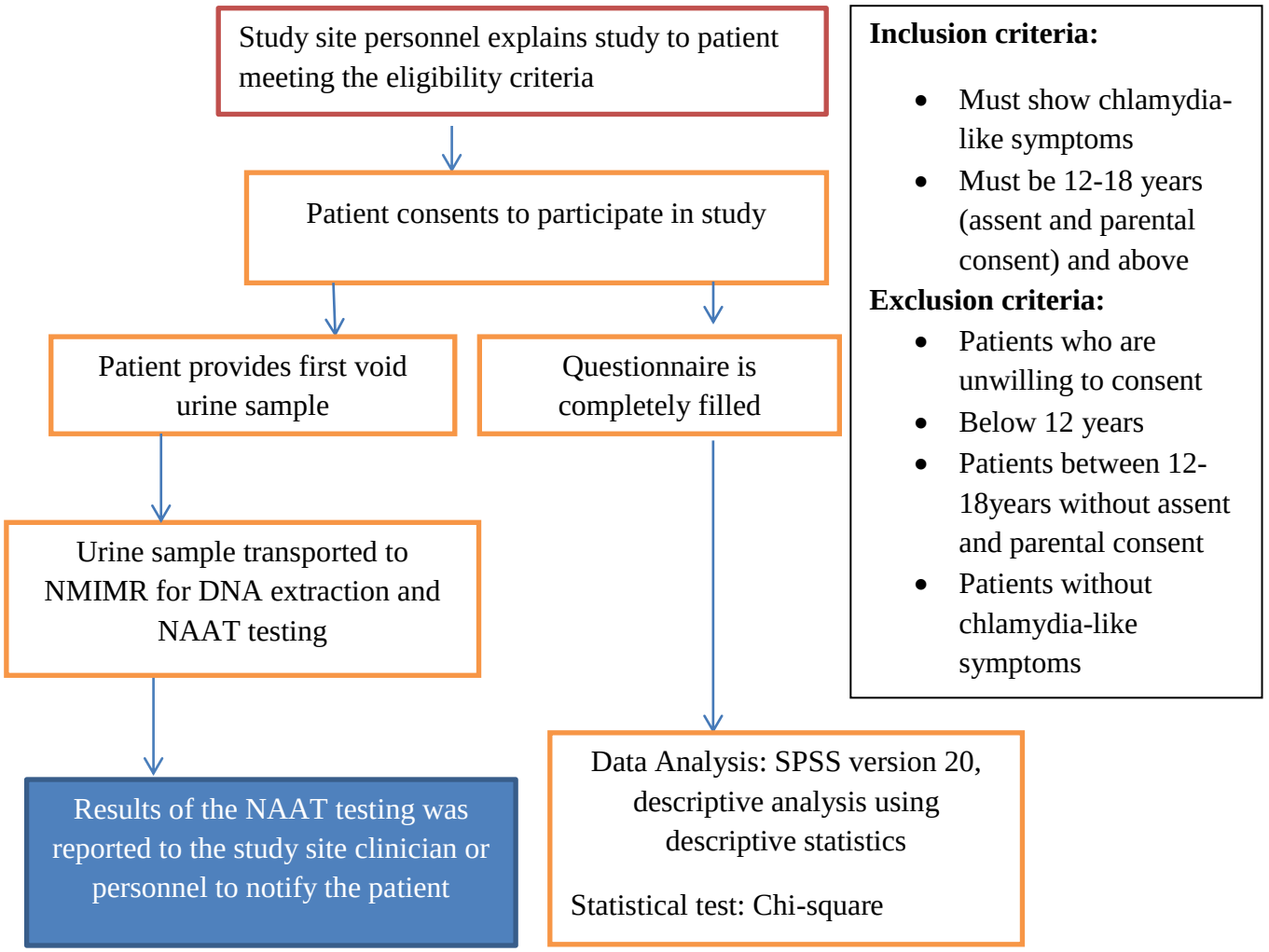


**Figure 2: Sample Processing for 37 Military Hospital, Adabraka and Takoradi**

# CHAPTER 4

# RESULTS

## 4.1 Characteristics of the Participants

The mean age of the participants was 29 years with a standard deviation of 7 with a minimum age of 18years and a maximum age of 61.

**Table 4: Demographic characteristics of patients enrolled in the study**

| | | |
|---|---|---|
| **Age group (years)** | | |
| | **Number** | **Percentage (%)** |
| 18-25 | 108 | 31.8 |
| 26-33 | 161 | 47.4 |
| 34-41 | 49 | 14.4 |
| 42-49 | 17 | 5 |
| 50- above | 5 | 1.5 |
| Total | 340 | 100 |
| **Marital status** | | |
| Single | 196 | 57.6 |
| Divorced/Separated | 11 | 3.2 |
| Married | 133 | 39.1 |
| **Gender** | | |
| Male | 151 | 44.4 |
| Female | 189 | 55.6 |
| **Religion** | | |
| Christian | 304 | 89.4 |
| Muslim | 36 | 10.6 |
| **Education** | | |
| No school | 14 | 4.1 |
| Primary/ Preparatory school | 58 | 17.1 |
| Secondary school & above | 268 | 78.8 |

The age group that showed the highest attendance in the hospitals was 26-33 years group. Patients who were single, 57.7% (196/340) showed the highest attendance rate as compared

to the divorced/ separated, 3.2% (11/340) and married, 39.1% (133/340). More females, 55.6% (189/340) were seen than males, 44.4% (151/340). Whiles 89.4% (304/340) Christians were enrolled; only 10.6% Muslims (36/340) were enrolled. Patients, who had their educational level of Secondary school & above, showed a higher attendance of 78.8 % (268/340) in the study as compared to 4.1% (14/340) participants who had no basic education.

