GENITAL *Chlamydia* AND HIV CO-INFECTION: ADVERSE PREGNANCY OUTCOMES

Dibua, Uju M.E.[1], Ugonabo, J.A.C.[2,] Oladepo, D. [3], Iroha, I.R.[4], Odimegwu, N. D.[5]

[1,2,3,]Department of Microbiology, University of Nigeria Nsukka
[4]Department of Microbiology, Ebonyi State University, Abakaliki
[5] Department of Molecular and Medical Virology, Ruhr University Bochum, Germany
*__Corresponding__ author:
Email: oby2112@yahoo.co.uk; essuju@gmail.com
Phone: +2348037792951

Abstract

In the remote and poor-resource areas of Nigeria, there is paucity of data on the prevalence of HIV, genital *Chlamydia trachomatis* (CT) infections and their squeal among women of reproductive age. The cohort study of 303 female volunteers was done by investigation of their HIV status from serum samples using the Determine HIV-1 and HIV-2 (Abbot Laboratories Japan). Chlamydia antigen was screened for using Chlamydia Rapid Test Device – Swab (Inter-chemical Ltd China). Coinfecting pathogens were assayed by cultivation of endocervical smears on appropriate bacteriological media using standard methods. Infertility was assessed by hysterosalpingography; pelvic ultrasound was used to diagnose pelvic inflammatory diseases (PID) following historical and clinical findings. Pearson Chi–Square was used for statistical analysis at $P<0.05$ (95% Confidence Level). High prevalence of CT infection (56.1%) was observed; the most vulnerable age group was 15-19 years (80%). Single women were at greater risk of CT infection; with multiple sex partnership being the most implicated mode of transmission. Overall prevalence of infertility was 75.6: primary (26.4%), secondary (35.3%).

Preponderance of PID was 67.8%. Approximately 61% of participants reactive to *C. trachomatis* antigen were HIV seropositive; with preponderance in the 15-19, and 20-24 years age bracket. Isolated coinfecting pathogens among the dually infected included *Neisseria gonorrhea* and *Staphylococcus aureus.* This study observed a synergy between HIV, CT and other sexually transmitted infections (STIs), which disproportionately affected people in poor resource areas, with severe adverse outcomes calling for early diagnosis and treatment of STIs as a critical option for HIV control.

Key words: Genital Chlamydia infection, HIV/CT co-infection, endocervical smears,

Infertility, PID

{**Citation:** Dibua, Uju M.E., Ugonabo, J.A.C., Oladepo, D., Iroha, I.R., Odimegwu, N. D. Genital *Chlamydia and HIV* co-infection: adverse pregnancy outcomes. American Journal of Research Communication, 2013, 1(12): 470-500} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Genital Chlamydia trachomatis infection (CT) has become the most prevalent among the bacterial STDs. The Centers for Disease Control and Prevention (CDC), reported an approximate one million global cases of *C. trachomatis* infections among sexually active young people aged 15-25 years, more than half of these were in females (CDC, 2005). In parts of Nigeria, varying prevalence rates have been reported: a 51% prevalence among pregnant and non-pregnant women and their spouses attending pre and antenatal clinic at the University of Lagos (Okoror *et*

al., 2000); a lower prevalence of 38.3% occurred in Zaria (Tukur *et al.,* 2006). The asymptomatic cases account for up to 50% in men and 70-80% in women and makes it spread latently among the risk population (Ikeme *et al.,* 2001). The diagnosis of CT infection poses serious difficulties as a result of its fastidiousness, neither does clinical history give reliable diagnostic evidence of the infection, which is often asymptomatic. The consequence of untreated cases is migration of the bacterium to the upper genital tract of and subsequent elaboration of tubal dysfunction including tubal blockage, pelvic inflammatory disease (PID), ectopic pregnancy, abortion/preterm delivery, infertility among women of child bearing age in whom high mortality and morbidity are common (Manju *et al.,* 2011)**.** However, infertility and other associated adverse outcomes of CT could be preventable and treatable if detected early enough.

C. trachomatis therefore poses a serious public health problem not only as a result of its asymptomatic infections but also due to its ability to change the epitope of its major antigen. This is due to the domains with variable nucleotide sequence in the plasmid encoding for its major outer membrane proteins (MOMP), - omp1 (Zhang *et al.,* 1995). This variability accounts for the various epitopes presented at different times and at different locations. Antibodies produced against these epitopes are usually escaped due to these changes.

Sexually transmitted infections (STI) are among the most well-established risk factors for HIV infection. Whether ulcerative or nonulcerative, STIs facilitate the transmission and acquisition of other STIs, including human immunodeficiency virus (HIV), through local inflammatory processes; by breaching protective mucosal barriers and recruiting susceptible immune cells (eg, CD4 T-helper cells, macrophages) to the site of infection (Ward and Ronn, 2010). Their diagnosis thus signals sexual health risk because coexisting STIs increase susceptibility of acquiring and transmitting HIV by two- to fivefold (*HIV prevention through early detection and*

treatment of other sexually transmitted diseases—United States). The prevalence of STIs including CT, gonorrhoea and *S. aureus* among people living with HIV/AIDS has implications for containing the spread of HIV in general and the effectiveness of HIV treatments for prevention in particular. Growing evidence indicates that active CT infection is an important risk factor facilitating sexual transmission of HIV infection, and several observed high rates of CT assumes significance in view of risk of HIV transmission and spread. Further observation indicated that HIV positivity significantly correlated to CT infection; the combined epidemiology of these infections may partly be due to the fact that STDs including HIV and CT have common sexual/behavioural risk factors including premarital sex and multiple partnership, therefore, it may be appropriate to conclude that all sexual/behavioural factors could potentially interplay for the acquisition of these infections (Brunham *et al.,* 1996).

CT infection and HIV infection have interrelationship independent of the sexually transmissible risk factors. These include: the invasive intracellular pathogenesis of CT which can cause substantial damage to the genital epithelial layer thereby facilitating HIV infection; immunological changes due to HIV infection which may favour CT infection and the interrelationship between the two infections and mutually associated transmission pattern (Debattista *et al*., 2002; Hitchcock, 1999).

