KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,
KUMASI
COLLEGE OF ALLIED HEALTH SCIENCES
DEPARTMENT OF CLINICAL MICROBIOLOGY

PREVALENCE OF CHLAMYDIA INFECTION AMONG ASYMPTOMATIC FEMALE COMMERCIAL SEX-WORKERS IN THE KUMASI METROPOLIS

BY
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DECEMBER, 2005

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Prevalence of Chlamydia infection among asymptomatic female commercial sex-workers in the Kumasi Metropolis

Thesis Submitted to the Department of Clinical Microbiology, College of Allied health Sciences, Kwame Nkrumah University Of Science and Technology (KNUST), Kumasi in partial fulfillment of the requirement of the award of Master of Science degree in Clinical Microbiology

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December, 2005

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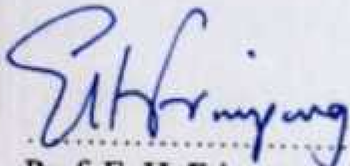
DECLARATION

I, hereby declare that, apart from references to other people's work which have been duly cited, this thesis is the result of an original research work and that the material has not been presented either in part or whole elsewhere for another degree.



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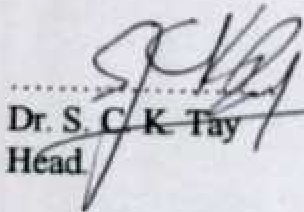

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DEDICATION

This work is fully dedicated to A.K. Kankam and my wonderful Son, Stanley Akwasi Kankam.

ACKNOWLEDGEMENT

1) To God be the glory honor and adoration. I am very much thankful to God Almighty for sustaining me throughout this work.

2) To my supervisor, Prof. E. H. Frimpong, I say may the Lord God richly bless you for being not only my Supervisor but a father, guiding and counseling me in a right direction and without whom, this work wouldn't have been in reality.

3) This is a good time for me to let everyone who will read this thesis know that I also owe Dr. Baffour Poku a great thanks. Dr. Baffour Poku may our almighty God keep you and bless you for every bit of help you gave me.

4) My next appreciation goes to all lecturers who taught me at the department, Prof. Ohene Adjei, Dr. S. C. K. Tay, Mr. Feglo and a lecturer whom I always see as a dear brother, Mr. Mohammed Mutochelu, who always encourages me to forge ahead.

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7) I will also wish to say bravo to Benjamin Payne in the SMS computer Library for typing and organizing this work, God bless you. Last but not the least my appreciation goes to all my study group members, Miss Akua Afriyie, Mr. Emmanuel Asenso Mensah and Mr. Ebo Okran all in the 5th Year Medical School for their immense contribution to a successful completion of this work..

ABSTRACT

Using a Rapid *Chlamydia* test known as the *QuickVue Chlamydia* test, the prevalence of *Chlamydia* infection among 100 asymptomatic female commercial sex-workers was studied at the Komfo Anokye Teaching Hospital (KATH). The symptomatic non-sex workers reporting for STI at the same hospital 50 of them were also screened as a control group. The study was conducted from January to April 2005 with the age of the women ranging from 18 – 35 years.

Two-endocervical swabs were collected from each participant, one was used for the *QuickVue Chlamydia* test and the other one was used for smear preparation and Gram staining. Culture on Thayer Martin's medium was also carried out, so as to establish any gonococcal infection. With the aid of a standard questionnaire, data on the age, marital status, educational background and profession of the participants were collected.

For the 100 asymptomatic female commercial sex workers screened, there were 19 positive cases giving a prevalence of 19.0% (19/100). In this group one gonococcal case was detected. Of the 50 symptomatic female non-sex workers screened, 4 were positive to *Chlamydia* infection given a prevalence of 8.0% (4/50) and no gonococcal case was detected. All the positive cases were within the age groups 20-30.

The outcome of the screening shows that those who are more at risk are the sex workers and the sexually active age group. It is therefore important for health personnel to educate them on the risk of acquiring STIs and the need to go for counseling and screening regularly.

TABLE OF CONTENTS

Declaration.....ii

Dedication.....iii

Acknowledgement.....iv

Abstract.....vi

Table of contents.....vii

List of Tables.....viii

List of plates.....ix

Chapter One: Introduction.....1

Chapter Two: Literature Review.....8

Chapter Three: Materials and Methods.....23

3.1.1 Study Design and Site.....23

3.1.3 Selection of Subjects.....23

3.1.4 Sample Collection and Processing.....24

3.2.0 Rapid Chlamydia test (*QuickVue* test principle).....24

3.3.0 Microscopic Examination of endocervical swab (ECS).....27

3.3.1 Gram staining.....28

3.3.2 Culturing of Specimen on Thayer Martin's Medium.....28

3.3.3 Statistical Analysis.....29

Chapter Four: Results.....30

Chapter Five: Discussion, Conclusion and Recommendation.....38

References.....46

Appendices.....55

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LIST OF TABLES

i. Demographic characteristics of asymptomatic commercial sex workers and symptomatic non-sex workers screened at KATH Microbiology Laboratory.

ii. Prevalence of *Chlamydia* and *Gonococcus* infection among both the women screened.

iii. Age range and prevalence of *Chlamydia* infection among both groups of women screened at KATH.

iv. A table showing the correlation of pus cells to *Chlamydia* and *Gonococcus* infection.

v. A chi-square table showing the difference between the two associations.

LIST OF ABBREVIATIONS

1. KATH - Komfo Anokye Teaching Hospital
2. STI - Sexually Transmitted Infection
3. ECS - endcervical swab
4. SCC - Squamous Cell Carcinoma
5. EIA - Enzyme Immunoassay
6. DNA - Deoxyribonucleic acid
7. RNA - Ribonucleic acid
8. NAAT - Nucleic acid amplification test
9. ATP - Adenosine Triphosphate
10. LGV - Lymphogranuloma Venereum
11. Ebs - Elementary bodies
12. TRIG - Trachoma – inclusion conjunctivitis
13. PID - Pelvic inflammatory diseases
14. LPS - Lippopolysaccharide
15. CF - Complement Fixation
16. DFA - Direct fluorescent antibody
17. PCR - Polymerase chain reaction
18. LCR - Ligase chain reaction
19. CI - Confidence interval

LIST OF PLATES

Photograph showing *QuickVue Chlamydia* test outcome.

i. Uninoculated Cassette.

ii. Positive case with two visible bands.

iii. Negative case showing one visible band.

CHAPTER ONE

INTRODUCTION

Genial *Chlamydia* infection is a sexually transmitted infection caused by an obligate intracellular parasite called *Chlamydia trachomatis* [CEG, 20001].

An estimated 3 million *Chlamydia trachomatis* infections occur annually among sexually active adolescents and young adults in the United States [Coombes, 2004].

The prevalence in Africa is low compared to the western world, which could be attributed to the more rampant use of unprescribed antibiotics such as doxycycline, azithromycin, erythromycin, tetracycline and oxytetracycline [Coombes, 2004].

A study in Kumasi, Ghana reported a prevalence of 3.6% for *Chlamydia* infection for both patients presenting for prenatal care and infertility, by using recently developed RNA Chlamydia Kit [Drescher et al. 1988].

In 2004, another study was conducted in Korle-Bu Teaching Hospital, Accra, Ghana, on the prevalence of STI's in the country and the prevalence of *Chlamydia trachomatis* was recorded as 3.0% when immunofluorescent monoclonal antibody technique was used [Apea-Kubi et al. 2004].

In Gambia a prevalence of 0.8-1.2% was observed in 2001 while in Benin a prevalence of 1.3%was recorded in the same year for *Chlamydia* infection [Buve et al. 2001].

In Cameroon when 1277 volunteer students were screened for *Chlamydia* infection, the prevalence was found to be 3.78% [Antoinette et al. 2003]. In the same year and the same place they carried out the same study on commercial sex workers and a prevalence of 38.3% was documented [Antoinette et al. 2003].

Research into the prevalence of *Chlamydia* infection is very appropriate in this 21st century when sexually transmitted infections are some of the major problems facing the world.

The majority of persons with *Chlamydia trachomatis* infection are not aware of their infection because they do not have symptoms (asymptomatic). Because of this reason, screening is necessary to identify and treat this infection.[CEG,2001].

Genital *Chlamydia* infection in women has serious sequelae. Untreated *Chlamydia trachomatis* infection can persist for long periods and up to 30% of inadequately treated women may go on to develop pelvic inflammatory diseases PID [Bolam et al. 997].

Up to half of all cases of PID's in developing countries can be attributed to *Chlamydia*. Among those with symptomatic PID, one fifth may become infertile and one tenth may suffer ectopic pregnancy [Simms et al. 1998].

Epidemiological studies suggest that *Chlamydia trachomatis* infection also confer increased risk for cervical squamous cell carcinoma (SCC). Of the specific *Chlamydia trachomatis* serotypes, serotypes G was mostly strongly associated with SCC. Other serotypes associated with SCC were D and I [Anttila et al. 2 001].

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Among men, urethritis is the most common illness resulting from *Chlamydia trachomatis* infection. In men complications may lead to epididymitis resulting in sterility. Men who engage in anal sex can have symptoms of proctitis or proctocolitis [CEG, 2001].

Diagnostic methods for *Chlamydia* infection

There are many methods used in diagnosing *Chlamydia* infections with varied sensitivity and specificity. In choosing any of the methods, factors that need to be considered are availability of test materials, qualified personnel, sensitivity and specificity of the test method, time, and quality of the test for a better result.

Culture which is the reference standard, which all other tests have been compared, is technically demanding and expensive [Groseclose et al. 1999]. The most common technique in cell culture involves inoculation of clinical specimens into cycloheximide-treated McCoy cells tissue culture [Ripa et al. 1977].

The basic principle involves centrifugation of the inoculum into the cell monolayer at approximately 3000x g for 1 hour, incubation is done for 48 to 72 hrs, and stained with iodine to detect the glycogen-positive inclusions. Use of fluorescein-conjugated antibodies represents the most sensitive method. Culture method for *Chlamydia* Screening is 100% specific and 92.1% sensitive but it is expensive and requires technical expertise [Stamm, 1983].

The next method is serology. However, serology has limited value in testing for uncomplicated genital *Chlamydia* and therefore not the best [Schachter, 1997].

Enzyme immunoassay (EIA) which is an improved form of serology could be used to detect *Chlamydia* antigens in clinical specimen.

E1A is inexpensive, suitable for large numbers and automatable, high specificity, variable sensitivity but needs much care [Chernesky et al. 1986].

Third method is fluorescent antibody test (FAT) which uses Fluorescein-conjugated monoclonal antibodies to detect elementary bodies (Ebs) in clinical specimen but is unsuitable for large numbers. It is labour intensive and requires skilled personnel. It is 80-85% sensitive and 98% specific [Tam et al. 1984].

The fourth method is Nucleic acid hybridization test, which detects *Chlamydia trachomatis* specific deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences. However, this test fails to detect a substantial proportion of infection and consequently, a new generation of nonculture test called nucleic acid amplification test (NAAT) was developed which is 44-100% sensitive and specificity less than 99%. It needs to be performed by well-trained personnel [Black et al. 1997].