**Table 5: Demographic information of patients enrolled at each site**

| | | | | |
|---|---|---|---|---|
| **Age group (years)** | | | | |
| | **Total** | **Adabraka** | **37 Military** | **Takoradi** |
| 18-25 | 108 | 42 | 47 | 19 |
| 26-33 | 161 | 64 | 58 | 39 |
| 34-41 | 49 | 20 | 19 | 10 |
| 42-49 | 17 | 7 | 8 | 2 |
| 50- above | 4 | - | 2 | 2 |
| **Marital status** | | | | |
| Single | 196 | 80 | 87 | 29 |
| Divorced/Separated | 11 | 5 | 2 | 4 |
| Married | 133 | 48 | 45 | 40 |
| **Gender** | | | | |
| Male | 151 | 44 | 79 | 28 |
| Female | 189 | 89 | 55 | 45 |
| **Religion** | | | | |
| Christian | 304 | 118 | 123 | 63 |
| Muslim | 36 | 15 | 11 | 10 |
| **Education** | | | | |
| No school | 14 | 6 | 7 | 1 |
| Primary/ Preparatory school | 58 | 33 | 12 | 13 |
| Secondary school & above | 268 | 94 | 115 | 59 |

### 4.2 Prevalence of the *C. trachomatis*

A total of 35 (10.3%) were positive for *C. trachomatis* out of the 340 patients who were tested. Adabraka STI clinic had 8 (6.0%) positive cases, 37 Military hospitals had 13 (9.7%) positives cases and finally the Takoradi sites had 14 (19.2%) positive cases. However, cumulatively the prevalence of the disease in among the Greater Accra clinics is 7.9%.

#### 4.2.1 Prevalence of *C. trachomatis* using the demographic information

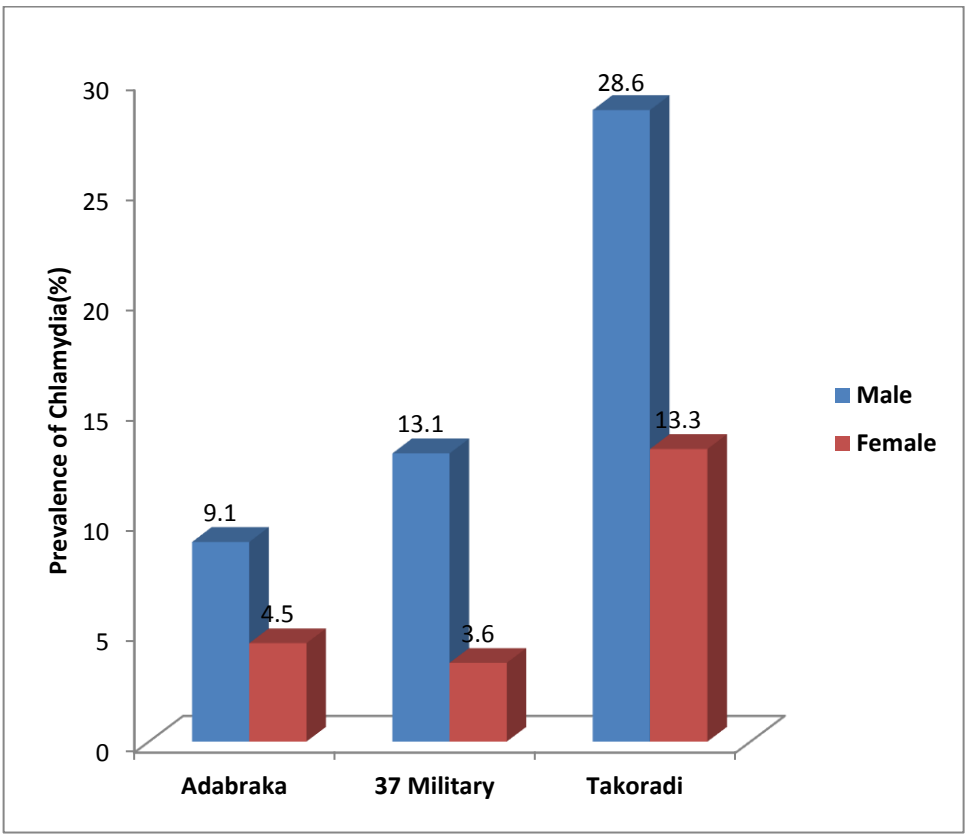


**Figure 3: A graph showing the prevalence of C. *trachomatis* in relation to gender**

Figure 3 shows that the prevalence of *C. trachomatis* infection was higher in males than in females in all three sites. Out of 151 male patients who attended the facilities, 23 (15.2%) tested positive for the disease while 12 out of the 189 females tested positive to the disease.

However, at 37 Military hospital, there was an association between gender and testing positive or negative to *C. trachomatis* (p-value=0.049).

Table 6 shows that in all the positive cases received, males showed higher numbers in relation to the various age groups, marital status, religion and education than females.

**Table 6: Prevalence Chlamydia by gender and demographic information**

| | | | | |
|---|---|---|---|---|
| **Age group** | | | | |
| | **Male** | **Percentage (%)** | **Female** | **Percentage (%)** |
| 18-25 | 7 | 15.9 | 2 | 3.1 |
| 26-33 | 11 | 14.5 | 9 | 10 |
| 34-41 | 5 | 27.8 | 1 | 3.2 |
| 42-49 | 0 | 0 | 0 | 0 |
| 50- above | 0 | 0 | 0 | 0 |
| **Marital status** | | | | |
| Single | 15 | 14.7 | 7 | 7.4 |
| Divorced/ Separated | 1 | 20 | 1 | 16.7 |
| Married | 7 | 15.9 | 4 | 4.5 |
| **Religion** | | | | |
| Christian | 19 | 14.5 | 11 | 6.4 |
| Muslim | 4 | 20 | 1 | 6.3 |
| **Education** | | | | |
| No school | 0 | 0 | 0 | 0 |
| Primary/ Preparatory school | 4 | 14.3 | 2 | 6.7 |
| Secondary school & above | 19 | 16.8 | 10 | 6.5 |

Table 7 shows that out of the total number of participants who tested positive, none had no education whiles those who had higher education (secondary school and above) were

reported having 29/268 (10.8%) positive for the disease and 6/58 (10.3%) positive for Primary/ Preparatory school education.

**Table 7: Prevalence of *C. trachomatis* in relation with education**

| | Total (%) | Adabraka (%) | 37 Military (%) | Takoradi (%) |
|---|---|---|---|---|
| **No school** | 0(0) | 0(0) | 0(0) | 0(0) |
| **Primary/Preparatory school** | 6(10.3) | 2(6.1) | 2(16.7) | 2(15.4) |
| **Secondary school and above** | 29(10.8) | 6(6.4) | 11(9.6) | 12(20.3) |

Table 8 shows that a high number in relation to marital status that tested positive in the study were the singles, 22/196 (11.2%). This was followed by the married having 11/133 (8.3%) patients testing positive for the disease. Divorced/ separated participants who tested positive for *C. trachomatis,* 2/11 (18.2%) showed the highest prevalence.