Gonorrhoea is one of the most deadly sexually transmitted infections (STIs) in developing countries and is a global public health problem, as undetected, untreated infections and co-infections with other STIs and especially HIV results to severe adverse outcomes in women of child bearing age, including infertility, pelvic inflammatory disease and abortions/miscarriage. Gonorrhoea, like CT, is an easily curable STI, but if remained undetected, untreated infections

and co-infections can lead to complications like pelvic inflammatory disease, ectopic pregnancy, tubal factor infertility, adverse pregnancy outcomes in females, and testicular and prostate infections and infertility in males (Laga *et al.*, 1993). It potentially increases the risk of both transmission and acquisition of human immunodeficiency virus (HIV). Asymptomatic patients, unaware of their infection, may serve as a reservoir of infection to their partners. Co-infection of HIV, CT and gonorrhoea has been severally reported: the Regional STD Teaching, Training & Research Centre, New Delhi, reporting on the high co-infection rate (14.4%) of gonorrhoea with other ulcerative (syphilis, chancroid, herpes, donovanosis), non-ulcerative STIs [chlamydiasis, trichomoniasis, bacterial vaginosis (BV), candidiasis] and HIV infection, highlighted the considerable burden of the diseases, and the need for urgent appropriate screening measures for STIs control (Ray *et al.*, 2006)

The realization of a subtle relationship between *C. trachomatis* IgG antibody in serum and tubal factor infertility has emanated to several research on the use of Chlamydia antibody testing (CAT) as a significant screening protocol for both infertility and other related tubal complications. This simple and cost effective test is considered valuable in sub-fertile women and may also serve as surrogate marker for other at risk women presenting with tubal and/or infertility problems especially in the remote and resource limited areas of Nigeria.

So far, very little or no information has been documented on the coinfectivity of CT, HIV and other non-HIV sexually transmitted infections and their association with infertility and related complications in Nsukka. The increasing cases of CT-associated coinfection with HIV and other STIs as well as the adverse pregnancy outcomes and tubal dysfunctions among the local populace in Nsukka therefore necessitates this study which aims at assessing the prevalence of coinfection of CT and other sexually transmitted infections including HIV among women of

child bearing age in Nsukka, with the view to determine the risk factors of the diseases, their paradigm of infectivity in the population, and their association with adverse pregnancy complications including infertility, ectopic pregnancy, preterm delivery and abortion in the area as well as its co-infection with other organisms.

Genital Chlamydia infection whose global impact and prevalence has been on the increase in recent times has remained a severe public health concern, being linked to serious sequelae among women of child bearing age including tubal infertility, pelvic inflammatory disease (PID), ectopic pregnancy, and other related pelvic pain. CT has become a disease of young people, with sexually active young adults remaining at highest risk of infection. C. trachomatis is a successful human parasite; a success facilitated by its ability to cause asymptomatic infection, which reckons it as a silent killer-disease in women. The general health impact and paucity of data on this deadly disease and its association with heterosexual transmission of HIV infection in Nsukka area of the southeast Nigeria, has resulted in an upsurge of interest in the prevalence of the disease and the associated complications and/or adverse outcomes.

MATERIALS AND METHODS

Study Population: This was a 2-year cohort study of 303 female volunteers (symptomatic and asymptomatic), aged 14-47 years investigated at the Bishop Shanahan Hospital, Nsukka between March 2009 and August, 2011. Exclusion criteria were pregnant women and those in antibiotic use prior to enrolment in the study. Inclusion criteria were those presenting with pelvic infertility diseases and other infertility profiles. Detailed history and physical examination

of all participants were carried out prior to commencement of study by physicians and nurses in attendance.

Ethical Consideration: Ethical approval was obtained from the Ethical Committee of the Bishop Shanahan Hospital and all appropriate boards at the University of Nigeria prior to commencement of research. Informed consent of each participant or ward was requested and obtained following explanation of the research objectives before participant's inclusion into the research.

Questionnaire and Oral interviews:

Appropriately structured and vetted questionnaires were administered to participants using the Kish formula at 10.5%, at 95% Confidence interval, and authenticated at 0.05 level of accuracy. Details of the questionnaire include the following: socio-demographic details, sexual behaviour, history of STI including Chlamydia infection and allied predisposing factors, and urogenital symptoms including cases of pelvic inflammatory disease (PID), infertility and abortion. Oral interviews were similarly conducted on participants based on details used for the questionnaire.

HIV Screening

Screening for HIV antibodies was carried out on patients' sera samples according to Manufacturers' instructions following pre-test counseling and informed consent, using the *in vitro* test: the Determine HIV-1 and HIV-2 (Abbot Laboratories Japan,) an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2.

Post HIV counseling was then given to all HIV positive participants with assurance and maintenance of confidentiality.

Screening for *C. trachomatis* Antigen:

Sample collection:

Endocervical swabs of the subjects were aseptically collected by female nurses in attendance using sterile plastic-shaft Dacron swabs. The swabs were pressed firmly and rotated against the wall of the tube to extract the fluid before use.

Rapid screening test for *C. trachomatis*: Sample analysis was carried out using the Chlamydia Rapid Test Device – Swab (Inter-chemical Ltd. China), a qualitative, lateral flow immunoassay for the detection of Chlamydia antigen from swab samples.

Test procedure/principle: This is an antibody-antigen reaction in which antibody specific to the Chlamydia antigen was coated on the test line region of the test device. The extracted antigen solution reacted with the Chlamydia antibody solidly coated onto particles; there was then migration of the mixture and subsequent reaction with the Chlamydia antibody on the membrane, generating a coloured line in the test-line region.

Test interpretation:

Coloured line in the test-line region was indicative of a positive result; absence of such colour showed a negative reaction. However, coloured lines in the control-line region authenticated the experiment, and indicated adequacy of specimen addition as well as accuracy of experiment.

Assessment of infertility profile and PID

Infertility was assessed by hysterosalpingography (by radiography) at the University of Nigeria Teaching Hospital (UNTH) following historical (oral interviews) and clinical investigations. Infertility profile was grouped into primary and secondary infertility. Primary infertility here refers to women who have not hitherto become pregnant after at least 1 year of sexual intercourse. Secondary infertility on the other hand, refers to failure to conceive following a previous pregnancy. Pelvic ultrasound was used to diagnose pelvic inflammatory diseases (PID) following historical and clinical findings: lower abdominal tenderness, bilateral adnexal tenderness, uterine tenderness, and cervical motion tenderness as described by Hager *et al*., (1983). Laparoscopy or endometrial biopsy were not carried out. Abortion was diagnosed by checking for Human Chorionic Gonadotrophine (HCG) level by attending physicians.

