ORIGINAL ARTICLE

Seroprevalence of *Chlamydia trachomatis* in Enugu, Nigeria

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Abstract

Background: *Chlamydia* infections in women cause pelvic inflammatory disease, which often results in devastating consequences of infertility, ectopic pregnancy, or chronic pelvic pain. The infection is largely asymptomatic.
Objective: To determine the seroprevalence of *Chlamydia trachomatis* in Enugu, South Eastern Nigeria.
Materials and Methods: A population-based prospective study comprising female residents of Enugu, South Eastern Nigeria. Indirect solid phase enzyme immunoassay of *Chlamydia* antibodies was done using ImmunoComb *C. Trachomatis* IgG Kit (Orgenics).
Results: The population comprised 136 female undergraduate students and 150 non-student women. The overall prevalence of *C. trachomatis* in the population studied was 29.4%. The percentage of subjects who admitted to be having multiple sexual partners was higher among the student population (71.2%) compared to those from the non-student population (28.8%). The highest percentage of seroprevalence was 28 (33.3%) in the age group of 20-24 years for the student population and 18 (21.4%) in the age group of 25-29 years for the non-student population. The highest seroprevalence of *C. trachomatis* antibodies (69.0%) in both populations was observed in females without any history of infection. Females that had pelvic inflammatory disease, sexually transmitted infection, and secondary infertility assayed for *C. trachomatis* had seroprevalence levels of 19%, 9.5%, and 2.4%, respectively. There was a positive correlation between positive *Chlamydia* assay and the type of subject population (student or non-student) with r^2 value of 1.55 at $P < 0.01$.
Conclusions: *C. trachomatis* infection is largely underdiagnosed and remains a silent disease in the apparently healthy population of Enugu, South eastern Nigeria.

Key words: *Chlamydia trachomatis*, infection, seroprevalence

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Introduction

With an estimated 90 million cases worldwide, *Chlamydia trachomatis* is a major causative agent of sexually transmitted disease (STD).[1]

C. *trachomatis* is an obligate intracellular bacterium with 15 immunotypes, which are as follows: Types A-C cause trachoma (chronic conjunctivitis endemic in Africa and Asia); D-K cause genital tract infections; and serotypes L1-L3, *Lymphogranuloma venereum* (associated with genital ulcer disease in tropical countries).

Genital infection caused by C. *trachomatis* is generally asymptomatic. Approximately 50% of infected males and 80% of infected females show no symptoms, but infection may cause a mucopurulent cervicitis in females and urethritis

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in males.[2] Commonly unrecognized and often poorly or inadequately treated, *Chlamydia* infections can ascend the reproductive tract resulting in pelvic inflammatory disease (PID) and, consequently, lead to chronic pelvic pain, ectopic pregnancy, and infertility.[3]

Women with a *Chlamydia* infection (especially serotype G) are 6.5 times more likely to develop cervical cancer than those without infection.[4] In women with recent or invasive *Chlamydia* infection, indicated by the presence of 1 gM antibody against C. *trachomatis*, increased rates of preterm delivery, premature rupture of membranes, low birth weight, and still birth have been observed.[5] Infection with C. *trachomatis* is also implicated in postabortal, post Cesarean section, and postpartum maternal infections.

C. *trachomatis* in the cervix may be transmitted to a neonate during vaginal delivery, resulting in conjunctivitis and neonatal pneumonia. *Chlamydia* infection develops in 60% of neonates born vaginally to infected mothers and untreated neonatal conjunctivitis can lead to blindness.[6]

Premarital sexual intercourse and intercourse with multiple partners have been shown to be significant risk factors for C. *trachomatis* as well as HIV infection.[7] The invasive intracellular pathogenesis of C. *trachomatis* can cause significant damage to the genital epithelial layer, which may facilitate HIV infection. Conversely, the immunological changes due to HIV infection may favor C. *trachomatis* infection.[8]

Methods of testing for *Chlamydia* include cell culture, direct fluorescent antibody, enzyme immunoassay, and the newer nucleic acid amplification technique. There is a wide variation in the costs, sensitivities, and specificities of these methods.