**Table 8: Prevalence of *C. trachomatis* in relation with marital status**

| | Total (%) | Adabraka (%) | 37 Military (%) | Takoradi (%) |
|---|---|---|---|---|
| **Single** | 22(11.2) | 6(7.5) | 11(12.6) | 5(17.2) |
| **Divorced/Separated** | 2(18.2) | 1(20) | 0(0) | 1(25) |
| **Married** | 11(8.3) | 1(2.1) | 2(4.4) | 8(20) |

.

**Figure 4: Prevalence of *C. trachomatis* in relation to Religion**

Figure 4 shows that there were 30/ 304 (9.9%) Christians testing positive for *C. trachomatis* whereas 5/36 (13.9%) Muslims tested positive.

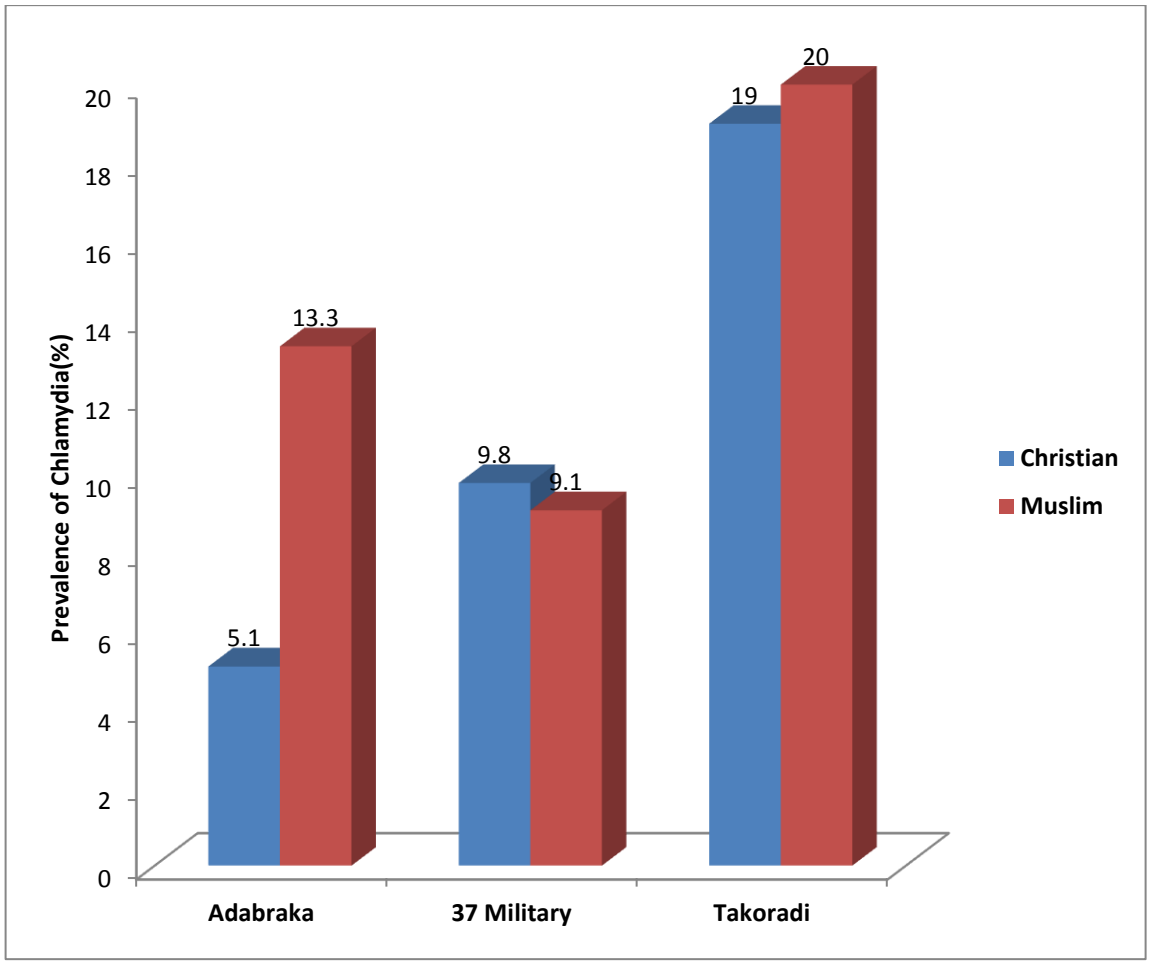


Table 9 shows that the age range, 26-33 showed the highest number of participants and positive cases (20/161) in the study, followed by age range 18-25 presenting with 9/108 positives and 6/49 positives for the age range of 34-41.

**Table 9: Prevalence of *C. trachomatis* in the various age groups**

| Age range | Total (%) | Adabraka (%) | 37 Military (%) | Takoradi (%) |
|---|---|---|---|---|
| 18-25 | 9(8.3) | 1(2.4) | 5(10.6) | 3(15.8) |
| 26-33 | 20(12.4) | 6(9.4) | 5(8.6) | 9(23.1) |
| 34-41 | 6(12.2) | 1(5) | 3(15.8) | 2(20) |
| 42-above | 0(0) | 0(0) | 0(0) | 0(0) |

**Note**: The percentages (prevalence) in each of the sites are based on the number of patients enrolled (Table 5). Denominators were calculated based on the number of patients enrolled with the demographic information they presented**.**

### 4.3 Patients screened and diagnosed with Chlamydia by gender and symptoms in Adabraka

Burning sensation during urination and discharge from penis/ vagina were the symptoms in both males and females that were the most presented at Adabraka. Most of the positive cases that were recorded fell within the category of these two symptoms among both males and females.