Investigation of other co-infecting pathogens:

Smears of the endocervical samples were prepared and Gram-stained (Cheesbrough, 2004) prior to cultivation in growth media. Diagnosis of *N. gonorrhoea* was carried out using standard laboratory protocols; by direct cultivation of endocervical smears on chocolate agar and saponin-lysed blood agar, supplemented with vancomycin, colistin, nystatin, trimethoprim (VCNT). The endocervical samples were further cultivated on duplicate plates of Blood and McConkey Agar for investigation of other co-infecting pathogens. The Blood and MacConkey agar media were incubated aerobically at 37^{o}C for 24 hours, and the Chocolate agar media incubated in a carbon dioxide atmosphere using a candle jar canister. Confirmatory test of the isolates was then done using standard biochemical tests: indole citrate utilization, catalase, coagulase, urase activity, and oxidase tests.

Statistical analysis:

Pearson Chi–Square was used to test differences between proportions. Statistical significance was accepted at $P<0.05$ (95% Confidence Level).

RESULTS

High prevalence rate of *C. trachomatis* infection was apparent in the study population (56.1%): 170 of the 303 tested were positive for the infection. The age specific prevalence of *C. trachomatis* infection, with variations across the different age brackets is presented (Figure 1, Table 1). The age group 15-19 were the most infected, with prevalence of 56 (80%); followed by those within the age bracket 20-24, with prevalence of 43 (74%). However, there was no statistical difference in the prevalence rates between the age groups 25-29 years (68 %) and 30-34 years (55%). Nonetheless, the infectivity was lower among those in the 40-44 (22%) and > 45 (13%) age brackets.

Table 1: Age Specific Characteristics of Participants

Participants Characteristics	No Tested (n = 303)	No Positive	%
< 15	2	1	50
15-19	70	56	80
20-24	58	43	74
25-29	44	30	68
30-34	36	20	55
35-39	33	9	27
40-44	31	7	22
>45	29	4	13

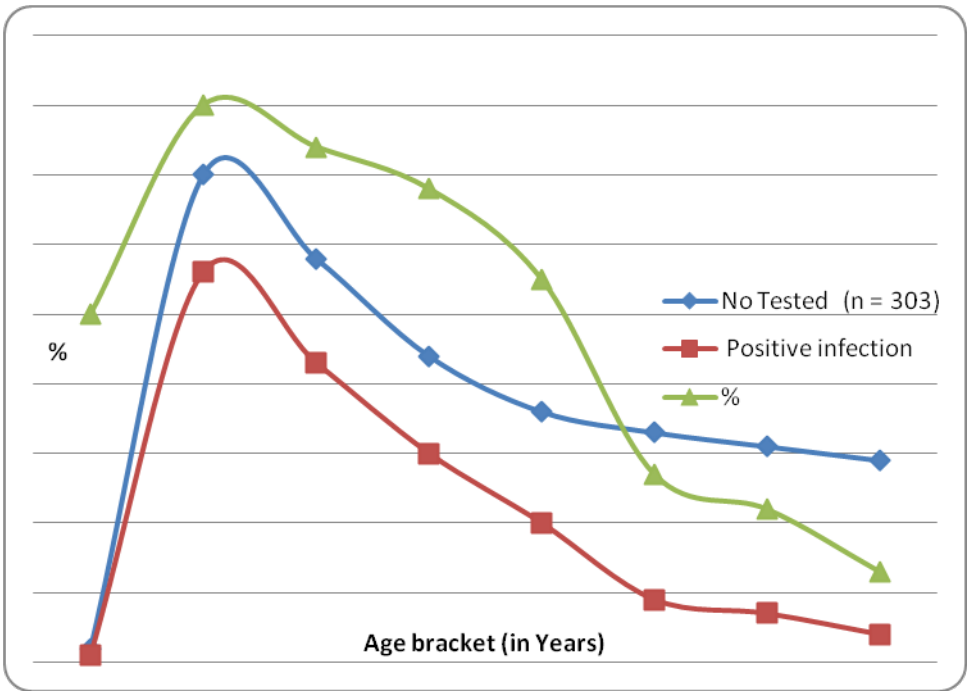

Figure 1: Age specific prevalence of *C. trachomatis* infection.

A relationship was established between marital status and preponderance of *C. trachomatis* infection. Higher prevalence of the infection was observed among the single participants across different age brackets, in comparison with the married individuals, implicating marital status as an important risk factor for the transmission of *C. trachomatis* infection (Table 2). Preponderance of the infection was however higher in 15-19 years age range: out of the 56 participants screened for the infection, 39 were single while only 17 were married. Infectivity was nonetheless observed to decrease with age of participants: >45 years age group were associated with lower prevalence of the infection.

Table 2: Distribution of *C. trachomatis* infection among different marital status Marital

Marital status:	Total positive (N = 170)	Single	Married
< 15	1	1	-
15-19	56	39	17
20-24	43	32	11
25-29	30	22	8
30-34	20	17	3
35-39	9	7	2
40-44	7	6	1
>45	4	3	1

On the educational levels, individuals with no formal education had an increased prevalence of *C. trachomatis* infection (70%), followed by those in primary educational level (45.5%). However, prevalence rates were lower for those in secondary and attempted tertiary educational levels, thus associating lower educational levels with increased prevalence of *C. trachomatis* infection (Table 3).

Table 3: Educational Levels of Participants

Educational levels:	No Screened	No Positive	%
Primary Education	134	61	45.5
Secondary Education	22	9	40.9
Tertiary Education (Attempted)	6	1	16.6
No formal Education	141	99	70

Sexual activities were generally initiated before the age of 30 years, indicative of early onset of menarche; adolescence sexual exposure was thus implied. Greatest sexual activity commenced between the ages of 15-19 years: 171 participants attested to having had sex between the ages of 15-19 years; 107 of these had positive *C. trachomatis* infection; while 37 individuals indicated initial sexual encounter mostly with their male counterparts at <15 years of age; 33 of those in this age category were infected with C. trachomatis infection. The observation therefore that the later the age of first sexual activity, the lower the chances of infection with *C. trachomatis* and is established: 9 out of the 20 individuals between the ages of 25-29 were positive for the infection (Table 4, Figure 2).