QUIDEL Corporation has developed a *QuickVue Chlamydia* test for a rapid *Chlamydia* screening. *QuickVue Chlamydia* test is a rapid immunoassay using antibodies to detect *Chlamydia* antigens. This type of screening method has been chosen for this study because, it does not require expensive or sophisticated equipment, takes few minutes to get result; It is 82.1% - 82.5% sensitive and has a specificity of 99.3% - 99.5%. [Miller, 1985].

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Many studies are looking for better ways to diagnose, treat, and prevent *Chlamydia* infection. Developing topical microbicides (preparations that can be inserted into the vagina to prevent infection) that are effective and easy for women to use is also a major research focus.

Justification for the study

Due to the asymptomatic nature of *Chlamydia* infection routine screening for it is not usually done hence periodic studies must be carried out to determine the current prevalence. This current study involved asymptomatic female commercial sex workers and symptomatic female non-sex workers presenting for STI at KATH.

The previous study on the prevalence of *Chlamydia* infection by Drescher et al. was carried out in 1988 in Kumasi, which involved symptomatic males and females. Since prevalence keeps on changing it is about time another study is undertaken to determine the current prevalence.

Most women infected with *Chlamydia* are asymptomatic and before they are aware it has resulted in complications. These complications may include bladder infections and other serious pelvic inflammatory diseases (PID), ectopic pregnancy and sterility. Most women presenting with tubal blockage are as a result of *chlamydia* infection. Symptoms of both *Chlamydia* and *gonococcus* infections are almost the same and therefore the later is normally diagnosed when a person shows these symptoms.

A study on *Chlamydia* infection conducted by Antoinette et al. 2003 recorded a prevalence of 38.3% among commercial sex workers in Cameroon.

Sex workers are exposed to multiple sexual partners therefore the likelihood of acquiring the infection is high.

A control group of 50 women who presents at the Microbiology laboratory of KATH with complaints of sexually transmitted infection were also screened in addition to investigation for *gonorrhoae* to give the prevalence of *Chlamydia* among STI patients.

OBJECTIVE OF STUDY

General objective

To determine the prevalence of *Chlamydia* infection among asymptomatic female commercial sex-workers and symptomatic female non- sex workers in Kumasi.

Specific objective

To determine the prevalence of *Chlamydia* infection among 100 asymptomatic female commercial sex-workers between the ages 18-35 in the Kumasi Metropolis using the *QuickVue Chlamydia* test Kit.

To determine the prevalence of *Chlamydia* infection among 50 symptomatic female non-sex workers presenting with STD at KATH, using the *QuickVue Chlamydia* test kit.

To determine the prevalence of *Gonococcus* infection among the asymptomatic female commercial sex-workers and symptomatic female non-sex workers by endocervical specimen culture, on Thayer Martin's medium.

The outcome of this study will help health workers and other policy makers on how to educate young women and encourage them to take screening of *Chlamydia* infection seriously to prevent its future complications. When this is achieved, money used in treating complications that it causes could be channeled to other things.

CHAPTER TWO

LITERATURE REVIEW

Common STIs that manifest in women are usually easily diagnosed; however *Chlamydia* infection is often asymptomatic, leading to complications including infertility before it is detected [Jensen et al. 2004].

Biology of the organism

Chlamydia infection is a curable but most prevalent sexually transmitted infection caused by a bacterium known as *Chlamydia trachomatis*. The word *Chlamys* is Greek for ''cloak draped around the shoulder''. This described how the intracytoplasmic inclusion caused by the bacterium is ''draped'' around the infected cell's nucleus. It is an obligate intracellular parasite that exclusively infects humans because it cannot synthesize its own ATP or grow on artificial medium. It was once thought to be a virus because of its unique developmental cycle. It has a genome size of approximately 500-1000 kilo bases and contains both RNA and DNA. *Chlamydia* is also extremely sensitive to temperature and must be refrigerated at 4°C as soon as the sample is obtained [Hatch and Thomas, 1996].

Chlamydia trachomatis exist as 15 different serotypes. These serotypes cause different diseases in humans: endemic trachoma (caused by serotypes A, B, Ba and C), sexually transmitted disease and inclusion conjunctivitis (caused by serotypes D-K), and lymphogranuloma venereum (LGV) (caused by serotypes L1, L2 and L3). Serotypes D-K may be acquired in neonates from the birth canal, giving rise to inclusion conjunctivitis and pneumonia [Hatch and Thomas, 1996].

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Chlamydia trachomatis exists in two main forms, elementary body and reticulate body.

Elementary body (Ebs) is the dispersal form and is analogous to a spore. It is approximately 0.3mm in diameter and induces its own endocytosis upon exposure to target cells. It is formed intracellularly in the cell; it prevents phagolysosomal fusion and hence allows for intracellular survival. The glycogen produced causes the elementary body to ''germinate'' into the vegetative form (Reticulate body).

Reticulate body is referred to as the vegetative form. It divides by binary fission at approximately 2-3 hours per generation. It has an incubation period of 7-21 days in the host. After division the reticulate body transforms back to the elementary body and is released by the cell by exocytosis. One phagolysosome usually produces 100-1000 elementary bodies) [Hillis and Susan, 1995].

Transmission:

Chlamydia is transmitted through infected secretions only. It infects mainly mucosal membranes, such as the cervix, rectum, throat and conjunctiva.

The serotypes of *Chlamydia trachomatis* causes diseases at different parts of the body and are transmitted differently: serotypes A, B, Ba, and C causes endemic trachoma, which is transmitted from one person to another through infected discharge from the eye, by contaminated hands, clothing or towels. [Hillis and Susan, 1995].

Serotypes D-K cause paratrachoma, a mild or severe eye infection that is transmitted by direct contact, usually sexual. The term Trachoma-inclusion conjunctivitis (TRIC) is sometimes used to describe sexually transmitted *Chlamydia trachomatis* strains that cause eye infection

Infants born of mothers with *Chlamydia trachomatis* infection can become infected during delivery and develop conjunctivitis (inclusion-conjunctivitis). Occasionally infection during delivery can cause neonatal pneumonia. [Hillis and Susan, 1995].

Serotypes L1, L2, and L3 causes lymphogranuloma venereum usually referred to as LGV. It is sexually transmitted from one person to another. A small ulcer forms at the site of infection followed by an inflammation and swelling of lymph glands in the groin [Hillis and Susan, 1995].

Symptoms due to *Chlamydia* infection are quite variable. Most women are asymptomatic after the infection but few show signs and symptoms with varied degrees. Many women discover they have *Chlamydia* infection only when their partners are found to be infected and others discover that they might have had it for sometime when they are treated for the infertility it can cause. After contact with the infection, symptoms appear within 7-21 days which include discharge from the vagina, pain or burning while urinating, more than usual urination, excessive vaginal bleeding during menstruation, painful intercourse for women, spotting between periods or after intercourse, abdominal pains, nausea, fever and inflammation of the rectum or cervix [Bolam et al. 1997].

One very serious illness it can cause is pelvic inflammatory disease [PID] in women, which can lead to early labour and delivery in the case of pregnant women. Infection can also be passed from mother to child during birth, which may cause blindness and pneumonia if not properly treated. It can also infect the throat from oral sexual contact with an infected partner. *Chlamydia* infection is usually confused with *gonorrhea* because the symptoms of both diseases are similar and they sometimes occur together [Buve et al 2001].

The most reliable way to find out whether the disease is a *Chlamydia* infection is through laboratory investigation. A doctor or other healthcare worker will send a sample of endocervical swab to the laboratory to be examined for the presence of the bacteria. It is very important for persons that have *Chlamydia* infection to have the other partners treated.

Diagnosing methods for *Chlamydia* infection:

There are several methods used in diagnosing *Chlamydia* infected patients. Certain factors like cost, expertise, time quality control and availability of screening materials must be taken into consideration when screening for *Chlamydia* infections,

Methods of screening *Chlamydia* include, cell culture, nonculture and serology.

Cell culture method:

Cell culture test for *Chlamydia* infection has been the reference standard against which all other test have been compared [Groseclose et al. 1999].

However other tests have been needed because culture methods for *Chlamydia* infection screening are difficult to standardize, technically demanding, and expensive. Culturing is associated with problem in maintaining the viability of organisms during transport and storage in the diverse settings in which testing is indicated. The most common technique in cell culture involves inoculation of clinical specimens into cyclohexamide-treated McCoy cell tissue culture as discussed in page 3 [Ripa et al. 1977].

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Nonculture methods:

Nonculture tests, including those that can be automated have been developed by manufacturers, and do not require viable organisms. Nonculture tests include Enzyme immunoassays, Direct fluorescent antibody test and Nucleic acid hybridization test.

Serological diagnosis:

The most widely used serological test for diagnosing *Chlamydia* infection is the complement fixation test (CF). The test is not useful in diagnosing uncomplicated lower genital tract infection, which represent the majority of the tests. Although manufactures may claim that their tests are specific for *Chlamydia trachomatis* and that titers of a specific level are diagnostic of current infection, neither of those claims is likely to be accurate. The test will detect antibodies not only to *Chlamydia trachomatis* but also to *Chlamydia psittaci* or *Chlamydia pneumonia* because Lippopolysaccharides (LPS) are genus-specific [Grayston, 1989].

Serology has limited value in testing for uncomplicated genital C*hlamydia* infection and is not advisable to be used in screening because there is infrequent rise and fall in IgG and IgM making test result less meaningful [Schachter, 1997].

Enzyme immunoassays (EIAs)

A number of commercially available products can detect *Chlamydia* antigens in clinical specimens by using enzyme immunoassay (E1A) procedures. The tests include either monoclonal or polyclonal antibodies to the Lipopolysaccharide (LPS) and thus theoretically could detect all *Chlamydiae*. For *Chlamydia trachomatis*, EIA appears to be slightly more

sensitive and slightly less specific. EIA takes more hours to perform, inexpensive, suitable for large numbers and automatable [Chernesky et al. 1986].

Fluorescent antibody tests (FATs):

Most of the early experience with immunofluorescence procedures used polyclonal antibodies in either direct or indirect fluorescent antibody procedures. With this, there are no commercial sources, and laboratories had to prepare their own reagents.

More recently, manufacturers have made fluorescein-conjugated monoclonal antibodies available, which detect elementary bodies (Ebs) in clincal specimen, in contrast to previous efforts to detect inclusions [Tam et al. 1984].

There were problems associated with early commercial direct fluorescent-antibody (DFA) reagents. They show cross-reaction with *staphylococcus aureus* and other bacteria. DFA reagents have now been drastically improved and current experience indicates that the test has approximately 80 to 85% sensitivity and 98% specificity as compared with culture. Although it is highly sensitive and specific and can accommodate all specimen types, it is unsuitable for large numbers, labour intensive and needs skilled personnel [Dean et al. 1998].

Nucleic acid hybridization tests also detect *Chlamydia trachomatis* specific deoxyribonudeic acid (DNA) or ribonucleic acid (RNA) sequences [Black et al. 1997].