**Table 10: *C. trachomatis* prevalence in Adabraka STI clinic in relation with symptoms**

| Symptom | Total number of cases | Positive (%) | Negative (%) | p value |
|---|---|---|---|---|
| **Males n=44** | | | | |
| Burning during urination | 37 | 4(10.8) | 33(89.2) | 0.362 |
| Discharge from penis | 27 | 3(11.1) | 24(88.9) | 0.557 |
| Bleeding from penis | 3 | 0(0) | 3(100) | 0.57 |
| Foul smell from urine | 18 | 2(11.1) | 16(88.9) | 0.698 |
| Pain in penis | 16 | 1(6.3) | 15(93.8) | 0.62 |
| Pain when having sex | 3 | 0(0) | 3(100) | 0.57 |
| Ulcers on genital parts | 1 | 0(0) | 1(100) | 0.749 |
| Warts on genital parts | 0 | 0(0) | 0(0) | 0 |
| Other | 2 | 15(0) | 1(50) | 0.039 |
| **Females n=89** | | | | |
| Burning during urination | 58 | 3(5.2) | 55(94.8) | 0.362 |
| Discharge from vagina | 79 | 4(5.1) | 75(94.9) | 0.467 |
| Bleeding from vagina | 29 | 1(3.4) | 28(96.6) | 0.741 |
| Foul smell from urine | 23 | 2(8.7) | 21(91.3) | 0.259 |
| Pain in vagina | 25 | 2(8.0) | 23(92.0) | 0.318 |
| Pain when having sex | 6 | 0(0) | 6(100) | 0.582 |
| Ulcers on genital parts | 4 | 0(0) | 4(100) | 0.657 |
| Warts on genital parts | 2 | 0(0) | 2(100) | 0.756 |
| Other | 3 | 0(0) | 3(100) | 0.702 |

### 4.4 Patients screened and diagnosed with Chlamydia by gender and symptoms in 37 Military hospital

From Table 11, there were more positive cases (10/79) males with the symptom of burning sensation during urination than all the other symptoms that were reported during the enrollment. The common symptom that was recorded was discharge from penis (64/79),

followed by burning during urination in the males. The highest symptom recorded among the females was also discharge from the vagina (48/55).

**Table 11: *C. trachomatis* prevalence in 37 Military hospital in relation with symptoms**

| Symptom | Total number of cases | Positive (%) | Negative (%) | p value |
|---|---|---|---|---|
| **Males n=79** | | | | |
| Burning during urination | 52 | 10(19.2) | 42(80.2) | 0.059 |
| Discharge from penis | 64 | 9(14.1) | 55(85.9) | 0.941 |
| Bleeding from penis | 0 | 0(0) | 0(0) | 0 |
| Foul smell from urine | 3 | 0(0) | 3(100) | 0.478 |
| Pain in penis | 18 | 5(27.8) | 13(72.2) | 0.053 |
| Pain when having sex | 3 | 0(0) | 3(100) | 0.478 |
| Ulcers on genital parts | 9 | 0(0) | 9(100) | 0.2 |
| Warts on genital parts | 1 | 0(0) | 1(100) | 0.686 |
| Other | 18 | 3(16.7) | 15(83.3) | 0.702 |
| **Females n=55** | | | | |
| Burning during urination | 21 | 1(4.8) | 20(95.2) | 0.726 |
| Discharge from vagina | 48 | 1(2.1) | 47(97.9) | 0.107 |
| Bleeding from vagina | 2 | 0(0) | 2(100) | 0.78 |
| Foul smell from urine | 15 | 0(100) | 15(100) | 0.378 |
| Pain in vagina | 14 | 0(0) | 14(100) | 0.4 |
| Pain when having sex | 15 | 0(0) | 15(100) | 0.378 |
| Ulcers on genital parts | 2 | 0(0) | 2(100) | 0.78 |
| Warts on genital parts | 0 | 0(0) | 0(0) | 0 |
| Other | 27 | 2(7.4) | 25(92.6) | 0.142 |

### 4.5 Patients screened and diagnosed with Chlamydia by gender and symptoms in Takoradi

From Table 12, patients who reported at the Takoradi sites also recorded those who presented with symptoms of burning sensation during urination and discharge from the penis/ vagina. They recorded the highest numbers in the aforementioned presentations and also the number of positive cases in both males and females.

**Table 12: *C. trachomatis* prevalence in Takoradi in relation to symptoms**

| Symptom | Total number of cases | Positive (%) | Negative (%) | p value |
|---|---|---|---|---|
| **Males n=28** | | | | |
| Burning during urination | 19 | 7(36.8) | 12(63.2) | 0.159 |
| Discharge from penis | 26 | 7(26.9) | 19(73.1) | 0.486 |
| Bleeding from penis | 3 | 0(0) | 3(100) | 0.246 |
| Foul smell from urine | 8 | 1(12.5) | 7(87.5) | 0.234 |
| Pain in penis | 14 | 5(35.7) | 9(64.3) | 0.403 |
| Pain when having sex | 5 | 2(40.0) | 3(60.0) | 0.533 |
| Ulcers on genital parts | 1 | 0(0) | 1(100) | 0.52 |
| Warts on genital parts | 3 | 1(33.3) | 2(66.7) | 0.847 |
| Other | 2 | 0(0) | 2(100) | 0.353 |
| **Females n=45** | | | | |
| Burning during urination | 18 | 2(11) | 16(88.9) | 0.72 |
| Discharge from vagina | 39 | 5(12.8) | 34(87.2) | 0.796 |
| Bleeding from vagina | 6 | 0(0) | 6(100) | 0.302 |
| Foul smell from urine | 5 | 1(20.0) | 4(80.0) | 0.642 |
| Pain in vagina | 11 | 2(18.2) | 9(81.8) | 0.586 |
| Pain when having sex | 13 | 2(15.4) | 11(84.6) | 0.796 |
| Ulcers on genital parts | 2 | 0(0) | 2(100) | 0.57 |
| Warts on genital parts | 1 | 0(0) | 1(100) | 0.692 |
| Other | 3 | 2(66.7) | 1(33.3) | 0.005 |

Table 13 shows that most of the males who tested positive corresponding with the symptoms they presented for *C .trachomatis* had a mean age of between 28 and 30.