Table 4: Age of Initiation of Sexual Activity

Age of First sexual encounter (Years)	No Screened	No Positive
<15	37	33
15-19	171	107
20-24	75	21
25-29	20	9
Total	**303**	**170**

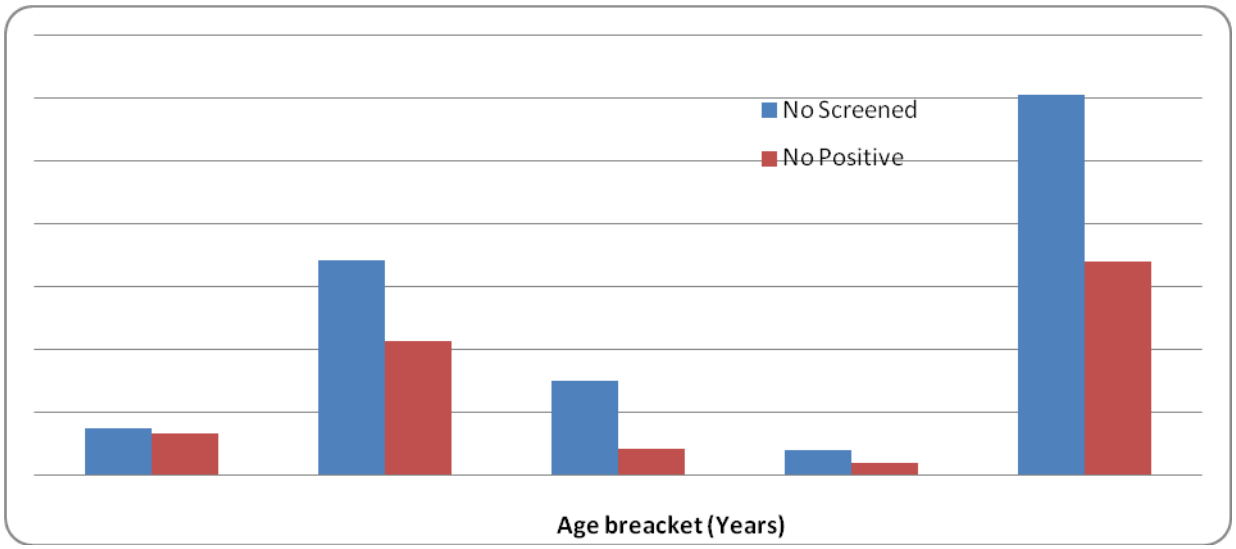

Figure 2: Profile of Age of Commencement of Sexual Activity (Years).

Preponderance of *C. trachomatis* infection in the surveyed areas was intrinsically linked to sexual behavioural attitude in form of premarital/multiple sex partnering, (frequent partner change). Individuals in multiple sex partnership were therefore observed to be hardest hit by *C. trachomatis* infection: out of the 303 persons screened for the infection, 139 of those in the multiply sex partnership were infected with *C. trachomatis*, while only 31 (10.2%) of those in single sex partnership were positive for the infection (Table 5).

Table 5: Associated Sexual Behaviour and *C. trachomatis* infection

Sexual behaviour:	No. of Participants	No. infected with *C. trachomatis*	%
Multiple sex partnering	303	139	45.8
Single sex partner	303	31	10.2

Table 6: *C. trachomatis*-associated complications in surveyed population

Complications/Adverse Outcome	No screened	No Positive for C. trachomatis
PID	87	59
Infertility:	98	74
Primary	28	21
Secondary	70	53
Abortion/miscarriage	53	14
STDs excluding *C. trachomatis*	65	23

Adverse health outcomes

Result of the questionnaire responses indicated several adverse health outcomes implicated as serious consequences of *C. trachomatis* infection. About 98 (75.6%) cases of infertility were reported; primary infertility was observed in 28 respondents (26.4%;) 21 of these had *C. trachomatis* infection; while secondary infertility occurred in 70 (35.3%) participants; 53 were infected by *C. trachomatis*. Similarly, 87 (67.8%) cases of pelvic inflammatory diseases (PID) were reported; 59 had C. trachomatis infection. Prevalence of abortion and miscarriages were observed in the population studied: 14 out of the 53 persons with cases of abortion and miscarriages were similarly infected with *C. trachomatis*. Furthermore, previous exposure to other STIs excluding *C. trachomatis* infections was observed in 65 individuals, 23 of these were co-infected with *C. trachomatis* (Table 6).

The paradigm of HIV and *C. trachomatis* coinfection as well as association with other sexually transmitted infections in the study population, establishing that C. trachomatis is growing alongside HIV and other sexually transmitted pathogens in the rural communities is here presented. Out of the 170 individuals reactive to *C. trachomatis* antigen, 103 (61%) were HIV seropositive, while 67 (39%) were seronegative. Preponderance of the coinfection in young people is observed. High frequency of both infections occurred in the age categories: 15-34 years of age, with increased prevalence among those within 15-19 years age group followed by those between 20-24 years. Similarly, co-infection of *C. trachomatis* with other sexually transmitted pathogens other than HIV was also apparent from the study. Implicated pathogens of medical importance isolated included *Neisseria gonorrhea* and *Staphylococcus aureus.* Among the 170 individuals reactive to the *C. trachomatis* antigen, 23 were dually infected with *N. gonorrhea* (17) and *S. aureus* (6) (Table 7, Figure 3).