Owing to varied characteristics of the study population and different methods used for *Chlamydia* detection, there is a wide variation in prevalence rates of *Chlamydia* infection.[9,10] Approximately 4 million cases of *Chlamydia* infection are reported per year in the US, with an overall prevalence of 5%.[11] In Ethiopia,[10] the prevalence rate for *Chlamydia* infection of the cervix was 5.9%. Low gravidity and age <30 years were independently significant risk factors for cervical antigen positivity. Among unsuspecting women attending antenatal clinic in Benin City, Nigeria,[12] a prevalence rate of 13.3% was noted, while Nwanguma *et al*.[13] reported a prevalence of 33% in asymptomatic volunteers in a population of Nigerians living in two cities in the South Eastern part of the country.

However, morbidity caused by the sub-clinical nature of *Chlamydia* infection has been recognized in developed countries and screening methods adopted to prevent consequences of untreated infection such as PID, the same cannot be said of the developing societies because reliable assays are too expensive and complex for routine use.[14,15]

Brunham *et al*.[16] suggested that in the absence of strategies to alter sexual network, vaccine would be needed to halt spread of infection at the population level.

This study was conducted to estimate the prevalence of C. *trachomatis* infection in Enugu and South-Eastern Nigeria, as well as suggest ways to limit the spread and complications of this largely asymptomatic disease.

Materials and Methods

This was a population-based prospective study conducted at University of Nigeria Teaching Hospital, Enugu, South-Eastern Nigeria, over a 3-month period.

Enugu, an old regional capital has a population of 717,291 according to the 2006 national census figure. Over the past two decades, Enugu has undergone rapid urbanization. The major occupations include civil service, trading, and animal husbandry by the Hausa minority at the periphery of the town. The major ethnic group is the Ibos.

The study group (non-Student) comprised 150 women aged 20-34 years attending the gynecological clinic for secondary infertility, PID, and STD.

The control group comprised 136 female undergraduate volunteers in the same age bracket.

For all participants, a standardized questionnaire, which gathered demographic and behavioral information such as age, marital status, and number of sex partners was completed.

Only those who had not been on azithromycin, erythromycin, doxycycline, or tetracycline in the past three months before sampling were included in the study. Each subject gave her consent and the study was approved by the Ethical Committee of the hospital. Data were analyzed using tables and simple percentages. Chi-square test was used to compare the variables and $P \leq 0.05$ was considered statistically significant.

Venous blood was aseptically collected from the antecubital fossa or dorsal veins of the arm of 286 female subjects into clean plain test tubes. The blood samples were allowed to clot and centrifuged at 3,000 rpm (Hettich Universal). Thereafter, the sera were separated into plain bottles and preserved at 2-8°C. The materials used included ImmunoComb *Chlamydia trachomatis* IgG kit (Orgenics) and precision pipette.

The solid phase is a comb with 12 projections (teeth); each

tooth is sensitive at two positions-an upper and a lower spot.

At the start of the test, serum specimens were added to the diluents in the wells of row A of the developing plate. The comb was then inserted into the wells of row A. Anti-C. *trachomatis* antibodies, if present in the specimens, will specifically bind to respective *Chlamydia* antigens on the lower spot of each tooth of the comb. Simultaneously, immunoglobulins present in the specimens were captured by the anti-human immunoglobulin on the upper spot (internal control). Unbound components were washed away in row B. In row C, the human IgG captured on the teeth will react with alkaline phosphatase-labeled anti-IgG. In the next two rows, unbound components were removed by washing.

In row F, the bound alkaline phosphatase will react with chromogenic components. The results were visible as gray-blue spots on the surface of the teeth of the comb.

All components, developing plates, reagents, and specimens were brought to room temperature (25°C) and the tests were performed at room temperature.

Row A of the developing plate was perforated and 0.10 ml of each serum sample was placed into the wells using a micropipette and mixed repeatedly and the pipette tip discarded. The comb was then inserted into the wells of row A containing the specimens, mixed several times and incubated at room temperature for 10 minutes in row A. Towards the end of 10 minutes, the foil of row B was perforated, the comb was removed from row B and the adhering liquids from the tips of the teeth absorbed on a clean absorbent paper. The comb was inserted into the wells of row B and agitated for 2 minutes. Row C was perforated and the comb transferred into the wells and allowed to stand for 20 minutes. It was absorbed and inserted into row D for 2 minutes and row E for 2 minutes. The comb was inserted in row E for one minute for a stop reaction and then allowed to dry in air. The controls were carried along with the test.