**Table 13: Average age of male participants screened and diagnosed with Chlamydia**

| Symptom | Patients screened | | | Patients with positive diagnosis | | | |
|---|---|---|---|---|---|---|---|
| | Number of cases | Mean age | S. D | Positive diagnosis | Mean Age | S.D | Prevalence(%) |
| **Males n=151** | | | | | | | |
| Burning during urination | 108 | 29.2 | 6.28 | 21 | 28.3 | 6.16 | 19.44 |
| Discharge from penis | 117 | 29.0 | 6.46 | 19 | 29.4 | 5.98 | 16.24 |
| Bleeding from penis | 6 | 28.7 | 5.05 | - | - | - | - |
| Foul smell from urine | 29 | 28.1 | 4.40 | 3 | 33.70 | 2.31 | 10.34 |
| Pain in penis | 48 | 29.0 | 5.57 | 11 | 28.82 | 7.09 | 22.92 |
| Pain when having sex | 11 | 29.0 | 3.36 | 2 | 32.5 | 3.54 | 18.18 |
| Ulcers on genital parts | 11 | 31 | 7.16 | - | - | - | - |
| Warts on genital parts | 4 | 29 | 3.70 | 1 | 30.00 | - | 25 |
| Other | 22 | 30.2 | 8.12 | 4 | 31.25 | 7.63 | 18.18 |

Table 14 shows that most of the females who tested positive corresponding with the symptoms they presented for *C .trachomatis* had a mean age of between 24 and 30.

**Table 14: Average age of female participants screened and diagnosed with Chlamydia**

| Symptom | Patients screened | | | Patients with positive diagnosis | | | |
|---|---|---|---|---|---|---|---|
| | Number of cases | Mean age | S. D | Positive diagnosis | Mean Age | S.D | Prevalence(%) |
| **Females n=189** | | | | | | | |
| Burning during urination | 97 | 29.0 | 6.88 | 6 | 27.5 | 3.94 | 6.19 |
| Discharge from vagina | 166 | 29.0 | 7.03 | 10 | 29.0 | 5.17 | 6.02 |
| Bleeding from vagina | 37 | 27.2 | 6.53 | 1 | 27.0 | - | 2.07 |
| Foul smell from urine | 43 | 29.8 | 7.65 | 3 | 30.7 | 8.08 | 7 |
| Pain in vagina | 50 | 29.0 | 6.98 | 4 | 27.3 | 2.5 | 8 |
| Pain when having sex | 34 | 29.0 | 6.14 | 2 | 29.5 | 5.0 | 5.88 |
| Ulcers on genital parts | 8 | 25.8 | 5.01 | - | - | - | - |
| Warts on genital parts | 3 | 24.0 | 1.73 | - | - | - | - |
| Other | 33 | 28.8 | 7.16 | 4 | 26.3 | 5.5 | 12.12 |

Patients who reported burning sensation during urination among the males who were enrolled in all the sites had the highest number of *C. trachomatis* positive cases (21/151) in the study with a mean age of 28.3 and a standard deviation of 6.16. This was followed by the symptom of discharge from penis (19/151) with a mean age of 29.4 and a standard deviation of 5.98. Discharge from the vagina, among the females cumulatively recorded the highest number of positive cases of the disease (10/189) with a mean age of 29 and a standard

deviation of 5.17 whiles burning sensation during urination recorded 6/189 positives. The prevalence of the disease was dominant among the 26-33 age range.

**Table 15: Prevalence of Chlamydia based on symptoms**

| Symptom | Total number of cases | Prevalence | P value |
|---|---|---|---|
| Burning during urination | 205 | 27/205(13.2%) | 0.031 |
| Discharge from penis/vagina | 283 | 29/283(10.2%) | 0.95 |
| Bleeding from Penis/vagina | 43 | 1/43(2.3%) | 0.066 |
| Foul smell from urine | 72 | 6/72(8.3%) | 0.537 |
| Pain in Penis/Vagina | 98 | 15/98(15.3%) | 0.053 |
| Pain when having sex | 45 | 4/45(8.9%) | 0.739 |
| Ulcers on genital parts | 19 | 0/19(0%) | 0.129 |
| Warts on genital parts | 7 | 1/7(14.3%) | 0.725 |
| Other | 55 | 8/55(14.3%) | 0.257 |

The prevalence of the disease based on the symptom, discharge from penis/ vagina recorded the rate of 10.2% (29/283) followed by burning sensation during urination with a rate of 13.2% (27/205) and finally pain in penis/vagina, 15.3% (15/98). The prevalence of the disease based on pain in penis/vagina presented the highest prevalence of 15.3% (15/98). Other symptoms which included warts on genital parts also recorded a significant 1/7 positive case with a prevalence of 14.3%.

During the enrolment, patients who met the eligibility criteria were asked to bring their partners for testing and out of the total 340 people enrolled, 3/130 cases from Adabraka who were asymptomatic, were brought in by their partners and they tested negative for *C.*

*trachomatis*. The other sites (37 Military hospital and Takoradi) were unable to enroll partners of patients who met the eligibility criteria.

# CHAPTER 5

# DISCUSSION

In 2008, *C. trachomatis* infection was estimated to be 100.4 million worldwide, with Africa having 9.1 million cases, with a prevalence of 3.9% in females, and 2.4% in males (WHO, 1999).

The mostly asymptomatic nature of the disease in both males and females normally leads to adverse conditions of urethritis and cervicitis which normally leads to infertility (Malhotra *et al*., 2013) Also, Pelvic Inflammatory Disease (PID) and other conditions have affected the reproductive health of many females worldwide (Malhotra *et al*., 2013). Infected women who are pregnant are at higher risks of getting their unborn children also getting infected or having complications as well (CDC, 2010). According to various reports that have been released, the prevalence rate of *C. trachomatis* is higher in women than men (WHO, 1999). This can also be attributed to a higher percentage of women seeking more reproductive and gynecological healthcare than men. This is normally the trend in many studies that involves both male and female patient enrollment.

According to the results deduced from this study, there was a high percentage of females (55.6%), attending the health facilities than males (44.4%). Prevalence of the disease was higher in males (15.2%) than in the females (6.3%) in the health facilities. In a study carried out in Rome to investigate the epidemiology of urogenital infections caused by *C. trachomatis* and characteristic features of patients at risk showed, 9.8% prevalence among females and 6.0% in males (Del Piano *et al*., 1994). The prevalence presented in this study

according to gender was far lower than a similar study that was carried out at by Nyarko at Tarkwa with a prevalence of 19.7% and 22.5% in females and males respectively (Nyarko *et al.*, 2014). However, the prevalence was higher than that reported by Opoku *et al*., 2010 who had a prevalence rate of 4.8% and Pepin *et al.,* 2004 with a prevalence of 3.2%. Although there was no overall association between the gender and patients testing positive for Chlamydia or not, one of the sites, 37 Military hospital showed that there was an association between gender and testing positive for the disease (p value=0.049). This means that there is a higher likelihood of eligible males being enrolled, to test positive for *C. trachomatis* than females enrolled at the 37 Military hospital.