Table 7: Age-related paradigm of HIV and *C. trachomatis* co-infection

Age Range	*C. trachomatis* positive	HIV positive
< 15	1	-
15-19	56	41
20-24	43	22
25-29	30	17
30-34	20	14
35-39	9	5
40-44	7	3
>45	4	1
Total	**170**	**103**

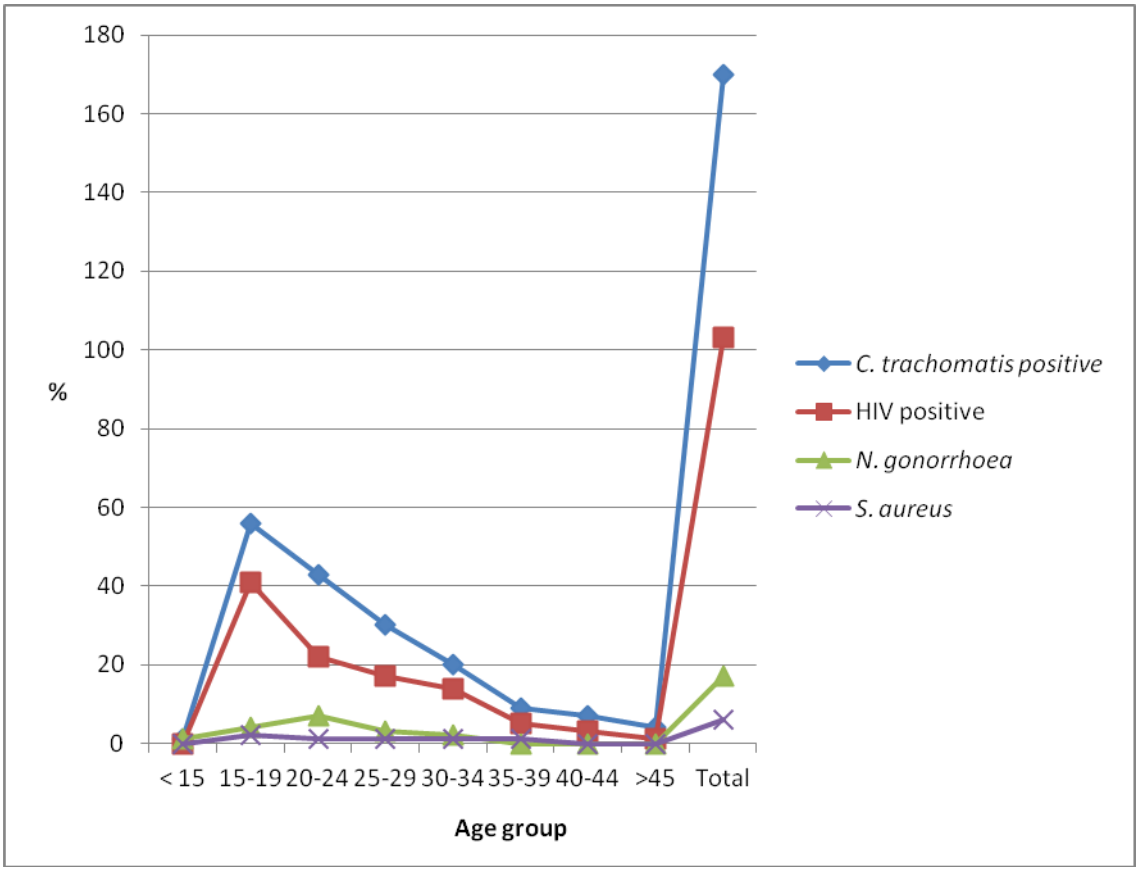

Figure 3: Age Distribution of HIV, *C. trachomatis and* associated STIs.

Participants Characteristics	**No Tested (n = 303)**	**No Positive**	**%**
< 15	2	1	50
15-19	70	56	80
20-24	58	43	74
25-29	44	30	68
30-34	36	20	55
35-39	33	9	27
40-44	31	7	22
>45	29	4	13
Marital status:	Total positive (N = 170)	Single	Married
< 15	1	1	-
15-19	56	39	17
20-24	43	32	11
25-29	30	22	8
30-34	20	17	3
35-39	9	7	2
40-44	7	6	1
>45	4	3	1
Educational levels:			
Primary Education	134	61	45.5
Secondary Education	22	9	40.9
Tertiary Education (Attempted)	6	1	16.6
No formal Education	141	99	70
Age of First sexual encounter (Years)	No Screened	No Positive	
<15	37	33	
15-19	171	107	
20-24	75	21	
25-29	20	9	
Total	**303**	**170**	
Complications/Adverse Outcome			
PID	87	59	67.8
Infertility:	98:	74:	75.6:
Primary	28	21	26.4
Secondary	70	53	35.3
Abortion/miscarriage	53	14	
STDs excluding C. trachomatis	65	23	
Sexual behaviour/and or frequency of partner change:			
Multiple sex partnering	303	139	45.8
Single sex partner	303	31	10.2

PROFILE OF PARTICIPANTS

Discussion

Preponderance of C. trachomatis (CT) infection was apparent in the study; with a high prevalence rate of 56.1% in the population, CT infection is established as a serious health problem. The reported high prevalence rate of CT in this study is in consonance with previously reported prevalence of over 51% among pregnant and non pregnant women and their spouses in Lagos, and a prevalence of 41% in South- Western Nigeria (Okoror *et al*., 20007); Okoror *et al.*, 2000).

High prevalence rates:

Expectedly therefore, the observed high prevalence CT infection in the current study could be intrinsically linked to its mutual relationship and/or co-infectivity with HI. Local inflammation of the genital tract caused by CT promotes HIV shedding, thereby increasing HIV infectiousness. A dose relationship therefore exists between leucocyte concentrations, a marker for inflammatory processes, and HIV viral shedding. Gonorrhoea and Chlamydia, for example, are associated with high concentrations of leucocytes in the genital tract consequently, there results greater HIV shedding with simultaneous increased prevalence of associated STI particularly, CT.

Another plausible reason for the increased prevalence observed in the study could be partly due to the subtle biologic interaction between HIV and CT. Chlamydia trachomatis as well as the Human Immunodeficiency virus-1 (HIV-1) are sexually transmitted pathogens; both can infect monocytes/ macrophages and have an obligate intracellular replication cycle. The present study underscores a significant association between ST and HIV spread. A mutual and/or synergistic interaction was therefore observed in the co-infectivity of both infections (as in

other STIs, especially the ulcerative STIs of the lower genital tract which facilitate the transmission of HIV) It is suggested that the joint epidemiology of the deadly double (HIV and CT infections) could be partly due to their common transmission modes; namely, sexual transmission and associated high risk behaviour including premarital sex and other forms of multiple sex partnership. An aspect of the biologic interaction that could also be responsible for the coinfectivity include the invasive intracellular pathogenesis of CT by which it induces aggressive genital epithelial layer and mucus membrane damage, which potentiates HIV entry and invasion, as well as some other subtle immunological modifications resulting from HIV subsequent invasion and colonization, which drastically encourages CT coinfection. On the other hand, immunosuppression resulting from HIV invasion could consequently grossly dispose infected persons to aggressive chlamydial diseases including pelvic inflammatory diseases and other associated adverse CT outcomes as observed in available results of the current study. Other reported mechanism supporting our finding indicates that CT enhances HIV-1 transmission by their common biologic mechanisms: polymorphonuclear leukocytes (PMNs) are recruited into the genital tract by CT, which serves not only to recruit PMNs, but also to interact with PMNs to increase HIV replication. HIV replication is thus triggered by contact of HIV-infected cells with PMNs, by the generation of reactive oxygen intermediates (ROIs), and by soluble factors such as TNF-alpha and IL-6 (Ho *et al.,* 1995). Consequently, the increased risk for acquiring HIV infection associated with CT may be related to the local recruitment of PMNs by CT and the induction of infectious virus from mononuclear cells present in semen via sexual transmission.