Results

Table 1 shows the age distribution of the subjects. The total distribution of subjects with respect to age was as follows: 120 of 286 subjects (42.0%) were observed for age groups of 20-24 and 25-29 years, whereas 46 of 286 subjects (16.1%) were observed for the age group of 30-34 years ($\chi^2 = 65.530$, $P < 0.001$). The correlation between age and type of subject was significant ($P < 0.01$), correlation coefficient $r^2 = 0.477$.

Similarly, the demography of the 286 study population showed that 218 were single and 68 were married. Of the 218 singles population, 124 (56.9%) were from the student population, while 94 (43.1%) were from the non-student population. Age and marital status of the subjects significantly correlated at $P < 0.01$, $r^2 = 0.408$.

The percentage of subjects who admitted having multiple sexual partners was higher among the student population (71.2%) compared with those from the non-student population (28.8%).

The seroprevalence of positive *Chlamydia* antibodies assay with respect to age in relation to the type of subject population is shown in [Table 2]. The highest percentage seroprevalence was 28 (33.3%) within the age group of 20-24 years and 18 (21.4%) in the age group of 25-29 years for student and non-student population, respectively. The χ^2 value for *Chlamydia* assay seropositivity was 9.751 at $P < 0.01$.

The overall percentage seroprevalence for positive *Chlamydia* antibodies in both populations was 36 (42.9%) each for the age groups of 20-24 years and 25-29 years and 12 (14.2%) for age group of 30-34 years. Matched age for age, there was a significant positive correlation between positive *Chlamydia* assay and the type of subject populations with r^2 value of 0.155 at $P < 0.01$.

In [Table 3], of the subjects with positive results, an incidence of 17.5% was observed in the student population, while 11.9% incidence was recorded for the non-student population. The χ^2 value for the summary of the incidence of *Chlamydia* antibodies in relation to subject population was 6.834 at $P < 0.01$.

Table 4 reveals the seroprevalence of positive *Chlamydia* antibodies with respect to subjects and type of infection or gynecological condition. It demonstrated that out of 84 sera obtained from both population, which tested positive for *Chlamydia* antibodies, 58 (69.0%) were observed in apparently healthy females assayed routinely, while 16

Table 1: Age distribution

Age (yr)	Student	Non student	Total
	No (%)	No (%)	No (%)
20-24	88 (30.8)	32 (11.2)	120 (42.0)
25-29	44 (15.4)	76 (26.6)	120 (42.0)
30-34	4 (1.4)	42 (14.7)	46 (16.1)
Total	136 (100.0)	150 (100.0)	286 (100.0)

Table 2: Distribution of positive *Chlamydia* antibodies with respect to age and type of subject population

Age (yr)	Student		Non-student		Student + non-student	
	Positive	Negative	Positive	Negative	Positive	Negative
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
20-24	28 (33.3)	60 (29.7)	8 (9.5)	24 (11.9)	36 (42.8)	84 (41.6)
25-29	18 (21.4)	26 (12.9)	18 (21.4)	58 (28.7)	36 (42.8)	84 (41.6)
30-34	4 (4.8)	Nil	8 (9.5)	34 (16.8)	12 (14.2)	34 (16.8)
Total	50 (59.5)	86 (42.6)	34 (40.5)	116 (57.4)	84 (100.0)	202 (100.0)

Table 3: Summary of *Chlamydia* antibodies assay result in relation to the subject

Chlamydia antibody	Type of student population		
	Students (%)	Non-students (%)	Total (%)
Positive	50 (17.55)	34 (11.9)	84 (29.4)
Negative	86 (30.1)	116 (40.6)	202 (70.6)
Total	136 (47.6)	150 (52.4)	286 (100.0)

Table 4: Seroprevalence of positive *Chlamydia* with respect to age in relation to provisional diagnosis of subject population