The primary drawback of these tests, chiefly for *Chlamydia* infection, is that they fail to detect a substantial proportion of infection and consequently, a new generation of nonculture tests, called Nucleic acid amplification test (NAAT'S), were developed that amplify and detect *Chlamydia trachomatis* specific DNA or RNA sequences. These tests are substantially more sensitive than the first generation nonculture tests [Chemesky, 1992].

Nucleic acid amplification test (NAATs):

Some nucleic acid probes are commercially available. The performance profile of these tests has not been well described. The tests clearly are less sensitive than culture and specificity less than 99%. It also needs particular care in the laboratory to avoid contamination.

NAAT's include:

The Roche Amplicor (manufactured by Roche Diagnostics Corporation, Basel, Switzerland) test uses polymerase chain reaction (PCR).

The Abbott LC x (Abbott laboratories, Abbott Park, Illinois) test uses ligase chain reaction (LCR).

Gen.-probe

The Gen.-Probe APTIMA (Gen.-Probe, Incorporated, and San Diego) assays for *Chlamydia trachomatis* uses transcription – mediated amplification (TMA) to detect a specific 23S ribo-RNA target [Black et al. 1997].

Polymerase chain reaction (PCR)

Polymarase chain reaction (PCR) and gene amplification refers to a highly sensitive technique by which minute quantities of specific DNA or RNA sequences can enzymatically amplified to the extend that a sufficient quantity of material is available to reach a threshold ''signal'' for detection. PCR is performed using automated, computerized ''hot block'' called a thermal cycler. PCR makes use of double stranded DNA (dsDNA) of interest, primers, and deoxyribonucleotide phosphate labeled with ^{32}p and Taq DNA polymerase. These are placed together in a small vial which, in turn, is placed in the hot block. The vials are heated and cooled in a manner that allows DNA denaturation, and then repeated cycles of DNA synthesis and denaturation, to occur. This leads to melting of the content of the vials, which allows the

single stranded DNA, oligonucleotide ''primers'' that flank the DNA sequence of interest to anneal to the denatured DNA strands. Extension of the primers by DNA synthesis is done by the Taq polymerase. Repetition of the denaturing–primer annealing-primer extension reaction sequence result in the amplification of the DNA sequence that is located between the primers. The amplified sample is electrophoretically separated in acrylamide gels, followed by staining of the gel for 15 minutes in the running buffer containing drops of ethidium bromide. The gel separation can then be visualized using UV radiation.

***QuickVue Chlamydia* test**

The *QuickVue Chlamydia* test developed by *Quidel* Corporation San Diego, U.S.A is a rapid immunoassay using antibodies to detect *Chlamydia* antigens in clinical specimen.

It is a lateral flow immunoassay intended for the rapid qualitative detection of *Chlamydia* directly from endocervical swab. The test is intended for use as an aid in the presumptive diagnosis of *Chlamydia* infection. It does not require expensive or sophisticated equipment, and is packed as single units. The result is read some few minutes after the test has been performed. It is referred to as a ''point–of–care'' test because it offers advantages in physicians offices, small clinics and hospitals, detention centers and other settings where result are needed immediately (e.g. when decisions need to be made regarding additional testing or treatment while the patient is still present) [Miller, 1985].

Limitation of *QuickVue Chlamydia* test:

The test employs genus-specific monoclonal antibodies and will not specifically differentiate *C. trachomatis*, *C. psittaci* and *C. pneumonia*.

Detection of *Chlamydia* in clinical specimen is affected by age, history of STI, and the presence or absence of symptoms.

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Prevention and Treatment:

Chlamydia infection can be prevented by the correct use of male or female condom every time one had sexual intercourse and others include personal strategies, community-based strategies and health-care provider strategies [Andrews et al. 1997].

WHO recommends syndromic management for symptomatic STI in resource poor settings in both developing and industrialized courtiers .In most countries selective screening in high-risk groups is recommended largely because testing for *Chlamydia* infection is expensive and requires considerable expertise [Saad et al. 2004].

If someone is infected without symptoms (asymptomatic), the person may pass the bacteria to the other sex partner(s) unnoticed, therefore doctors recommend that anyone who has more than one sexual partner, especially women between the ages of 18-35 be screened for *Chlamydia* infections regularly even if they do not have symptoms [Golden et al. 2000].

Chlamydia infection can be treated with antibiotics such as azithromycin 1gm (taken for one day only) or doxycycline 100mg 2 times a day (taken for seven days) or erythromycin 500mg 4 times a day (taken for fourteen days), deteclo 300mg 2 times a day (taken for seven days), ofloxacin 200mg 2 times a day or 400mg once a day (taken for seven days). Pregnant women may be treated with azithromycin or erythromycin or alternatively with amoxycillin [Hillis and Susan 1995].

Prevalence of *Chlamydia infection* in Ghana and some other countries:

The prevalence of urogenital *Chlamydia* infections among selected patients in Kumasi, Ghana was evaluated using an immuno-flourescent monoclonal antibody technique. *Chlamydia* infection was identified in 4 of 110 patients presenting for prenatal care, 2 of 55 female patients with infertility giving a prevalence of 3.6% in each case [Drescher et al. 1988].

Another research on *Chlamydia trachomatis* and *Nesseria gonorrhoea* were undertaken by Apeah-Kubi et al. Among 517 women attending STI clinic in Korle-Bu Teaching Hospital in Accra, Ghana, by using endocervical swabs and RNA detection kit, the prevalence was recorded as 0.6% and 3.0% for *Nesseria gonorrhoeae* and *Chlamydia trachomatis* respectively [Apea-Kubi et al. 2004].

A study conducted in Accra, Ghana recorded a prevalence of 1.6% for *Chlamydia* infection among 280 women screened for the infection [Asamoh-Adu et al. 2001].

In Burkina Faso 645 pregnant women were screened for *Chlamydia* and *Gonococcus* infections and prevalence rates of 3.1% and 1.6% were respectively recorded [Meda et al. 997].

A similar study for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in Benin showed 5.7% for *Neisseria gonorrhoea* and 2.1% for *Chlamydia* infection [Alary et al. 1998].

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Other studies conducted in 2001 by Buve et al, recorded a prevalence of 0.9% for *Neisseria gonorrhoeae* and 1.3% for *Chlamydia trachomatis* in Cotonou in Benin, and 2.7% for Neisseria *gonorrhoeae* and 9.4% for *Chlamydia trachomatis* in Yaounde in Cameroon.

In Gambia a low level of cervical infections was also noted in surveys of women of reproductive age in Gambia. Where as a prevalence of 0.8-1.2% was recorded for *Chlamydia trachomatis*, there was no isolation of *Neisseria gonorrhoeae* [Walraven et al. 2001].

The prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among women between the ages of 15-19 years was surveyed in Masaka and Rakai, Uganda and Mwanza, Tanzania. The prevalence for *N. gonorrhoeae* was 1.8-2.3% and 1.6-13.0% for *Chlamydia trachomatis* [Orroth et al. 2003].

In Cameroon when volunteer students and commercial sex workers were screened for *Chlamydia* infection between May and July 2001. The prevalence rates were found to be 3.78% and 38.3% respectively [Antoinette et al. 2003].

A research was carried out in the Abu Dhabi Emirate, Middle East to determine the prevalence of *Chlamydia* infection among women yielded 2.6% positive of the 1039 women screened with ages between 16-40. It was realized that those women within the age 16- 19 were showing 0.01% and those who were over 40years showed 30% positives [Saad et al. 2004].

In Honolulu, Hawaii prevalence of *Chlamydia* infection was determined by using pelvic examination-based STI screening to all female detainees. Endocervical swabs were taken and

tested for the presence of *Chlamydia trachomatis* using a DNA probe (Genprobe) and a prevalence of 13.9%(14/101) was observed [Katz et al. 2004].

In Jordan University hospital urology clinic, the study of *Chlamydia* infection was carried out among 230 patients, 103 with signs or symptoms associated with urethritis (symptomatic) and 100 showed no signs or symptoms (asymptomatic). The result was that prevalence of *Chlamydia* infection was 4.6% among symptomatic patients with urethritis [Awwad et al. 2003].

In UK Simms et al. 2003 carried out a study on *Chlamydia* infection using endocervical swabs. The prevalence of *Chlamydia* infection was determined by the ligase chain reaction. Of the 45 PID patients tested, 12 were positive to *Chlamydia* infection given a prevalence of 27% (12/45).

In New Orleans, USA, 5,877 students were screened for *Chlamydia and gonorrhea* using ligase chain reaction assay and overall 451 students had *Chlamydia*, 117 had *gonorrhea* and 50 had both *Chlamydia and gonorrhea* [Nsuami et al. 2004].

A research conducted in England for *Chlamydia* infection in women army recruits showed that, nearly 1 out of 10 female new recruits in the army is infected with *Chlamydia trachomatis*. The result of *Chlamydia* screening in 13,204 women showed a prevalence of 9.2% but the figure jumped to more than 12%, if only 17-year-olds were considered. This suggests that, the disease then was highest for the youngest recruits [Gaydos et al. 1998].

Patient screening programs for *Chlamydia* infection

For any screening to be done, the disease should be medically important and clearly defined, and its prevalence reasonably known. The natural history of the disease should be known and an effective intervention must exist and *Chlamydia* infection perfectly fits into this description [Grimes and Schulz, 2002].

Prevalence of *Chlamydia* infection varies extensively depending on the healthcare setting, it is low in primary care but very much higher among women attending STI clinics. For this reason to be able to establish the prevalence in any community, screening should be undertaken at a community level [Paul et al. 1999].

McNulty has suggested that opportunistic testing and screening for genital *Chlamydia* infections in sexually active women under the age of 25years can lead to reduction in *Chlamydia* infection and related morbidity [McNulty et al. 2004].

National *Chlamydia* screening program in England was established in 2002 after a successful pilot study in Portsmouth and various publications of the government's national sexual health and HIV/AIDS strategy [Fenton et al. 2004].

In 1995, a total of 477,638 cases of *Chlamydia* infection were reported to Center for Disease Control and Prevention U.S.A., representing a rate of 182.22 cases per 100,000 population, state–specific rate for women ranged from 46.4 to 622.0 per 100,000 population. The reported rate for women (290.3) was nearly six times more than that for men (52.1). More cases are been reported due to increase *Chlamydia* screening and the use of more sensitive tests and improved reporting as well as high burden of the disease [CDC, Atlanta, U.S. 1995].

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Health policy organization guidelines in the United States recommend annual *Chlamydia* screening of sexually active adolescent and young adult females because symptoms due to *Chlamydia* infection are quite variable and about 75% of female with this infection show no symptoms at all [Hillis and Susan, 1995].

Madison's laboratory school of hygiene and Milwaukee's Bureau of laboratories have come out with a control program (Wisconsin Control Program) in the US, which gives selective screening, low-cost and high volume testing notification and treatment to *Chlamydia* infected partners. The Control Program is also involved in educating the healthcare providers on how to identifying at risk groups [Hillis and Susan, 1995].