The present study on the other hand, contradicts the lower prevalence rates of 10% in Ibadan and 9% in Maiduguri, respectively (Sanders *et al.,* 1994; Darougar *et al.,* 1982). The lower

prevalence rates could represent its endemicity and subsequent silent horizontal and vertical transfer between sex partners as well as under-diagnosis of cases. Since CT is often asymptomatic and is found in latent infections, it is therefore, frequently unreported for diagnosis. However, many women who are at risk in the study location are still not being screened—reflecting, in part, the lack of awareness in the area by appropriate authorities and the limited resources available to support these screenings. Reported lower prevalence of CT in developed countries, in contrast to the high rates presented in this study, could be as a result of access to diagnosis and treatment procedures, low or improved sexual risk lifestyles, and increased knowledge of sexually transmitted infections including CT (Navarro *et al*., 2002).

CT infection is predominantly a disease of adolescent girls and young women. The study underscores the preponderance of CT among adolescents and young adults, who have been shown to consistently be associated with increased risk of chlamydial infection in the population. Age-based analysis of the prevalence of CT infection highlighted a significant relationship between age and chlamydial infection: younger people had higher prevalence estimates than older participants; highest prevalence occurring among those within the ages 15-19 years, with prevalence of 56 (80%); followed by those within the age bracket 20-24, with prevalence of 43 (74%). Age is thus considered a significant risk factor for CT, which has a predilection for the columnar cells of the cervix of young women. According to research findings, the incidence of CT infection in women decreases substantially after 30 years of age, likely because the target cell for CT (i.e., the columnar epithelial cell, which is present on the ectocervix of young women [cervical ectopy]) is replaced by squamous epithelium through the process of squamous metaplasia that occurs with age (High and Calvet, 2013; Jacobson *et al.*, 2000). This implies that

younger women, are more susceptible to CT than adults. Several reasons have also been advanced on why adolescents are at greater risk for genital chlamydial infection than older people. These include their vulnerability to sexually transmitted infections, engendered by their physical development, which causes the persistence of columnar epithelium on the cervix, which therefore encourages the growth of CT, as well as changes in vaginal flora and mucus production (Berman and Hein, 1999). Additionally, differences in sexual behaviours, partly explain this trend in youth vulnerability to CT: greater numbers of adolescent women engage in premarital sex; as well, sexual debut during early adolescence is often associated with greater numbers of sex partners, less ability to discuss sexuality issues as well as less likelihood to recognize the dangers of premarital or unprotected sex or attend health education summits. Worthy of note also is the opinion that older women are less disposed to CT because they acquire partial immunity following initial or previous serial infections. These views are in consonance with previous reports on highest prevalence of CT among those <19 years and <25 years (Armando Brito de Sá *et al.*, 2002), and also consistent with reports of high rates among disadvantaged youth and young people (Dyani *et al.*, 2012).

Marital and socioeconomic status are sturdily linked. Evaluation of the questionnaire responses indicated the preponderance of CT among single people within all the age brackets, with highest prevalence among the 15-19 and 20-24 age groups, representing the most vulnerable and sexually active age bracket. The observed trend of events in the surveyed area is attributable to socioeconomic levels, chiefly poverty, emanating to lack of educational and job opportunities which make it difficult for several young people within the indicated age brackets to engage in marital union. Many unmarried (single) young people, therefore, due to economic constraints

live alone and fend for themselves and their families by engaging in "any available Jobs", including premarital and/or multiple sex partnering. This situation therefore heightens their vulnerability to various forms of criminal tendencies and promiscuous behaviours and related high risk lifestyles that grossly predispose them to sexually transmitted infections including HIV and CT, and explains the observed trend in the higher prevalence of CT among single people. Similar findings indicated that socioeconomically disadvantaged women had a significantly higher prevalence due to poverty and/or economic hardship and associated sexual promiscuity and multiple sex partnering (Dibua, 2010). This view is however, contradicted by other reports which showed preponderance of CT among married rather than single people (Mawak *et al.*, 2011).

This study further emphasizes the importance of age of first sexual intercourse or activity as an implicating factor for sexually transmitted infections, including CT/HIV infection. Our questionnaire responses highlighted the importance of this phenomenon, and indicated that more women in the study area had first sexual encounter at the adolescent age, 15-19 years. This suggests that the adolescent age group has the utmost likelihood of predisposition to gross CT infection and the attendant HIV/AIDS in the area of study, and apparently, elsewhere in Nigeria and abroad compared with older age groups. The reasons are obvious: this age bracket is characterized by intense sexual activity; including sexual expression, exploitation and experimentation, which, most often go without parental control and/or consultation, but on exclusive collaboration with, and adherence to the opinion of peers (peer pressure). Several promiscuous activities predisposing to sexually transmitted infections and especially, HIV/AIDS and CT are therefore unconsciously and unwittingly indulged in. The implication therefore, is that, the probability of getting infected with CT has a direct relationship with the age of

commencement of sexual intercourse; in other words, predisposing to CT is a function of age of onset of sexual activity. The most plausible explanation therefore, is that; the earlier the age of onset of sexual activity, the greater the chances of acquisition of CT. This finding is in consonance with previous reports, which showed the age of onset of sexual activity as a factor of CT transmission (Navarro *et al.*, 2002; Kirby *et al.*, 1994).