Age (yr)		Provisional diagnosis before assay				
		Routine	PID[a]	Std[b]	Infertility[c]	Total
		No (%)	No (%)	No (%)	No (%)	No (%)
20-24	Positive[d]	30 (40.5)	2 (2.4)	-	-	36 (42.9)
	Negative[e]	72 (35.6)	8 (4.0)	4 (2.0)	-	84 (41.6)
25-29	Positive[d]	20 (23.8)	10 (11.9)	6 (7.1)	-	36 (42.9)
	Negative[e]	46 (22.8)	16 (7.9)	14 (6.9)	8 (4.0)	84 (41.6)
30-34	Positive[d]	4 (4.8)	4 (4.8)	2 (2.4)	2 (2.4)	12 (14.3)
	Negative[e]	8 (4.0)	14 (6.9)	4 (2.0)	8 (4.0)	34 (16.8)
Total	Positive[d]	58 (69.0)	16 (19.0)	8 (9.5)	2 (2.4)	84 (100.0)
	Negative[e]	126 (62.4)	38 (18.8)	22 (10.9)	16 (7.9)	202 (100.0)

a - Pelvic inflammatory disease; b - Sexually transmitted disease; c - Secondary infertility; d - Pearson Chi-square value- 30.695 at $P < 0.001$; - Pearson Chi-square value- 52.114 at $P < 0.001$

(19.0%), 8 (9.55%), and 2 (2.4%) were observed in females with PID, STD, and secondary infertility, respectively.

The highest seroprevalence rate of 69.0% observed in routine assays showed a distribution of 34 (40.5%), 20 (23.8%), and 4 (4.8%) in the age groups of 20-24 years, 25-29 years, and 30-34 years, respectively.

Pearson Chi-square tests of relationship between provisional diagnosis and prevalence of *Chlamydia* antibodies showed $\chi^2 = 30.695$ at $P < 0.001$. There was a significantly positive correlation between age and provisional diagnosis with r^2 value of 0.45% at $P < 0.01$.

The relationship between the type of subject population (student and non-student) and provisional diagnosis was significant, with r^2 value of 0.614 at $P < 0.01$. However, no significant correlation was observed between positive *Chlamydia* and provisional diagnosis.

Discussion

This study demonstrated that asymptomatic women tested positive for *Chlamydia* more than women attending a gynecological clinic for PID, STD, and secondary infertility. This is surprising, as these conditions should usually be associated with increased incidence of reproductive tract complication arising from *Chlamydia* infection.

The diagnosis of C. *trachomatis* has been problematic due to labor-intensive culture techniques. The method used in this study has been documented as an inexpensive and effective screening method in developing countries.[17,18] The more accurate test like nucleic acid amplification for detection of infected genital secretions was not available. A case is hereby made for more networking between the developed and developing world in the provision of these more accurate test kits. It will in the long run be more profitable both in the research and management of patients.

There is a wide regional variation in the incidence of C. *trachomatis* and this variation depends on the age, marital status, clinical condition, sensitivities of the methods used, and various other factors.[9,10]

The *Chlamydia* assay result in relation to the subject population (student and non-student) found in this study showed a 29.4% positive incidence of C. *trachomatis* infections, while 70.6% were negative in both populations. This result showed a high incidence of asymptomatic C. *trachomatis*, which agrees with findings of other reports.[13,19]

This study also revealed that the overall seroprevalence of positive C. *trachomatis* was higher in the student population than in the non-student population. This is in agreement with the previously reported association of C. *trachomatis* infection with young age groups.[20] This may indicate higher sexual activity, multiple sexual partners, and low or no use of barrier (condom) method of contraception that is prevalent in this group.[21]

In this study, the majority of patients who were positive to C. *trachomatis* were tested routinely during a sexually transmitted infection and general gynecological clinic and were not associated with a diagnosis of PID, STD, and secondary infertility. This finding is in agreement with previously reported data, which indicated that 70% of *Chlamydia*-associated genital infections are asymptomatic.[2] The asymptomatic infections are pivotal to persistence and ongoing transmission on a population level.[22]

Widespread utilization of more accurate tests like nucleic acid amplification for detection of infected genital secretions in those attending the sexually transmitted infection or general gynecological clinics for infertility, whether symptomatic or asymptomatic, should still be practiced, especially in developing countries. This is perhaps a better way of detecting and treating asymptomatic C. *trachomatis*, which is still highly prevalent in apparently healthy population, especially the more sexually active student population.

This is perhaps a prospective useful epidemiological eradication measure for the control of C. *trachomatis* infection. Other measures, such as the use of barrier method during sexual

intercourse, limiting the number of sexual partners, public awareness, school involvement, and peer educators may also help in reducing the incidence and prevalence of this infection.

Chlamydia infection remains largely underdiagnosed and a silent disease in apparently healthy populations in developing countries.

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