There have also been a higher prevalence of Chlamydia observed amongst female adolescents, mostly ≤20years (24.1%-27%) and the association with young age highlight the importance of screening sexually active persons below twenty years (WHO, 2001). In this study, the lowest age enrolled was 17 years and majority of the patients who were enrolled in the study fell within the 26-33 years age bracket, however, it was this age group that had the highest prevalence (12.4%) which is similar to the results deduced by Okoror *et al*., 2014. However, the expected age group (18-25 years) still showed a lower prevalence rate (8.3%) as compared to work done by Opoku *et al.*, 2010 (66.6%).

In relation to marital status and the prevalence of *C. trachomatis,* the singles, similar to work done by Okoror *et al.*, 2014 in Nigeria, showed the higher number of cases (11.2%) testing positive for the disease as compared to the married (8.3%).

It was noted that burning sensation during urination and urethral discharge in men was a major symptom that was reported in all the study sites. Nyarko *et al*'s (2014) study showed

results whereby majority of the male positive (10%) cases presented urethral discharge and burning sensation as symptoms. However, there was an association between burning during urination and one having the disease in both males and females (p value= 0.031). This is therefore a good symptomatic signal to give Clinicians the clues in helping to diagnose patients who present such cases in order to acquire the right treatment. Vaginal discharge in female patients presented with a high number (6%) of *C. trachomatis* cases. This finding will aid in the diagnosis of cases of vaginal discharge commonly presented in women which is mostly suspected to be Candidiasis. This correlates with the work carried out by Pepin *et al* where majority of the women who were investigated showed vaginal discharge. Another symptom that was also commonly reported was pain in the vagina which showed the highest prevalence (8%) and pain in the penis (22.92%) with a total prevalence rate of 15.3% in both males and females. This means that majority of the patients who tested positive for *C. trachomatis* had a common complaint of pain in the penis or the vagina.

Another result worth noting was the mean ages that were associated with the symptoms presented with *C. trachomatis* positive cases. Among the males, the highest mean age was 33.7 whiles the lowest was 28.8. The highest mean age among the females was 30.7 whiles the lowest was 26.3. This attests to the fact that the age group, 26-33 years had the highest numbers who tested positive for *C. trachomatis*. This confirms previous studies carried out by Opoku *et al* (2010) in the Kumasi Metropolis that chlamydia infection falls with increasing age which was clearly illustrated in Table 9 (prevalence of *C. trachomatis* in the various age groups).

None of the 19 cases of patients presenting with genital warts tested positive for chlamydia whiles 1/7 (14.3%) positive *C. trachomatis* cases resulted from patients who reported with

genital ulcers at the health facilities. Genital ulcers and warts observed in the list of symptoms presented by some patients who enrolled could be indicative of STIs caused by viral and other bacterial pathogens which may warrant further surveillance of other STIs like Human Papilloma virus (HPV) and Herpes in Ghana.

Educationally, patients who had attended Secondary school and above had the highest numbers testing positive (10.8%) as compared to those who had Primary school education (10.3%). This is another surprising result because it is expected that the awareness to protected sex should be more with higher education. This clearly shows the need for more sensitization in terms of preventive measures (abstinence, condom use, etc.) needed for people to put in place to help reduce the rapid rate of transmission of various sexually transmitted infections circulating currently.

The prevalence of *C. trachomatis* was higher in Takoradi (19.8%) in the Western region than in Accra (7.9%), Greater Accra region. This could be due to the increasing numbers of offshore industries in those areas because of the recent oil find in that part of the country. This has contributed to the influx of foreigners leading to commercial sex workers engaging in their trade which has led to the increase of STIs in that region. The prevalence of Chlamydia was closer to the prevalence rate which was derived in the study that was conducted by Nyarko *et al* (2014) in Tarkwa , also in the Western region of Ghana.

With regards to the risk factors that were investigated in relation to the prevalence of Chlamydia, it can be noticed that not all the risk factors for STI are acceptable risk factors because none of the demographic information or symptoms showed some association between them. Therefore, a clinician or a health practitioner cannot diagnose patients based

on their demographic information or symptoms they present. The only symptom that showed a positive relationship cumulatively (Table 10) from all the sites was burning during urination giving clinicians to investigate further prior to treatment when a patient presents to the hospital with this particular symptom.

## 5.1 Conclusion

The overall prevalence of *C. trachomatis* in the study was 10.3% with 35 patients testing positive for the disease.

Chlamydia was higher in the males (15.2%) enrolled than the females (6.3%).

The sites at Takoradi showed a higher prevalence (19.17%) of the disease as compared the Accra sites (7.9%). 37 Military hospital showed a prevalence of 9.7% whiles Adabraka STI clinic showed a prevalence of 6.0%.

However, in relation with age, those within the age group of 26-33 years (12.4%) showed the highest prevalence of *C. trachomatis*.

Religion, age, educational level and marital status did not have any relationship with a patient testing positive for *C. trachomatis*.

There was however an association between burning sensation during urination and a patient testing positive for *C. trachomatis*.

## 5.2 Limitations

1. Using one method of testing for *C. trachomatis* prevented further laboratory analysis from being conducted.
2. Only symptomatic patients were enrolled, reducing the probability of investigating asymptomatic patients as well.
3. The scarcity, cost and unavailability of Chlamydia tests, makes it difficult for testing.

### 5.3 Recommendation

1. There is the need for more public awareness by Researchers and Clinicians of STIs including chlamydia due to its asymptomatic nature and therefore the need for its integration into routine health system for sexually active males and females to get tested.
2. More innovative ways must be found to test sexually active young people, since the disease remain subclinical. For example, integrating it in the form of routine checkups as a general hospital routine.
3. Clinicians should be encouraged to use NAATs as a diagnostic tool since they can be used without using invasive methods (urine) in acquiring samples.
4. In the absence of laboratory testing, syndromic approach (burning sensation during urination, pain and urethral discharge) could be used for diagnosis.

# REFERENCES


Barnes R.C. (1989) Laboratory diagnosis of human chlamydial infections. Clin Microbiol Rev; 2:119—36

Beatty W.L, Morrison R.P, Byrne G.I. (1994) Persistent Chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis. Microbiol Rev.; 58:689-99.