C. trachomatis has already been established as the major cause of a spectrum of lower and upper genital tract diseases in women. To the best of our knowledge, the prevalence of, course of CT infection and its associated complications in Nsukka area of southeastern Nigeria has not been well documented. Our study observed several adverse health outcomes as important risk factors for infertility; PID, abortion/miscarriage, evident from the identified CT specific antibodies from 74% cases of infertility (primary, 21% and secondary, 53%), PID, 59 (67%), abortion/miscarriage, 53 (13%) respectively. The underscored high prevalence of infertility in the current study, establishing CT as a very important etiological factor is supported by the findings of several researchers. A significantly high rate of CT infection with infertility cases were reported: chlamydial positivity was seen in 20 of the 74 (27%) women with primary infertility and in 11 of the 36 (30.6%) with secondary infertility (Malik *et al.*, 2006). Supportive evidence for the association of CT with miscarriage/abortion are also apparent (Wilkowska-Trojniel *et al.*, 2009). The observed high rates of the implicated outcomes/complications thus assumes significance as a result of HIV transmission and subsequent spread with and around the locality studied.

Identification of an STI is a pointer to a serious sexual health risk as this significantly enhances the transmissibility and high rate of acquisition of several other STIs, as well as human

immunodeficiency virus (HIV). Results of our research shows a mutual association between CT and HIV infection, such cases of co-infection and co-morbidity have the potential of enhancing an individual's vulnerability to HIV, by two- to fivefold evident from our study, which establishing CT as an important risk factor in facilitating the sexual transmission of HIV in the population. Suggested explanations for the high prevalence of the coinfection observed have previously been demonstrated, and include the mutual mode of sexual spread of the deadly duo, HIV and CT, the incriminated aetiologies, and their subsequent synergistic interactions, which mutually potentiates the invasion and spread of each other. Other established biologic evidence of the interaction which gives credence to our findings include the opinion on the invasive intracellular pathogenesis of CT, which induces significant damage to the genital epithelium, thereby enhancing HIV invasion and spread. Further to this are the immunological changes resulting from HIV infection, which favours CT invasion. Similarly, immunosuppression emanating from HIV infection drastically leads to the establishment and progression of CT diseases (in CT infected persons), including pelvic inflammatory diseases and tubal infertility among others (Hitchcock, 1999; Siemer *et al*., 2008). The implications of these observations evident from results of this study therefore, is that serological proof of infection with CT (demonstrated by the detected IgA), or HIV infection was far higher in individuals co-infected with both CT and HIV, indicative of the subtle, but mutual interaction of the deadly duo which potentiate each other's infectivity.

This study further demonstrates the importance of *N. gonorrhoea* as a severe public health risk chiefly by its sexual relationship with HIV and CT in the population surveyed. With a prevalence of 61% HIV/CT coinfection, and 23 persons dually infected with *N. gonorrhea* (17), it is established that both bacterial infections are growing alongside HIV in the rural

communities here represented. The age most affected in each case of the disease however, are adolescents and young adults who are in their productive and sexually active years (15-34 years of age). This has grave consequences for both individual and the community; triggering further spread of the infections, especially HIV/AIDS, reduced labour force and associated unproductivity and poverty, as well as persistence of the infections. The comparatively low prevalence rate of *N. gonorrhoea* in this study is in contrast to previously reported high rates of CT and *N. gonorrhoea* coinfection in India and the US (Divekar *et al*., 2000; Lyss *et al.*, 2003). Our result is nevertheless in consonance with reported low prevalence rates of CT and *N. gonorrhoea* among HIV-infected women in Thailand (Srifeungfung *et al.*, 2009). The observed low prevalence rate of *N. gonorrhoea* could be partly due to the population size and category of individuals studied, indiscriminate use of antibiotics to which the organism could have become resistant, or to the screening procedure used; a more recent and optimized procedure with high sensitivity such as the polymerase chain reaction (PCR)] would have given a much sensitive and specific results. However, this procedure is not cost effective for use in the local communities investigated.

In spite of the paucity of information on the inter-relationship between *S. aureus* and the deadly duo HIV and CT in the area of study, our results however further underscores their mutual association and importance as serious risk factors either singly or in consortium in immune suppression, degeneration of genital epithelium and subsequent advancement of pelvic diseases including PID, tubal infertility, abortion/miscarriage and other associated adverse conditions. The low prevalence of *S. aureus* notwithstanding, its impact in the mutual interaction in enhancing CT and HIV disease progression has serious public health consequences, as the pathogen also shares similar sexual behavioural pattern in modes of transmission, invasion and

establishment. Available results of this study is supported by the reported co-infectivity of CT and other STIs including *S. aureus* (Okonko *et al*., 2012), however, their specific association with CT disease conditions such as PID and infertility was not established

Available results of this study highlights the sexual association of *N. gonorrhoea and S. aureus* in CT and HIV infection, and consequently their mutual interaction in the progression of HIV to AIDS. Both have been implicated as aetiologic agents of several disease conditions including STIs (salpingitis, epidymitis), and their presence in diseases of the urogenital track is often demonstrated as cross-infections, which get to significant levels particularly in conditions of immunodeficiency, evident from results of this study. It is suggested that involvement of *S. aureus* in urogenital tract infection and consequently, sexual transmission, could be attributed to its ability to elaborate serious complications and ulcerations of the genital epithelium, which ultimately would enhance the invasiveness and infectivity of CT and HIV among immunocompromised persons. Consequently therefore, the impact by the invading pathogens, either singly or in combination would be progressive immune dysfunction and or down-regulation.

This study has succinctly demonstrated the subtle link that exists between HIV, CT and other traditional STDs via their common mode transmission modes, namely; sexual contact and associated behavioural risk factors. The distinct mechanisms by which these pathogens elaborate infection are noteworthy, and have been advanced to further elucidate their inter-relationship in enhancing disease processes. These include their "epidemiological synergy", which is a mutual interaction between HIV and STIs at the community level. It is suggested here that co-infection with HIV prolongs or increases infectiousness of the infected person; while the STD on the other hand facilitates HIV transmission. This mechanism is applicable to chancroid, genital herpes and

syphilis **(**Wasserheit,1991**).** Reported biological mechanisms of HIV transmission by STDs, (both ulcerative and non-ulcerative) affect *both* HIV infectiousness and susceptibility. These have been indicated: firstly, other STDs facilitate HIV shedding in the genital tract, which probably promotes HIV infectiousness; secondly, STDs increase also susceptibility to HIV, by recruiting HIV susceptible inflammatory cells to the genital tract and by disrupting mucosal barriers to infection. The established relation between STDs such as *N. gonorrhoea* and *S. aureus*, and genital HIV and CT shedding is strictly dependent on immunosuppression, since advancing immunosuppression may promote STD acquisition as opined (WHO, 2010)