In the US *Chlamydia* infection is responsible for an estimated 43%, 70%and 50% of ectopic pregnancies, tubal infertility and pelvic inflammatory disease respectively. *Chlamydia* is the major cause of infant pneumonia and neonatal conjunctivitis [Oakeshott et al. 1998].

Many women discover that they have *Chlamydia* infection only when their partners are diagnosed of the infection. Other women discover that they must have had the infection for sometime when they are being treated for the infertility it has caused [Jensen et al. 2004].

Risk factors:

A research carried out on female genital mutilation (FGM) in the Northern part of Ghana shows that girls who are genitally mutilated are more prone to infections like *Chlamydia trachomatis*, *Bacterial vaginosis* and *candidiasis* [Odoi-Agyarko and Batong, 2001].

Having multiple sexual partners can increase a person's chance of acquiring *Chlamydia* infection.

Association with other STI's

In San Francisco County Jail, (10%) (113/1149) women screened for *Chlamydia* were positive and 5% (55/1142) were positive for gonococcal infection, including 1.0% positive for both infections [Puisis et al. 1998].

There is a positive relationship between the number of *Chlamydia* present in the genital tract and the presence of pus cells; the character of the discharge and likelihood of pelvic inflammatory diseases gives clue for the presence of infection [Geisler et al. 2001].

It is important to distinguish between gonococcal and *Chlamydia* infections, as they require different antibiotic therapy. Unfortunately the diagnosis of *Chlamydia* infection can only reliably be made using diagnostic kits [Domeika, 1997].

Since *Chlamydia* infection is the most common sexually transmitted infection its presence facilitate a person's chance of acquiring other STI's [Oakeshott et al. 1998].

Limitation for *Chlamydia* screening

It is difficult to have a simple routine screening for *Chlamydia* infection.

Although in the United State routine screening of all sexually active adolescents female for *Chlamydia trachomatis* infection is recommended at least annually, no national or state-specific population-based estimates of *Chlamydia* screening coverage are known to exist [Bauer et al. 2004].

CHAPTER THREE

MATERIALS AND METHODS

3.1.0 STUDY DESIGN

3.1.1 STUDY SITE

The study was conducted at the Microbiology Laboratory of Komfo Anokye Teaching Hospital (KATH), Kumasi. The Hospital is well patronized due to its location in the city center of Kumasi, and also being the second largest Hospital in Ghana after Korle Bu Teaching Hospital in Accra, Ghana. It is open to patients from all walks of life 24 hours a day and the doctors and the nurses work on shift basis.

3.1.2 SUBJECTS

One hundred and fifty female were recruited for the study, comprising 100 commercial sex workers and 50 symptomatic non-sex workers used as a control group. The commercial sex workers were considered the asymptomatic because they showed no symptoms of the infection while the symptomatic women were referred by a doctor to be screened due to one or more symptoms suspected to be *Chlamydia* infection. The commercial sex workers reported at the laboratory for their routine checkups.

3.1.3 SELECTION OF SUBJECT

Verbal consent was sought from both the commercial sex workers and the symptomatic non-sex workers and those who gave their consent were included in the study.

Asymptomatic female commercial sex workers who had no clinical complaints were recruited for the study. From February to April 2005 those who reported at the laboratory each day were screened for *Chlamydia* infection.

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For the symptomatic female non-sex workers, those who attended the STI clinic at KATH with clinical complaints suspected to be *Chlamydia* infection were also recruited as the control group. Doctors always referred a fresh case to the Microbiology laboratory for *Chlamydia* screening.

3.1.4 SAMPLE COLLECTION AND PROCESSING

A well-designed questionnaire (Appendix 1) was administered to each of the women recruited for the study, which contains, the patients number, age, date on which sample was taken, marital status, sex life, number of children, educational background and profession. Because the quality of specimen obtained is of much importance and determines the quality of the test, the specimen was taken with great care. First swab was taken to remove excess mucus from the exocervix by a well-trained nurse under the supervision of a doctor. After opening the vagina with speculum to see the endocervix, a second sterile cotton swab was inserted into the endocervical canal, past the squamocolumnar junction until most of the tip was no longer visible. The swab was then firmly rotated for 15-20 seconds and then withdrawn without contamination with exocervical or vaginal cells. The specimen was then tested immediately after collection.

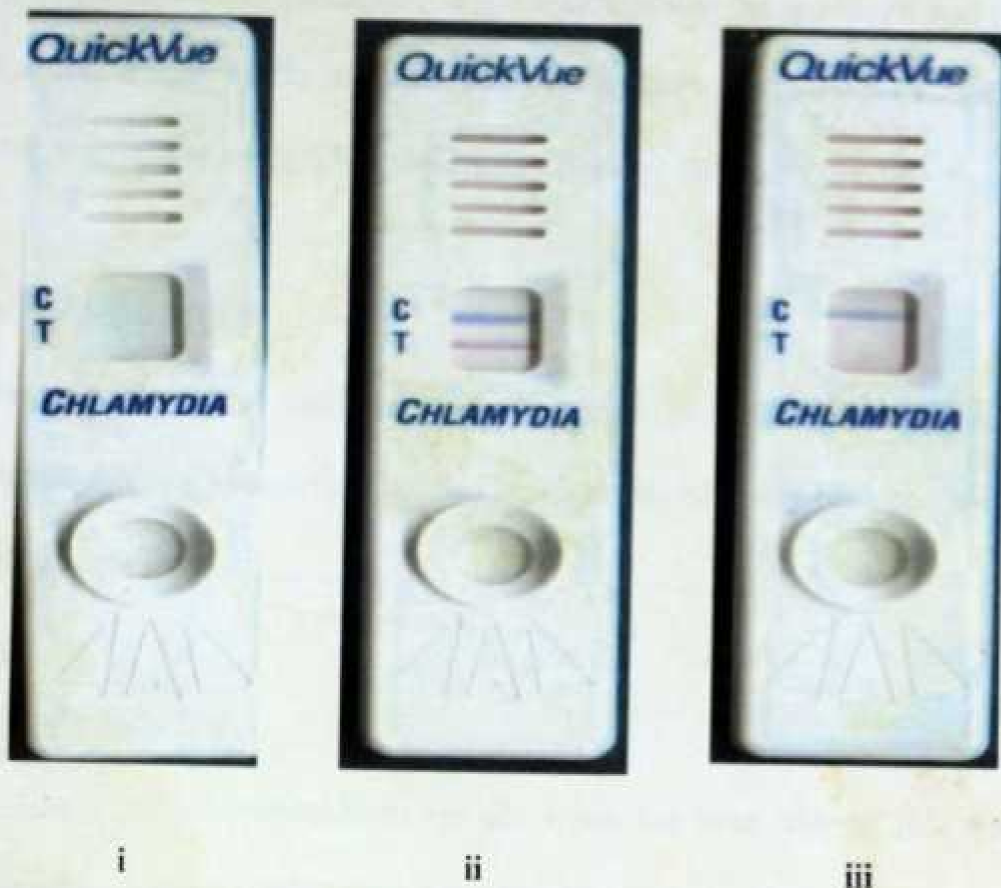
3.2.0 *RAPID CHLAMYDIA TEST*

3.2.1 ***QuickVue Chlamydia test***

To perform the test, the endocervical specimen obtained was placed into a tube-containing reagent A (Extraction solution); after 2 minutes, reagent B (Neutralization solution) was added to the tube. After extraction and neutralization, 3 drops of the extracted sample were added to the test cassette sample well. The sample migrated

through a label pad containing a monoclonal anti-*Chlamydia* antibody conjugated with a pink-Colorado test label and a blue-coloured label. If the sample contained *Chlamydia* antigen, the antigen bound to the antibody coupled to the pink coloured test label which in turn, bound to a second monoclonal anti-*Chlamydia* LPS antibody spotted on the membrane. As the *Chlamydia* antigen antibody complex was captured, a faint-to-dark red test line was visible. A blue control line also appeared in the result window indicating that right volume of clinical sample entered the test cassette and capillary flow occurred. If *Chlamydia* antigen was not present or present at very low levels, only a blue control line was visible. If the blue control line did not develop, the assay was considered invalid. The *QuickVue Chlamydia* test is 82.1% - 82.5% sensitive and has a specificity of 99.3% - 99.5%.

PLATE 3:1 - Photograph showing *QuickVue Chlamydia* test outcomes



i ii iii

LEGEND: (i) Uninoculated Cassette

(ii) Positive Case; two visible bands

(iii) Negative Case one visible band

C-band = Control Band

T-band = Test Band

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3.2.2 POSITIVE RESULT

A positive *QuickVue Chlamydia* result indicates that the specimen was positive for the presence of *Chlamydia* antigen. The appearance of any faint-to--dark red test line along with a blue control line indicated a positive result.

3.2.3 NEGATIVE RESULT

A negative QuickVue result indicates that the specimen is a presumptive negative for the presence of *Chlamydia* antigen. The appearance of only blue control line indicated negative results.

3.2.4 INVALID RESULTS

The test result was considered invalid when the blue control line was not visible within 10 minutes. Whenever this happens the test was repeated by using the remaining extract in the tube.

3.3.0 MICROSCOPIC EXAMINATION OF ENDOCERVICAL SWAB (ECS)

Gram stain was performed on each sample taken, to be able to compare some characteristics of microscopic features such as the presence or absence of pus cells and *Gonococcus* among the women recruited for the study. Two endocervical swabs were taken. One swab was used to do the *Chlamydia* test while the other swab was also used to prepare a smear on a clean, dry microscopic slide. It was then fixed with heat, and a gram stain was performed, and after drying it with a blotting paper 2-3 drops of oil was added and then observed under the microscope.

3.3.1 GRAM STAINING

The endocervical swabs that were taken were each rolled on a sterile dry slide. The smear was then evenly spread on the slide. The smear on the slide was then kept to dry in the air and then fixed by passing it fast through a flame. After the smear was carefully fixed with heat, it was covered with crystal violet for one minute. It was washed with clean water. Gram's iodine normally called the double strength iodine was used to cover the smear and allowed to stay for 1–2 minutes and again washed with clean water. The smear was decolorized for 10–30 seconds with gentle agitation in acetone (30ml) and alcohol (70ml). It was then washed with clean water and safranine was allowed to run over it for 10–30 seconds (2.5% solution in 95% alcohol) and then it was washed with clean water and then dried with a blotting paper. The smear was then examined with the 100 x oil immersion lens of the microscope.

3.3.2 CULTURING OF SPECIMEN ON THAYER MARTIN'S MEDIUM FOR *GONOCOCCUS*

Inoculation was done on Thayer Martin's medium by rolling the cotton tip endocervical swab across a segment of a plate or preferably large 'Z' pattern. This prevents clustering of the inoculum at a particular point, giving adequate area of the plate for growth. The plates were then streaked with a sterile loop to ensure adequate dispersion of the gonococcal colonies. The inoculated plates were then incubated at 37°C in a sealed jar with at least 70% humidity and 5 – 10% carbon dioxide by the introduction of a candle into the jar. The plates were examined after 48hours of incubation.