Byrne G.I, Ojcius D.M. (2004) Chlamydia and apoptosis: life and death decisions of an intracellular pathogen. Nat Rev Microbiol; 2(10):802-8.

Bentsi C., Klufio C. A., Perine P. L., Bell T. A., Cles L. D., Koester C. M., and Wang S. P.( 1985) Genital infections with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Ghanaian women. Genitourin Med; 61(1): 48-50.

Buve A., Carael M., R.J. Hayes, Auvert B., Ferry B., Robinson N.J., Anagonou S., Kanhonou L., Laourou M., Abega S., Akam E., Zekeng L., Chege J., Kahindo M., Rutenberg N., Kaona F., Musonda R., Sukwa T., Morison L., Weiss H.A. and Laga M., for the Study Group on heterogeneity of HIV Epidemics in African Cities (2001). Multicenter study on factors determining differences in rate of spread of HIV in sub-Saharan Africa: methods and prevalence of HIV infection. AIDS Suppl 4: S127-31.

Cates W. Jr., Wasserheit J.N. (1991) Genital chlamydial infections: epidemiology and reproductive sequelae. American Journal of Obstetrics and Gynecology; 164:1771-81.

Centers for Disease Control and Prevention (CDC). (2010.)Sexually transmitted diseases treatment guidelines. Morbidity and Mortality Weekly Report. Vol. 59, No. RR-12

Center for Disease Control (CDC), Morbidity and Mortality Weekly Report (MMWR); Screening Tests to Detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections (2002), Recommendations and Reports; Vol.51, No. RR-15

Chemesky A. Max, (2005) The laboratory diagnosis of Chlamydia trachomatis infections. Can J Infect Dis Med Microbiol; 16(1): 39–44.

Crotchfelt K.A, Welsh L.E, DeBonville D., Rosenstraus M., and Quinn T.C. (1997). Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genitourinary specimens from men and women by a coamplification PCR assay. J Clin Microbiol; 35:1536—40

Del Piano M., Magliano E.M, Latino M.A, Nicosia R., Pustorine R., Sanitino I., Gordini C., Clerici P., Colombo R., and Sessa R. (1994) Epidemiology of urogenital infections caused by *Chlamydia trachomatis* and outline of characteristics features of patients at risk. J Med Microbiol.; 41:168–172.

Deceuninck G., Asamoah-Adu C., Khonde N., Pépin J., Frost E.H., Deslandes S., Asamoah-Adu A., Bekoe V., Alary M. (2000) Improvement of clinical algorithms for the diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by the use of Gram-stained smears among female sex workers in Accra, Ghana. Sex Transm Dis: 401-10.

Drescher C., Elkins T.E., Adkeo O., Akins-Bekoe P., Agbemadzo T., Foster R.L. and Martey J.O. The incidence of urogenital *Chlamydia trachomatis* infections among patients in Kumasi, Ghana. - Int. J. Gynecol. Obstet., 27: 381-383

Dzidzo Y.T.; Annang, T. N; Apea-Kubi, K.A; L. George; Mensah, D.; Akyeh, L.; Bosompem, K. M.

(2014) *Chlamydia trachomatis* and *Neisseria gonorrhoeae* prevalence among women of reproductive age living in urogenital schistosomiasis endemic area in Ghana.BMC Research Notes doi:10.1186/1756-0500-7-349.

Dimitrakov J. (2002) Chlamydia: A journey from embryonated Egg to LCR; http://www.prostatitis.org/chlamydia.html 12-11-05

Everett K. D., Bush R. M., and Andersen A. A. **(**1999). Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. Int. J. Syst. Bacteriol. 49 Pt 2**:**415-440.

Emerson P.M, Burton M., Solomon A.W., Bailey R., Mabey D. (2006) The SAFE strategy for trachoma control: using operational research for policy, planning and implementation. Bulletin of the World Health Organization; 84:613-619

Gordon F. B., and Quan A. L**., (**1965). Isolation of the Trachoma Agent in Cell Culture. Proc. Soc. Exp. Biol. Med. 118**:**354-359.

Ikeme A. C., Ezegwui H.U., Ikeako L.C., Agbata I., Agbata E. (2011) Seroprevalence of Chlamydia trachomatis in Enugu, Nigeria,. Niger J Clin Pract :176-80. doi: 10.4103/1119-3077.84010

Ingrid J. G. Rours., Liesbeth Duijts ., Henriette A. Moll., Lidia R. Arends ., Ronald de Groot., Vincent W. Jaddoe ., Albert Hofman .,Eric A. P. Steegers ., Johan P. Mackenbach ., Alewijn Ott ., Hendrina F. M. Willemse., Elizabeth A. E. van der Zwaan ., Roel P. Verkooijen., Henri A. Verbrugh. ( 2011) Chlamydia trachomatis infection during pregnancy associated with preterm delivery: a population-based prospective cohort study. European journal of epidemiology; 26:493-502.

Jonsson M., Karlsson R., Rylander E. (1997) The associations between risk behaviour and reported history of sexually transmitted diseases, among young women: a population based study. Int J STD AIDS; 8(8):501-5

Johnson L., Coetzee D., and Dorrington R. (2005) Sentinel surveillance of sexually transmitted infections in South Africa: a review. Sex Transm Infect. 81(4): 287-293.

Kuo C.C., Jackson L.A., Campbell L. A., Grayston J.T. (1995) Chlamydia pneumoniae (TWAR), Clinical Microbiology Revised. 8(4):451-61

Malhotra M., Sood S., Mukherjee A., Muralidhar S., Bala M. (2013) Genital Chlamydia trachomatis: An update: Indian J Med Res. 138(3): 303-316

Mayaud P., Ka-Gina G., Cornelissen J., Todd J., Kaatano G., West B., Uledi E., Rwakatare M., Kopwe L., Mnoko D., Laga M., Grosskurth H., Hayes R., Mabey D. (1998) Validation of WHO algorithm with risk assessment for the clinical management of vaginal discharge in Mwanza, Tanzania. Sex Transm Infect; 74 Supp 1:577-84.

Miller K. E. (2006) Diagnosis and Treatment of *Chlamydia trachomatis* Infection. (Am Fam Physician; 73:1411-6.