Sexually transmitted infections (STIs) are associated with increased human immunodeficiency virus type 1 (HIV-1) susceptibility and viral shedding in the genital tract, but the mechanisms underlying this association are poorly understood. Genital epithelial cells (GECs) are the first cells in the female genital tract to encounter sexually transmitted pathogens. It has been suggested that STIs may increase HIV-1 replication in the female genital tract via pro-inflammatory signaling pathways directly and indirectly by their effects on genital epithelial cells (GECs). In response to interactions with microbes, GECs have been shown to produce pro-inflammatory cytokines, including tumor necrosis factor (TNF-α) and interleukin 6 (IL-6), which can increase HIV replication. Therefore, HIV replication may be up-regulated in infected cells exposed to pro-inflammatory cytokines produced by GECs, leading to impairment of the mucosal epithelial barrier in the genital tract thus facilitating viral and bacterial translocation (Nazili *et al.*, 2010). This invariably implies that STIs especially *N. gonorrhoea* or *S. aureus* could enhance HIV replication by inducing production of pro-inflammatory cytokines and chemokines from GECs in the genital tract, and this may be sufficient to enhance HIV replication. It further suggests that inhibition of inflammatory pathways may also be effective in

reducing HIV replication in co-infected individuals in addition to antimicrobial treatments (Poli *et al.,* 1990).

Conclusion

This study observes that the HIV epidemic has disproportionately affected people living in economically-deprived areas, establishes a clear synergy between HIV, Chlamydia and other bacterial STIs resulting from their mutual heterosexual transmission modes, and underscores the high risk of the multiple infections to individuals and the community as the infected, but untreated women (in their sexually active age), constitute a reservoir of the infections for continuous transmission to the entire community. Impacts of this epidemiological synergy, which range from increased potential of further dissemination of HIV and faster progression to the active disease, AIDS, additionally predispose the infected individuals who are mostly young people in their reproductive and economically most productive age to societal ills including ostracization and stigmatization. The infected women further face the challenge of several gynaecological and obstetric complications and/or adverse outcomes including PID, infertility, abortion and/or miscarriage, which has serious crippling consequences at the individual and community levels. The study therefore suggests enactment of efficient comprehensive HIV prevention strategy directed to the most vulnerable group; the young people and young adults at the grassroots. Policy makers and HIV programme managers, should emphasize health education programmes for clinicians and the local people on the prevalence of, and sequel of HIV/CT and other STIs , improve access to efficient STI clinical services, promote early diagnosis and establish epidemiologic surveillance systems for proper monitoring and management of infected persons.

References

Okonko IO, Okerentugba PO, Adejuwon AO, and Onoh CC (2012). Prevalence of sexually transmitted infections (stis) among attendees of lead city university medical centre in Ibadan, Southwestern, Nigeria. Archives of Applied Science Research, 4 (2):9 80-987

Mawak J.D, Dashe N, Agabi Y.A, Panshak B.W (2011). Prevalence of Genital Chlamydia Trachomatis Infection among Gynecologic Clinic Attendees in Jos, Nigeria. Shiraz E-Medical Journal Vol. 12, No. 2,

Sanders JW, Hook EW, Welsh LE, et al. Evaluation of an enzyme immunoassay for detection of Chlamydia trachomatis in urine of asymptomatic men. J Clin Microbiol. 1994; 32: 24-27.

Darougar S, Forsey T, Osoba A O, Dines R J, Adelusi B, Coker G O. Chlamydial genital infection in Ibadan, Nigeria. A seroepidemiological survey. Br J Vener Dis. 1982; 58: 366-369.

Dyani Lewis, Danielle C Newton, Rebecca J Guy, Hammad Ali, Marcus Y Chen, Christopher K Fairley, and Jane S Hocking (2012). The prevalence of *Chlamydia trachomatis* infection in Australia: a systematic review and meta-analysis. BMC Infect Dis. 12: 113.

Cheesbrough, M., 2004. District Laboratory Practice in Tropical Countries Part 2. Cambridge Low Price Edn., Cambridge University Press, London

Hager WD, Eschenbach DA, Spence MR, Sweet RL. Criteria for diagnosis and grading of salpingitis. Obstet Gynecol 1983; 61: 113-114.

Dibua U (2010). Socio-Economic And Socio-Cultural Predisposing Risk Factors To Hiv/Aids: Case Study Of Some Locations In Eastern Nigeria. The Internet Journal of Tropical Medicine Volume: 6, Issue: 2, Pages: 1-11

Moorman DR, Sixbey JW, Wyrick PB.(1986). Interaction of Chlamydia trachomatis with human genital epithelium in culture. J Gen Microbiol. ;132(4):1055–67

Berman SM, Hein K. (1999). Adolescents and STDs. In: Aral SO, Sparling PF, Mardh PA et al, eds. Sexually Transmitted Diseases. New York: McGraw-Hill, 129-42.

Ho JL, He S, Hu A, Geng J, Basile FG, Almeida MG, Saito AY, Laurence J, Johnson WD Jr. (1995). Neutrophils from human immunodeficiency virus (HIV)-seronegative donors induce HIV replication from HIV-infected patients' mononuclear cells and cell lines: an in vitro model of HIV transmission facilitated by Chlamydia trachomatis. J Exp Med. 1995 Apr 1;181(4):1493-505.

Okoror, L.E, Omilabu, S.A., Fadojutimi, J.and Nsongkhai, V. (2000): Seroepidemio-logical survey of Chlamydia in patients attending pre and post natal clinic at the Col-

lege of Medicine of the University of Lagos, Nigeria. In: Book of Abstract of the 24th annual conference of the Nigerian Society for Microbiology.

Jorn Siemer*,Oliver Theile,Yaw Larbi, Peter A. Fasching, K. A. Danso, Rolf Kreienberg and Andreas Essig (2008). *Chlamydia trachomatis* Infection as a Risk Factor for Infertility among Women in Ghana, West Africa. *Am J Trop Med Hyg February 2008 vol. 78 no. 2 323-327.*