3.3. 3 STATISTICAL ANALYSIS

Chi-square table and confidence intervals between the two proportions (asymptomatic and symptomatic) were used to analyze the result statistically.

CHAPTER FOUR

RESULT 4.0

TABLE 4: 1 Demographic characteristics of the 100 asymptomatic commercial sex workers and 50 symptomatic non sex-workers screened at the microbiology laboratory of KATH for the prevalence of *Chlamydia* infection.

		Asymptomatic Commercial sex-workers	Symptomatic Non sex-workers
		Frequency (%)	Frequency (%)
Age(years)	18 - 22	56 (56)	14 (28)
	23 - 27	29 (29)	14 (28)
	28 - 32	8 (8)	12 (24)
	33 - 37	7 (7)	10 (20)
	Total	100 (100)	50 (100)
Marital Status	Married	0 0	28 (56)
	single	100 100	20 (40)
	Widow	0 0	2 (4)
	Total	100 100	50 (100)
Educational Background	Literate	59 (59)	28 (56)
	Illiterate	41 (41)	22 (44)
	Total	100 (100)	50 (100)
Profession	Student	19 (19)	11 (22)
	Trader	36 (36)	30 (60)
	Housewife	0 0	4 (8)
	Professional	5 (5)	5 (10)
	"Purely Sex-work"	40 (40)	0 0
	Total	100 (100)	50 (100)

Table 4.1 shows the age range, marital status, educational background, and profession, with percentage frequencies.

Of the 100 asymptomatic female commercial sex workers screened 56% (56/100) of them were between the ages 18-22, 29% (29/100) were between the ages 23-27, 8% (8/100) were also between the ages 28-32 and finally those who were between the ages of 33-37 were 7%(7/100). None of the asymptomatic female commercial sex workers was married or widowed rather they were all single.

Considering educational background, 59% (59/100) of them were literate and 41% (41/100) were completely illiterate. 19% (19/100) out of the 100 them were students in different institutions, 36% (36/100) were traders, none of them was a housewife, 5% (5/100) were professionals in different professions and 40% (40/100) of the women were engaged in purely sex-work.

Of the 50 symptomatic female non sex workers screened the ages ranging from 18-22 and 23-27 were 28% (14/50) in each range, 24% (12/50) was observed between the ages 28-32 and between the ages 33-37 was 20% (10/50).

From the table, 56% (28/50) of the symptomatic female non-sex workers screened were married, 40% (20/50) were single and 4% (2/50) were widowed. Considering educational background, 56% (28/50) were educated and 44% (22/50) were illiterate. 10%(5/50) of the symptomatic female screened were professionals with various professions, 22% (11/50) were students in various institutions, 60% (30/50) were traders and 8% (4/50) were housewives.

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TABLE 4: 2 Results showing prevalence of *Chlamydia* and *Gonococcus* infections among both the 100 asymptomatic female commercial sex-workers and 50 symptomatic non sex-workers screened at the microbiology laboratory of KATH.

Group of women	Total number screened	Positives	
		Chlamydia	*Gonococcus*
Commercial sex-workers	100	19 (19%)	1 (1%)
Symptomatic non sex-workers	50	4 (8%)	0

Of the 100 asymptomatic female commercial sex-workers screened, 19.0% (19/100) were positive to *Chlamydia* infection and only 1.0% (1/50) was positive for *Gonococcus* infection.

Of the 50 symptomatic female non-sex workers screened 8.0% (4/50) of them showed positive to *Chlamydia* infection and none of them was positive for *Gonococcus*.

TABLE 4:3 Age range and prevalence of *Chlamydia* infection among both the 100 asymptomatic female commercial sex workers and 50 symptomatic female non-sex workers screened at the microbiology laboratory of KATH.

Age (years)	Prevalence of Chlamydia infection (%)	
	Asymptomatic commercial sex-workers	Symptomatic non-sex workers
18 – 22	12% (12/100)	0
23 – 27	6.0% (6/100)	4.0% (2/50)
28 – 32	1.0% (1/100)	4.0% (2/50)

From the table, the age group with the highest prevalence rate of infection was found to be between 18–22 years in the asymptomatic commercial sex-workers, followed by 23–27 year group and 28–32 year group having the prevalence of 6.0% (6/100) and 1.0% (1/100) respectively. Among the symptomatic female non sex workers, the ages between 23–27 and 28–32 years both had 2 positive cases each, given a prevalence of 4.0% (2/50) in each case and nothing was recorded for the ages between 18–22 years among the symptomatic female non-sex workers.

TABLE 4:4 A table showing the correlation of pus cells to *Chlamydia* and *Gonococcus* infections.

Result	*Chlamydia*		*Gonococcus*	
Pus Cell	++ ($\geq$ 5 –10)	+++ (>10)	++ ($\geq$ 5 –10)	+++ (>10)
Asymptomatic Sex workers (100)	9	4	0	1
Symptomatic non-sex workers (50)	0	3	0	0

When the number of pus cells is greater or equal to (5-10)it is reported as (++), when it is greater than (10) it is written as (+++) . Of the 100 asymptomatic female commercial sex workers screened 16.0% had pus cells with either ++ or +++ and out of this number 81.3% (13/16) showed positive to *Chlamydia* infection and 6.3%(1/16) gonococcal infection as seen in table 4.4.

Of the 50 symptomatic female non-sex workers screened 8.0% (4/50) had pus cells with either ++ or +++ and out of this number 75% (3/4) were positive to *Chlamydia* infection and none was recorded for gonococcal infection as can also be seen in table 4.4.

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ANALYSIS I

TABLE 4 :5 chi-square (χ^2) of the difference between two Association.

Group of women	*Chlamydia*				Total
	Positive		Negative		
	observation	Expectation	Observation	Expectation	
Asymptomatic sex workers	19	15.3	81	84.7	100
Symptomatic non sex workers	4	7.7	46	42.3	50
Total	23		127		150

$$\chi^2 = \sum \frac{(0-E)^2}{E}$$

Degree of Freedom (df) = k – 1

df = 4 – 1 = 3

from standard tables;

$\chi_{0.05}$ (3) = 7. 81 5 as the critical value. But from the results obtained $\chi^2 = 3.16$ which is lower compared to 7.815.

The prevalence of *Chlamydia* infection was tested statistically using the a chi-square table.

The prevalence of *Chlamydia* infection among symptomatic female non-sex workers is not statistically different from that of the asymptomatic female commercial sex workers.

ANALYSIS II

Using confidence intervals of the two proportions (Asymptomatic and Symptomatic) to determine the difference between the two.

$$\left(\hat{P}_2 - \hat{P}_1\right) \pm Z\left(1 - \frac{\alpha}{2}\right) \times \sqrt{\frac{\hat{P}_2(1-\hat{P}_2)}{n2} + \frac{\hat{P}_1(1-\hat{P}_1)}{n1}}$$

$\hat{P}_2$ = Proportion of Asymptomatic positive $\frac{19}{100} = 0.19$

$\hat{P}_1$ = Proportion of Symptomatic positive $\frac{4}{50} = 0.08$

$Z\left(1 - \frac{\alpha}{2}\right)$ = Coefficient of reliability at 95% level of significance.

$$(0.19 - 0.08) \pm 1.96 \times \sqrt{\frac{(0.19)(0.81)}{100} + \frac{(0.08)(0.92)}{50}}$$

$$0.11 + 1.95 \times \sqrt{0.001539 + 0.001472}$$

$$0.11 \pm 1.96 \times \sqrt{0.003011}$$

$$0.11 \pm 1.96 \times 0.548$$

$$0.11 \pm 0.10755$$

$$0.0024, 0.21755$$

The value P_1, P_2 (0.08, 0.19) is considered significant because, the difference between the two proportions is not zero and the proportion of the prevalence among asymptomatic commercial sex-workers is higher than that of the symptomatic non sex-workers.

This proves the fact that infection among asymptomatic sex workers is higher compared to that of symptomatic non sex workers.

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CHAPTER FIVE

5:0 DISCUSSIONS

Of the 100 asymptomatic female commercial sex-workers screened for *Chlamydia* infection between February to April 2005 at the Microbiology Laboratory of KATH, there were 19 positive cases giving a prevalence of 19.0% (19/100) as shown in the table 4.2.

Among the 50 symptomatic female non-sex workers screened at the same time and place, there were 4 positive cases giving a prevalence of 8.0 % (4/50).

The result obtained in this study is higher compared to the result obtained by Drescher et al. 1988, when a prevalence of 3.6% (4/110) was recorded among symptomatic female non sex workers attending STI Clinic in Kumasi by using immunofluorescent monoclonal antibody technique. The lower prevalence of *Chlamydia* infection in their study may be due to the different method they used and the type of subjects they selected.

Another study was conducted by Apea-Kubi et al. 2004 at Korle-Bu Teaching Hospital, Accra, Ghana, a prevalence of 3.0% was recorded for *Chlamydia* infection among 517 women screened which is low compared with the result in this study.
The difference in prevalence of *Chlamydia* infection in the two studies may be due to the fact that they used a recently developed RNA detection Kit as against QuickVue *Chlamydia* test Kit used in this current study.

Pepin et al. 2004 concluded in their study on sexually transmitted infections by saying that *Chlamydia* and Gonococcal infections are generally uncommon in West African women who are not sex workers.

Of the 280 women screened for *Chlamydia* infection in Ghana and Benin by the use of Polymerase chain reaction technique (PCR) 5 were positive to *Chlamydia* infection, giving a prevalence of 1.6%. The result is lower compared to the result obtained in this current study among both sex workers and non-sex workers and the difference could be attributed to the fact that their test may be species specific which could capture only one species of *Chlamydia* as compared to QuickVue test which is genus specific and can capture all species of *Chlamydia* and therefore giving a high percentage prevalence [Asamoah-Adu et al. 2001].

In the same year Asamoah-Adu et al. conducted a similar study in Burkina Faso, Mali and Guinea using the same method and a prevalence 6.1% (27/446) was documented which is considered higher compared with the prevalence among symptomatic non-sex workers but lower compared to that of asymptomatic commercial sex workers in this current study, and here too the selection of subjects could account for the difference in the result.

Mayaud et al. 1998 also conducted a study in Mwanza and Tanzania in the Eastern Africa among commercial sex workers and recorded a prevalence of 5-11% for *Chlamydia* infection, which is higher, compared to the result in this current study among symptomatic women but lower compared to the result among asymptomatic commercial sex workers.

The result is still lower among the symptomatic female non-sex workers compared to the results of a study conducted by Orroth et al. 2003, when prevalence for *Chlamydia trachomatis* was recorded as 1.6-13.0% among women in Masaka and Rakai, Uganda and Mwanza and Tanzania.

A study conducted in South Africa among 249 women on *Chlamydia* infection yielded a prevalence of 12% which is lower than the result obtained in this study for asymptomatic sex workers but higher than the result for symptomatic women.

In 2003, Antoinette et al. documented a prevalence of 3.96% for *Chlamydia* infection in Cameroon among 1277 volunteer students. The result in their study is about half the result obtained in this study among symptomatic female non-sex workers.
They extended their study to include asymptomatic sex-workers and recorded a prevalence of 38.3%, which is higher than the prevalence obtained in this study for asymptomatic female sex-workers.