Ngandjio A., Clerc M., Fonkoua M.C., Thonnon J., Njock F., Pouillot R., Lunel F., Bebear C., Barbeyrac B., and Bianchi A. (2003) Screening of Volunteer Students in Yaounde (Cameroon, Central Africa) for Chlamydia trachomatis Infection and Genotyping of isolated *C. trachomatis* strains. J. Clin Microbiol; 41(9): 4404–4407.

Nyarko C. C., Unson C., Nyarko K. P., Koduah M. (2014) Chlamydia trachomatis prevalence in Ghana- A study at a municipal district in Western Ghana. International Journal of Scientific and Technology research, 3, 1.

Obasi A.I., Balira R., Todd J., Ross D.A., Changalucha J., Mosha F., Grosskurth H., Peeling R., Mabey D.C.W., Hayes R.J. (2001) Prevalence of HIV and Chlamydia trachomatis infection in 15-19 year old in rural Tanzania. Tropical Medicine and International Health, 6, 7, 517-525.

Okoror L.E, Otoickian C., Eniolorunda T., Omoniyi F.H. (2014) Prevalence and risk of Chlamydia trachomatis in symptomatic patients attending clinics in South West Nigeria. Archives of Clinical Microbiology, 5, 5:2 doi: 10.3823/285.

Opoku B.K. and Sarkodie Y.A. (2010) Prevalence of genital Chlamydia and Gonococcal infections in at risk women in the Kumasi metropolis, Ghana. 44 (1).

Paavonen J. and Eggert-Kruse W. (1999) Chlamydia trachomatis: impact on human reproduction. Hum Reprod Update, 5(5):433-47.

Pepin J., Deslandes S., Khonde N., Kintin D., Diakite S., Sylla M., Meda H., Sobela F., Asamoah-Adu C., Agyarko-Poku T., Frost E. (2004) Low prevalence of cervical infections in women with vaginal discharge in West Africa: implications for syndromic management. Sex Transm Infect. 80(3): 230-5.

Richmond S. J., Hilton A. L, and Clarke S. K**. (**1972) Chlamydial infection. Role of Chlamydia subgroup A in non-gonococcal and post-gonococcal urethritis. Br. J. Vener. Dis. 48**:**437-444

Sangani P., Rutherford G., Wilkinson D. (2004) Population based interventions for reducing sexually transmitted infections, including HIV infection. Cochrane Database Syst Rev.

Scheibel J.H., Andersen J.T., Brandenhoff P., Geerdsen JP, Bay-Nielsen A, Schultz BA, Walter S. (1983) Chlamydia trachomatis in acute epididymitis. 17(1):47-50

Centers for Disease Control and Prevention. (2002) Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep; 51(RR-6):1-78

World Health Organization, Department of HIV/ AIDS. (1999) Global prevalence and incidence of selected curable sexually transmitted infections.

World Health Organization, Department of HIV/ AIDS, Geneva. (2001) Global prevalence and incidence of selected curable sexually transmitted infections. Overview and estimates. Pp:11

Yirenya-Tawiah D., Annang T. N., Apea-Kubi K.A., Lomo G., Mensah D., Akyeh L., Bosompem K.M.. (2014) Chlamydia Trachomatis and Neisseria Gonorrhoeae prevalence among women of reproductive age living in urogenital schistosomiasis endemic area in Ghana. 7:349. doi: 10.1186/1756-0500-7-349.

**Internet Sources**

http://www.nhs.uk/Conditions/Chlamydia/Pages/Complications.aspx(complications in men) Accessed on 11/04/2014 at 12:50pm

http://www.cdc.gov/std/pregnancy/stdfact-pregnancy . Accessed on 31/05/2015 at 2:30pm

http://www.cytologystuff.com. Accessed on 31/05/2015 at 10:30 pm

https://www.stockport.nhs.uk Accessed on 31/05/2015 at 11:15 pm

# APPENDICES

## Appendix 1: Materials and Reagents

Gloves

Biohazard bags

Cryovials

Sterile urine collection cups

Alcohol swabs

Reagents, primers, probes for molecular tests

-70°C freezer for storage of *N. gonorrhoeae* isolates

Refrigerator (4-8˚C)

-20˚C freezer

Roche Lightcycler 96 reaction well plates

Roche Lightcycler sealing foils

Roche Lightcycler for Nucleic Acid Amplification Tests

LightMix Chlamydia from TibMolbiol

## Other Supplies

Log books to record specimen identification and testing results

Data collection forms for epidemiologic and laboratory data

## Appendix 2: Standard operating procedure for the LightCycler 480II instrument

### Starting Experiment on LightCycler 480II

1. It is advised that the machine is on, ideally before template addition.
2. Select NEW EXPERIMENT if cycling conditions has not been saved as template.
3. If already saved go to EXISTING OBJECT and select template as per name saved.
4. In new Experiment, enter the cycling Conditions.
5. On SAMPLE EDITOR select ABSOLUTE AMPLIFICATION and select FILTER COMBINATIONS needed for the run.
6. For STI [465-510,498-640,498-660]

   On the same page, go to TOGGLE VIEW (Can switch between the normal plate view with filters)
7. Select the wells used according to the plate map, type in ID's of samples. Also do it for Negative control, Positive controls and standard row.

   NB: For Negative Control, Click on NEGATIVE CONTROL at the bottom on page

   For positive Control, Click on POSITIVE CONTROL/CALIBRATOR

   For standards, click on STANDARDS and key in concentration
8. Check all parameter and ID's are filled in.
9. Feed in plate and click on START RUN.

### REVIEWING RESULTS

1. At EXPERIMENT select DATA
2. Click the down arrow (Located just above the graphs), change FILTER to 498-640
3. Change AXIS to FLUORESCENCE OVER CYCLES

4. Reviews the graphs

**GENERATING CP VALUES**

1. At ANALYSIS select ABS QUANT/2ND DERIVATIVE MAX
2. FILTER COMBINATION 498-640, then CALCULATE.
3. Click the SAVE button.
4. Give a reason for changes and click the tick [ √]
5. Select REPORT and click on the parameters of interest
6. Eg. EXPERIMENT, PROTOCOL, SAMPLES, SETTING, RESULTS, STATISTICS,AMPLIFICATION and STANDARD CURVES
7. Then click GENERATE.
8. Scroll through to view CP values and record values.

The CP value that was considered positive for Chlamydia was between 18 and 35 with a sigmoid curve. Values below 18 and above 35 were considered negative for such samples.