Another study by Katz et al. 2004 recorded a prevalence of 13.9% (14/101) for *Chlamydia* infection in Hawaii, which is higher than the result obtained in this study among symptomatic non sex workers. This is because Katz et al. used a DNA probe technique as against QuickVue test used in this current study and both tests have different sensitivity and specificity.

In Gambia, Walraven et al. noted a prevalence of 0.8-1.2% for *Chlamydia* infection after a survey among women of reproductive age. This figure is lower compared with

the result in this study among both the symptomatic sex workers and symptomatic non-sex workers.

Simms et al. used liase chain reaction to carry out a research in UK on *Chlamydia* infection and reported a prevalence of 27% (12/45) which is very high compared to the research carried out in almost all the African countries discussed so far.

This could be attributed to the abuse of antibiotics in most African Countries where in some countries antibiotics could be acquired off the counter.
[Simms et al. 2003].

Another study conducted by the use Nucleic acid test in California, a prevalence of 14.6% and 13.0% were recorded for *Chlamydia* infection in different jail settings which is also higher than the result in this current study [Bauer et al. 2004].

One case of *Neisseria gonorrhoeae* was recorded among the 100 asymptomatic sex-workers giving a prevalence of 1.0% (1/100), but none was recorded among the symptomatic female non-sex workers.

Apea-Kubi et al. 2004 at Korle-Bu Teaching Hospital, Accra, Ghana, recorded a prevalence of 0.6% among 517 women screened for gonococcal infection which is lower compared with the results for gonococcal infection in this study.

Mayaud et al. 1998 also recorded a prevalence of 7-17% for gonococcal infection among commercial sex workers in Mwanza and Tanzania in the Eastern Africa, which

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is higher, compared to the result in this current study where a prevalence of 1.0% was recorded among the asymptomatic commercial sex workers.

Orroth et al. 2003 recorded a prevalence of gonococcal infection among symptomatic non-sex workers in Masaka and Rakai, Uganda and Mwanza, Tanzania as 1.8-2.3%, which is equally higher, compared to the prevalence of gonococcal infection recorded asymptomatic sex workers in this study.

Whereas Mayaud et al. 1998 recorded a prevalence of 11-20% among asymptomatic sex workers with both *Chlamydia* and gonococcal infections; none was recorded in this current study for both asymptomatic commercial sex workers and symptomatic non-sex workers.

From what have been discussed, the higher prevalence observed in some African countries could be attributed to increase in sex work and low awareness of STIs.

Generally prevalence of *Chlamydia* has been observed to be low in Africa and high in the Western countries, which could be due to: absence of screening programs and abuse of drugs in most African countries.

Most people do not know their status for the infection and women are normally screened when they complain of symptoms suspected to be *Chlamydia* infection.

One can observe from table 4.4 that of the 100 asymptomatic female sex workers screened 16.0% had pus cells with either ++ or +++ and out of this number 13.0% showed positive to *Chlamydia* infection and 1.0% gonococcal infection.

Of the 50 symptomatic female non-sex workers screened 8.0% had pus cells with either ++ or +++ and out of this number 6.0%were positive to *Chlamydia* infection and none was recorded for gonococcal infection. From this result one could therefore say that the presence of pus cells suggest the existence of an infection and this confirms the laboratory practice of suspecting patients with STI's presenting as discharges who do not grow any organism but show +++ pus cells of *Chlamydia.*
In this sense those with high pus cell count could have *Chlamydia.*(KATH Microbiology laboratory standard operating Manual).

From table 4:3 the ages ranging from 18-22 years had the highest prevalence of *Chlamydia* infection among the asymptomatic female commercial sex-workers and they are among the sexually active age group.

Among the asymptomatic female commercial sex workers, the ages ranging from 23-27years recorded a prevalence of 6.0% and1.0% was also recorded for the ages ranging 28-32. It is therefore very important for every woman attending STI clinic to be screened for *Chlamydia* infection.

The (χ_2) value obtained in analysis I is considered insignificant when compared to standard tables at 95% confidence interval (CI). This means that the prevalence of

Chlamydia infection in symptomatic female non sex workers is not statistically different from *Chlamydia* infection in asymptomatic sex workers.

The prevalence of *Chlamydia* infection in this study was found to be 0.19 and 0.08 among the asymptomatic female commercial sex workers and symptomatic female non- sex workers respectively. The difference between the two proportions is not zero and the proportion of the prevalence among asymptomatic commercial sex workers is higher than that of the symptomatic non-sex workers.

5:1 CONCLUSION

The result obtained in this study has clearly shown that *Chlamydia* infection still remains lower among women in Kumasi than women in the Western world.

The result also confirms a higher prevalence of *Chlamydia* infection among the sex-workers and the sexually active age groups compared to the non-sex workers.

5:2 RECOMMENDATIONS

From this study, it is therefore recommended that:

1. Annual screening programs must be instituted for commercial sex-workers, for the sexually active age group, they should be encouraged to go for testing from time to time and if possible seminars must be organized for them once every year.
2. Facilities for diagnosing and treating *Chlamydia* infection must be made available in at least all district hospitals.
3. Training of personnel on how to handle *Chlamydia* infections must be encouraged.
4. Puberty rites (Bragro) that prevent the teenage girl from engaging in early sex should be encouraged.

5. Parents must be encouraged to educate their wards on the avoidance of pre-marital sex and teenage pregnancies.
6. Latex condoms should be given freely to the sex-workers and the sexually active age group.

REFERENCES

1. Alary M., Baganizi E., Guedeme A. (1998). Evaluation of clinical algorithms for the diagnosis of *gonococcal* and *Chlamydial* infections among men with urethral discharge or dysuria and women with vaginal discharge in Benin. Sex Transm Infect; 74 (Suppl 1): S 44-9.

2. Antoinette Ngandjio, Maithe Clerc, Marie Christine Fonkoua, Jocelyn Thonnon, Friede Njock, Regis Pouillot, Francoise Lunel, Christiane Bebear, Bertille de Barbeyrac, and Anne Bianchi (2003). Screening of volunteer students in Yaounde, Cameroon, Central Africa for *Chlamydia trachomatis* infection and Genotyping of isolated *Chlamydia trachomatis* strain. J Clin Microbiol; 41(9): 4404-4407.

3. Anttila T., Saikku P., Koskela P., Bloigu A., Dillner J., Ikaheimo I., Jellum E., Lehtinen M., Lenner P., Hakulinen T., Narvanen A., Pukkala E., Thoresen S., Youngman L., and Paavonen J. (2001). Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. JAMA; 28:547-51.

4. Andrew WW, Lee HH, Roden WJ, Mott CW. (1997). Detection of genitourinary tract *Chlamydia trachomatis* infection in pregnant women by ligase chain reaction assay. J Obstet Gynecol; 87:556-60.

5. Apea-Kubi K A, Yamaguchi S, Sakyi B, Kishimoto T, Ofori-Adjei D, Hagiwara T. (2004). *Neisseria gonorrhoea, Chlamydia trachomatis* and *Treponema pallidum* infections in antenatal and gynecological patients at the Korle-Bu Teaching Hospital, Ghana. Jpn J Infect Dis; 57 (6): 253-6.

6. Asamoah-Adu C, Khonde N, Avorkliah M. (2001). HIV infection among Sex Workers in Accra: need to target new recruit entering the trade. J Acquired Immune Defic Syndr; 28:358-66.

7. Awwad ZM, Al-Amarat AA, Shehabi AA. (2003). Prevalence of genital *Chlamydia* infection in symptomatic and asymptomatic Jordanian patients. Int J infect Dis; 7 (3): 2006-9.

8. Bauer Heidi M., Chartier Maggie, Kessell Eric, Packel Laura, Brammeir Monique, Little Malaika, Bolan Gail (2004). *Chlamydia* screening of youth and young Adults in non-clinical settings throughout California. Sex Transm Dis; 31 (7): 409-414.

9. Black CM. (1997). Current methods of laboratory diagnosis of *Chlamydia trachomatis* infection. Clin Microbiol Rev; 10:160-84.

10. Bolam M.J, Stokes T., Shukla R., Bhaduri S. and Schober P. (1997). Controlling genital *Chlamydia infection*. BMJ; 314 (7072): 516-516.

11. Bridson E.Y (2006). Thayer Martin medium and variants. In: Oxoid Manual 9th ed. Wade Road, Basingstoke, Hampshire: Oxoid limited; 264.

12. Buve A, Weiss HA, Laga M. (2001). The epidemiology of *gonorrhoea*, *Chlamydia* infections and *syphilis* in four African cities. AIDS; 15 (Suppl 4): 579.

13. CDC. (1995). Ten leading nationally notifiable infectious diseases in the United State . MMWR 1996; 45-883-4.

14. Chenresky MA. (1999). Nucleic acid tests for the diagnosis of sexually transmitted diseases. FEMS Immunol Med Microbiol; 24:437-46.

15. Chernesky M.A., Mahony J.B., Castriciano S., Mores M., Stewart J.O Landis S.F, . Seidelman W., Sergeant E.J., and Lemon C. (1986). Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimen from symptomatic and asymptomatic men and women. J Infect Dis; 154:141-148.

16. Coombes R. (2004). Doctors demand national screening for *Chlamydia.* BMJ; 328:1397-0.

17. Dean D, Ferrero D, McCarthy M. (1998). Comparison of performance and cost effectiveness of Direct Fluorescent-antibody, Ligase Chain Reaction, PCR assays for verification of *Chlamydia* enzyme immunoassay results for populations with a low to moderate prevalence of *Chlamydia* trachomatis infection. J Clin Microbiol; 36:94-99.

18. Domeika M. (1997). Diagnosis of infection due to *Chlamydia trachomtis*. Acta Obstet Gynecol Scand; 164 (Suppl): 121-7.

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19. Drescher C, Elkins TE, Adkeo O, Akins-Bekoe P, Agbemadzo T, Foster RL, Martey J O. (1988). The incidence of urogenital *Chlamydia trachomatis* infections among patients in Kumasi, Ghana. Int J Gynaecol Obstet.; 27 (3): 381-3.

20. Fenton KA, Sara Randall, Scott D. La Montagne (2004). National Screening program for *Chlamydia* exists in England. BMJ; 329:172.

21. Frost E., Leclerc A., Collet M., Mavoungou D., Beneteau J.L., Gioanni G., Reniers J., Meheus A., Ivanoff B., (1987). The role of *Chlamydia trachomatis* in female infertility. Conference proceedings: African regional conference on STD's, Harare Zembabwe.231-233.

22. Gaydos C.A., Howell M.R., Pare B., Clark K.T., Elllis D.A., Hendrix R.M., Gaydos J.C., McKee K.T.Jr. and Quin T.C. (1998). *Chlamydia trachomatis* infections in female military recruits. N Engl J Med; 339:739-744.

23. Geisler W.M., Suchland R.J., Whittington W.L. and Stamm W.E. (2001). Quantitative culture of *Chlamydia trachomatis* relationship of inclusion-forming units produced in culture to clinical manifestations and acute inflammation in urogenital disease. J Infect Dis; 184:1350-1354.

24. Golden M.R., Schillinger J.A., Markowitz L and St Louis M.E. (2000). Duration of untreated genital infections with *Chlamydia trachomatis*: a review of the literature. Sexually Transmitted Diseases; 27 (6): 329-337.

25. Grayston J.T. (1989). *Chlamydia Pneumoniae*, strain TWAR. Chest; 95:664.

26. Grimes D.A and Schulz K.F. (2002). Uses and abuse of screening tests. Lancet; 359:881-4.

27. Groseclose SL, Zaidi AA, DeLisle SJ, Levine WC, St. Louis M.E. (1999). Estimated incidence and prevalence of genital *Chlamyida trachomatis* infection in the United States. Sex Transm Dis.; 26 (6): 339-44.

28. Hatch TP and Thomas P. (1996). Disulfide cross – linked Envelope proteins: the Functional Equivalent of Peptidoglycan in *Chlamydia*. J Bacteriol; 178:1-5.

29. Hillis S.D and Susan D (1995). Impact of a comprehensive *Chlamydia* prevention program in Wisconsin. Family planning perspectives; 27:108-111.

30. Jensen JS, Bjornelius E, Dohn B, Lidbrink P. (2004). Comparison of first void urine and urogenital swab specimens for detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* by polymerase chain reaction in-patients attending STD Clinics. Sex Transm Dis; 31 (8): 499-507.

31. Johnson AM, Lucia Grun, Andrew Haines (1996). Controlling genital *Chlamydia* infection. BMJ; 313:160-1161.

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32. Katz AR, Lee MV, Ohye RG, Effler PV, Johnson EC, Nishi S.M. (2004). Prevalence of *Chlamydia* and *gonorrhea* infections among females in a Juvenile detention Facility, Honolulu, Hawaii. J community Health, 29 (4): 265-9.

33. Levine WC, Linda W. Dicker, Owen Devine and Debra J. Mosure (2004). Indirect Estimation of *Chlamydia* screening coverage using public health surveillance data. Am J Epidemiol; 160:91-96.

34. Mayaud P, Ka-Gina G, Cornelissen J. (1998). Validation of a WHO algorithm with risk assessment for the clinical management of vaginal discharge in Mwanza, Tanzania. Sex Transm Infect; 74 (Suppl 1): 577-84.

35. McCNulty CA, Freeman E, Bowen J, Shefras J, Fenton K.A. (2004). Barriers to opportunistic *Chlamydia* testing in primary care. Br J Gen Pract; 54 (504): 508-514.

36. Meda N, Sangare L, Lankoandes S, Sanou PT, Campoare PI, Catraye J, Cartoux M, Soudre RB. [1997]. Pattern of sexually transmitted diseases among pregnant women in Burkina Faso, West Africa: Potential for clinical management based on simple approaches. Genitourinary Med; 73(3): 188-93.

37. Miller MJ. (1985). The Laboratory diagnosis of *Chlamydia trachoma's* and genital Mycoplasmas. Journal Med Tech; 2(8): 507-512.

38. Morency P., Dubois M.J. Gresenguet G, Frost E, Masse B, Deslandes S, Somse P,

Sammory A, Mberyo-Yaah F. and Pepin J. (2001). Aetiology of urethral discharge in Bangui, Central African Republic. Sex Transm Infect; 77:125-129.

39. Nsuami M, Cammarata CL, Brooks BN, Taylor SN, Martin DH. (2004). *Chlamydia* and *gonorrhoea* co-occurrence in a high school population. Sex Transm Dis; 31 (7): 424-7.

40. Oakeshott P., Kerry S., Hay S. and Hay P. (1998). Opportunistic screening for *Chlamydia* infection at time of cervical smear testing in general practice: prevalence study. BMJ; 316 (7128): 351-352.

41. Odoi-Agyarko K. and Batong A.A. (2001). Female genital mutilation and reproductive health morbidity in second cycle school girls in Bolgatanga District in Ghana. GMJ; Vol-35 No. 3.

42. Orroth KK, Korenramp EL, White RG. (2003). Comparison of STD prevalence in the Mwanza, Rakai and Masaka trial populations: the role of selection bias and diagnostic errors. Sex Transm Infect; 70:98-105.

43. Paul V.K, Singh M, Gupta U, Buckshee K, Bhargava VL, Takkar D, Nag VL, Bhan MK, Deorari AK. (1999). *Chlamydia trachomatis* infection among pregnant women: prevalence and parental importance. Natl Med J Ind; 12:11-4.

44. Pepin J., Deslandes S., khonde N., Kintin D.F., Diakite S., Sylla M., Meda H., Sobela F., Asamoah-Adu C., Agyarko-Poku T. and Frost E. [2004]. Low prevalence

of cervical infection women with vaginal discharge in West Africa: Implications for syndromic management. Sex Transm Infect; 80:230-235.

45. Puisis M, Levine WC, Mertz KJ. (1998). Overview of Sexually Transmitted disease. In: Puisis M ed. Clinical Practice in correctional medicine. St. Louis, Missouri: Mosby; 127-33.

46. Ripa K.T., and Mardh P. -A. (1977). Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. J. Clin. Microbiol; 6: 328-331.

47. Saad Ghazal –Aswaad, Badrinath P, Osman N, Abdul-Khaliq S, M C, Ilvennu S, Sidiky I. (2004). Prevalence of *Chlamydia trachomatis* infection among women in a Middle East Community, BMC Women's Health; 4:1472-6874.

48. Schachter J. (1997). DFA, EIA, PCR, LCR and other technologies: what tests should be used for diagnosis of *Chlamydia* infection? Immunol Invest; 26:157-61.

49. Schneider H, Coetzee DJ, Fehler HG, Bellingan A, Dangor Y, Rodebe F, Ballard RC. (1998). Screening for sexually transmitted diseases in rural South African women. Sex Transm Infect; 74 Suppl1: S147-52.

50. Simms I., Eastic K., Mallinson H., Thomas K., Gokhale R., Hay P., Herrings A. and Roger P.A. (2003). Association between *mycoplasma genitalium*, *Chlamydia trachomatis* and pelvic inflammatory disease. J Clin Path; 56: 616-618.

51. Simms I, Hughes G., Catchpole M (1998). Screening for *Chlamyida trachomatis*. BMJ; 317 (713-9): 680-630.

52. Stamm W.E. (1983). Detection of *Chlamydia trachomatis* inclusion in McCoy cell cultures with fluorescein-conjugated monoclonal antibodies. J Clin Microbiol; 17:666-668.

53. Sweet RL, Landers DV, Walker C, Schaster J. (1987). *Chlamydia trachomatis* infection and pregnancy outcome. Am J Obstet Gynecol; 156:824-833.

54. Tam M.R., Stamm W.E., Handsfield H.H., Stephens R., Kuo C. -C,. Holmes K.K, Ditzenberger K., Crieger M., and Nowinski R.C. (1984). Culture –independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. N Engl J Med; 310: 1146-1150.

55. Walravan G, Scherf C, West B. (2001). The burden of reproductive organ disease in women in the Gambia, West Africa. Lancet; 357: 1161-7.

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APPENDICES

APPENDIX 1

SAMPLE OF QUESTIONNAIRE

PERVALENCE OF CHLAMYDIA INFECTION AMONG ASYMPTOMATIC FEMALE SEX WORKERS AND SYMPTOMATIC FEMALE NON-SEX WORKERS IN THE KUMASI METROPOLIS

Patient number:........................ Asymptomatic [ ]

Symptomatic [ ]

Age:....................................

Date:...................................

Marital Status: Married [ ] Single [ ]

Boy Friend: Yes [ ] No [ ]

Number of sexual partners in the preceding 3 months: [ ]

Number of Children:..

Educational Background:..

....

Profession:...

CHLAMYDIA TEST RESULT		**GONOCOCCUS TEST RESULT**	
Positive	[ ]	**Positive**	[ ]
Negative	[ ]	**Negative**	[ ]

APPENDIX 2

TABLE 4:2 *Chlamydia* and *Gonococcus* test results for the 100 asymptomatic female commercial sex-workers screened at the Microbiology Laboratory of KATH.

Age (years)	Chlamydia	Gonococcus (GC)	Gram Result	Culture Result
19	Negative	Negative	Yeast Cell (+ +)	*Staphylococcus aureus*
19	Negative	Negative	Positive Rods	*Staphylococcus aureus*
19	Negative	Negative	No Organism Seen	No Bacteria growth
29	Positive	Negative	Pus cell (++)	No Bacteria growth
25	Negative	Negative	No Organism Seen	No Bacteria growth
25	Positive	Negative	Pus cell (+++)	α- haeumolytic *strept.*
20	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	No Organism Seen	No Bacteria growth
24	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	No Organism Seen	No Bacteria growth
23	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
22	Positive	Negative	Pus cell (++)	Coagulase Negative *Staph.*
22	Positive	Negative	Pus cell (+++)	α- haeumolytic *strept.*
20	Positive	Negative	Pus cell (+++)	*Staphylococcus aureus*
24	Positive	Negative	Yeast Cell (+++)	No Bacteria growth
31	Negative	Negative	Positive Rods	*Staphylococcus aureus*
21	Positive	Negative	Pus cell (++)	No Bacteria growth
21	Negative	Negative	No Organism Seen	No Bacteria growth
24	Negative	Negative	Yeast Cell (+)	*Staphylococcus aureus*
22	Negative	Negative	No Organism Seen	No Bacteria growth
20	Negative	Negative	No Organism Seen	No Bacteria growth
18	Negative	Negative	No Organism Seen	No Bacteria growth
23	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
33	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
21	Negative	Negative	No Organism Seen	No Bacteria growth
20	Positive	Negative	Pus cell (++)	α- haeumolytic *strept.*

24	Negative	Negative	No Organism Seen	No Bacteria growth
20	Negative	Negative	No Organism Seen	No Bacteria growth
20	Negative	Negative	No Organism Seen	No Bacteria growth
20	Negative	Negative	Yeast Cell (+++)	No Bacteria growth
27	Negative	Negative	No Organism Seen	No Bacteria growth
22	Positive	Negative	Yeast Cell (+ +)	*Staphylococcus aureus*
19	Negative	Negative	No Organism Seen	Coagulase Negative *Staph.*
24	Negative	Negative	No Organism Seen	No Bacteria growth
20	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
22	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	No Organism Seen	No Bacteria growth
20	Positive	Negative	Pus cell (++)	No Bacteria growth
20	Negative	Negative	No Organism Seen	No Bacteria growth
32	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	Pus cell (++)	Coagulase Negative *Staph.*
22	Positive	Negative	Pus cell (+++)	α- haeumolytic *strept.*
18	Negative	Negative	No Organism Seen	No Bacteria growth
23	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	Yeast Cell (+)	No Bacteria growth
24	Positive	Negative	Pus cell (++)	No Bacteria growth
28	Negative	Negative	Yeast Cell (+)	*Staphylococcus aureus*
22	Positive	Negative	Yeast Cell (+++)	No Bacteria growth
23	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
26	Positive	Negative	Pus cell (+++)	Coagulase Negative Staph.
23	Negative	Negative	Pus cell (++)	*Staphylococcus aureus*
25	Negative	Negative	No Organism Seen	No Bacteria growth
21	Positive	Negative	Yeast Cell (+ +)	*Staphylococcus aureus*
22	Negative	Negative	No Organism Seen	No Bacteria growth
19	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
23	Negative	Negative	No Organism Seen	No Bacteria growth
23	Positive	Negative	Pus cell (+++)	α- haeumolytic *strept.*
19	Negative	Negative	No Organism Seen	No Bacteria growth

33	Negative	Negative	No Organism Seen	No Bacteria growth
23	Negative	Negative	No Organism Seen	No Bacteria growth
20	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
25	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
20	Negative	Negative	Yeast Cell (++)	No Bacteria growth
23	Negative	Negative	No Organism Seen	No Bacteria growth
23	Negative	Negative	No Organism Seen	No Bacteria growth
31	Negative	Negative	Positive Rods	No Bacteria growth
24	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
19	Negative	Negative	Positive Rods	No Bacteria growth
20	Negative	Negative	Positive Rods	Coagulase Negative *Staph.*
23	Negative	Negative	No Organism Seen	No Organism growth
20	Positive	Negative	No Organism Seen	*Staphylococcus aureus*
20	Negative	Negative	No Organism Seen	No Bacteria growth
19	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	Yeast Cell (+++)	No Bacteria growth
24	Negative	Negative	Positive Rods	No Bacteria growth
23	Positive	Negative	Pus cell (++)	No Bacteria growth
22	Negative	Negative	No Organism Seen	No Bacteria growth
21	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
26	Negative	Negative	No Organism Seen	No Bacteria growth
27	Negative	Negative	No Organism Seen	No Bacteria growth
20	Positive	Negative	Yeast Cell (+ +)	*Staphylococcus aureus*
34	Negative	Negative	No Organism Seen	Coagulase Negative *Staph*
35	Negative	Negative	No Organism Seen	No Bacteria growth
24	Negative	Negative	No Organism Seen	No Bacteria growth
23	Negative	Negative	No Organism Seen	No Bacteria growth
18	Negative	Negative	No Organism Seen	Coagulase Negative *Staph.*
24	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
25	Negative	Negative	No Organism Seen	*Staphylococcus aureus*

21	Negative	Negative	No Organism Seen	No Bacteria growth
21	Negative	Negative	Positive Rods	*Staphylococcus aureus*
22	Negative	Negative	Yeast Cells (++)	*Staphylococcus aureus*
21	Negative	Negative	No Organism Seen	No Bacteria growth
24	Negative	Negative	Yeast Cells (+++)	*Staphylococcus aureus*
25	Negative	Negative	Positive Rods	*Staphylococcus aureus*
19	Negative	Negative	No Organism Seen	No Bacteria growth
21	Negative	Positive	Yeast Cells (+++)	No Bacteria growth
33	Negative	Negative	Pus cell (+++)	*Staphylococcus aureus*

Number of *Chlamydia* Positives = 19

Number of *Chlamydia* Negatives = 81

Number of *Gonococcus* Positives = 1

Number of *Gonococcus* Negatives = 99

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APPENDIX 3

TABLE 4:3 *Chlamydia* and *Gonococcus* test Result for the 50 symptomatic female non-sex workers screened at the Microbiology Laboratory of KATH. The table shows test outcomes for *Chlamydia, gonococcus* with gram and culture results.

Age	*Chlamydia*	*Gonococcus* (GC)	Gram Result	Culture Result
39	Negative	Negative	No Organism Seen	No Bacteria growth
28	Negative	Negative	No Organism Seen	No Bacteria growth
23	Positive	Negative	Pus cell (+++)	No Bacteria growth
27	Negative	Negative	Positive Rods	No Bacteria growth
33	Negative	Negative	No Organism Seen	No Bacteria growth
33	Negative	Negative	No Organism Seen	No Bacteria growth
20	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
24	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	No Organism Seen	No Bacteria growth
26	Negative	Negative	No Organism Seen	Coagulase Negative *staph.*
35	Negative	Negative	Yeast Cell (+ +)	No Bacteria growth
18	Negative	Negative	No Organism Seen	No Bacteria growth
19	Negative	Negative	No Organism Seen	No Bacteria growth
32	Negative	Negative	No Organism Seen	No Bacteria growth
25	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
30	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
28	Negative	Negative	No Organism Seen	No Bacteria growth
30	Positive	Negative	Pus cell (+ + +)	*Staphylococcus aureus*
30	Negative	Negative	No Organism Seen	No Bacteria growth
26	Negative	Negative	No Organism Seen	No Bacteria growth
18	Negative	Negative	Yeast Cell (+)	*Staphylococcus aureus*
22	Negative	Negative	No Organism Seen	No Bacteria growth
35	Negative	Negative	Pus cell (+ +)	*Staphylococcus aureus*
34	Negative	Negative	No Organism Seen	No Bacteria growth
18	Negative	Negative	Positive Rods	*Staphylococcus aureus*

27	Negative	Negative	No Organism Seen	No Bacteria growth
23	Negative	Negative	No Organism Seen	No Bacteria growth
26	Negative	Negative	Yeast Cell (+ +)	Coagulase Negative *Staph.*
19	Negative	Negative	No Organism Seen	*Staphylococcus aureus*
30	Negative	Negative	Positive Rods	No Bacteria growth
20	Negative	Negative	Positive Rods	No Bacteria growth
22	Negative	Negative	No Organism Seen	No Bacteria growth
35	Negative	Negative	Positive Rods	*Staphylococcus aureus*
21	Negative	Negative	No Organism Seen	No Bacteria growth
25	Negative	Negative	Positive Rods	*Staphylococcus aureus*
24	Negative	Negative	No Organism Seen	No Bacteria growth
23	Positive	Negative	Pus cell (+ + +)	*Staphylococcus aureus*
32	Negative	Negative	No Organism Seen	No Bacteria growth
30	Negative	Negative	Positive Rods	No Bacteria growth
27	Negative	Negative	No Organism Seen	No Bacteria growth
30	Positive	Negative	Yeast Cell(+++)	α- haeumolytic *strept.*
35	Negative	Negative	Positive Rods	*Staphylococcus aureus*
25	Negative	Negative	No Organism Seen	No Bacteria growth
33	Negative	Negative	Positive Rods	*Staphylococcus aureus*
23	Negative	Negative	No Organism Seen	No Bacteria growth
22	Negative	Negative	No Organism Seen	No Bacteria growth
21	Negative	Negative	Positive Rods	*Staphylococcus aureus*
21	Negative	Negative	Positive Rods	*Staphylococcus aureus*
32	Negative	Negative	No Organism Seen	No Bacteria growth
26	Negative	Negative	No Organism Seen	No Bacteria growth

Number of *Chlamydia* Positive = 4

Number of *Chlamydia* Negative = 46

Number of *Gonococcus* Positive = 0

Number of *Gonococcus* Negative = 50

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APPENDIX 4

NEISSERIA ISOLATION MEDIA AND PREPARATION TECHNIQUES

Thayer Martin's media preparation:

Media used for cultivation of Gonococci are agars of high peptone and starch content enriched with fresh horse blood (lysed) or soluble hemoglobin and GC growth supplements (yeast Autolysate supplement SR105 and Vitox SR90) which have been shown to stimulate growth from small inocula. Several conditions of selective antibiotics (VCNT, VCN, LCAT and VCAT) have been described that can be added to culture media in order to suppress Gram-positive and Gram negative contaminants [Bridson, 2006].

The choice of the selective supplement is dependent upon the preference of the laboratory as well as regional strain difference of the organism.

VCNT Selective supplement

Code: SR 91

An antibiotic supplements for the isolation of *N. gonorrhoeae* and *meningitis*.

Vial content (each vial is sufficient for 500ml of medium)

Vancomycine	1.5mg
Colistin methane sulpahate	3.75m
Nystatin	6,250 IU
Trimetoprim	2.5mg

Seth described a modification of Thayer Martin medium in which Trimethoprim 5mg/ml was added to the VCN antibiotics and was shown to be of value in preventing *Proteus* species swarming. Several other workers confirmed the non-inhibitory effect on *N. gonorrhoeae* and its value in preventing swarming.

Thayer Martin Medium with Vitox and VCNT Antibiotic Supplement

The above Thayer Martin's medium modification was used to culture the endocervical swab for the detection of gonococcal colonies in this current study.

1) 18 g of GC agar base suspended in 240ml of distilled water and brought to boil to dissolve the agar. It was sterilized by autoclaving at 121 ^{o}C for 15 minutes.
2) 2% solution of soluble Haemoglobin powder L53 was prepared by adding, 205ml of warm distilled water to 5g of Haemoglobin powder. It was continually stirred during addition of water. It was then sterilized by autoclaving at 121°C for 15 minutes.
3) The contents of the Vial of Vitox SR90 was dissolved as directed on the vial label,
4) The contents of the vial of either VCN Antibiotic supplement SR 101 or VCNT Antibiotic supplement SR91 was dissolved as directed on the vial label.
5) Vitox solution was aseptically added to 240 ml of sterile GC Agar Base cooled to 50°C.
6) Reconstituent antibiotic supplement VCN or VCNT was aseptically added to the GC agar base-Vitox solution.
7) 250ml of sterile haemoglobin solution, cooled to 50°C was aseptically added to the GC agar base Vitox-Antibiotic supplement solution. It was then gently mixed to avoid trapping of air bubbles and poured into sterile Petri dishes [Bridson, 2006].

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IDENTIFICATION OF ORGANISM

Gonococcus colonies were identified by gram stain, oxidase test and sugar fermentation test. Gonococcal colonies were pale to dark red colour and arranged in pairs with long parallel axes when observed under the microscope.

Oxidase test – Principle

A piece of filter paper was soaked with a drop of the oxidase reagent. A colony of the test organism was then smeared on the filler paper. If the organism is oxidase producing, the phenylenediamine in the reagent is oxidized to a deep purple colour within 3 seconds. Oxidase reagent, freshly prepared contains 10g/l solution of tetramethyl-p-phenylenediamine hydrochloride.

Principle of the carbohydrate vitalization test-containing carbohydrate-free discs.

This test is normally called the carbohydrate utilization test. The test was performed by adding 0.1ml of heavy saline suspension of the test organism to each of 70ml test tube containing a carbohydrate-free disc (included as a negative control). All the tubes were inoculated in water bath at 37°C and examined at 30 minutes intervals for up to 5 hours for a change in colour from red to yellow or red to yellow-orange, indicating carbohydrate utilization. The table below shows the result of carbohydrate utilization of the major pathogenic *Neisseria*.

Species	Glucose	Lactose	Maltose	Sucrose
N. gonorrhoeae	Produce acid	-	-	-
N. meningitis	Produce acid	-	Produce acid	-