Original article

Seroepidemiological survey of *Chlamydia* in North West zone of Nigeria

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Abstract

Objective: *Chlamydia* is made of organism responsible for respiratory as well as genital infections with very serious sequelae. In Nigeria there is paucity of information regards relative frequencies of *Chlamydia* infection of which this study reports in North West zone of Nigeria. **Methods**: Three hundred and thirty three (333) blood samples were collected from individuals attending various clinics in North West zone of Nigeria and tested for *Chlamydia* complement fixing antibody. Swabs collected from positive patients were re-tested using the Romanowsky-Giemsa staining technique. Statistical analysis were carried out in epi-info epidemiological software package. **Results**: From the total of 333 samples collected and tested for *Chlamydia* complement fixing antibody (CCFA) only 287 (86%) were positive. The culture showed that 215 (75%) were positive for *Chlamydia trachomatis* while only 135 (47%) were positive for *Chlamydia pneumoniae*. Seventy-one (71) females had symptomatic infection while 31 males were symptomatic. Of the 104 individuals who were asymptomatic 67 were females while 92 were males. Of the 31 symptomatic males were 22 positive to *Chlamydia pneumoniae* and the symptoms being that of respiratory syndrome while 9 had difficulty urinating. All the symptomatic women had symptoms resembling that of the pelvic inflammatory disease (PID) and vaginal discharge. Age groups 31-35 had the highest positive samples while the extreme ages had the lowest number of positive individuals and also the lowest number of samples. The number of samples as well as the positive results were validated using the epi-info statistical package version 3.4.1. There was no significant difference in the number of samples from both males and females ($\chi^2 = 1.360$, CI = 99%). **Conclusion**: A high percentage of positive result as validated by statistical analysis shows that *Chlamydia* infections are endemic in the population and efforts should be made to screen for the organism to avoid the "silent epidemics".

Keywords: *Chlamydia*; Serological survey; Infections

INTRODUCTION

Trachoma is the prototype *Chlamydia* infection and was recognized in the ancient times of Greece and Egypt and early twentieth century in specimens of persons with trachoma and thereafter in infants and their parents[1]. *Chlamydiae* were first cultured in the 1950s. The wide spread importance and frequencies of genital tract *Chlamydia* infections were first appreciated in the 1960s. *Chlamydia* were first thought to be viruses and were referred to as large viruses[2]. They look like bacteria by having cell wall which lack muramunic acid and are like viruses by being filterable[3].

Chlamydia includes organisms previously called

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the psittacisis- lymphogranuloma venereum-trachoma group (PLT organisms) or the trachoma-inclusion conjunctivitis (TRIC organisms). Chlamydia are non-motile coccoid looking like gram-negative bacteria ranging in size from 0.2 to 1.5 μm. For years *Chlamydia* were considered to be viruses but they are now considered to be a special kind of Gram negative bacteria. They also differ from viruses by containing both DNA and RNA[4]. They can only reproduce in the cytoplasmic vesicle of the host cell by a unique developmental life cycle involving the formation of elementary and reticulate bodies[5]. *Chlamydia* has three accepted species that infect man that includes *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Chlamydia psittacci*[2,6,7].

Chlamydia are rapidly inactivated by heat. They loose their infectivity completely after 10 minutes at 60°C and partly after 3 to 12 hours at 37°C. They can maintain infectivity for years at 5°C and 7°C. During the process of freeze-drying, much of the infectivity is lost, but successfully lyophylised preparations are stable for a long period. Chlamydiae are rapidly inactivated in the presence of phenols [3].

A mention of *Chlamydia* is often referred to a disease caused by *Chlamydia trachomatis*. *Chlamydia trachomatis* infections are mainly spread through sexual contact and most times neonatal. This includes from penis to vagina, penis to rectum and also from mother to child during birth[8] the sexually transmitted disease usually comes with no clear-cut symptoms. *Chlamydia trachomatis* threatens to cause reproductive damage and infertility in as many as 3 to 5 million people in America alone each year[8]. *Chlamydia trachomatis* still remains ahead of gonorrhea and syphilis in the list of most commonly transmitted sexual disease. *Chlamydia trachomatis* may result in urethritis, epididymitis, cervicitis, pelvic inflammatory disease (PID) and other conditions. Men and women infected with *Chlamydia* may have discharge from the penis or vagina and may notice burning urination. Infections in the rectum may cause problems or pains. In many instances, both men and women will not notice any symptoms (50 % of women and 25 % of men). If symptoms do occur, they usually show up within 1 to 2 weeks after been exposed. A person can be infected at any age, the age group been mostly affected being 15-19 years of age but Okoror et al. [9] reported age group 30-36 being mostly exposed but this they attributed to the fact that they sampled only women of child bearing age and their spouses. Okoror et al. [10] also reported that *Chlamydia trachomatis* infect all age groups. The discrepancies in their reports would have been because of difference in the populations sampled. Johnson et al. [11] reported that the adolescents are at high risk.

In women, signs can include unusual vaginal discharge or bleeding, burning during urination or lower abdominal pain. Men like women, may in addition to pain during urination develop swellings in the testicles. Without treatment 40 % of infected women develop pelvic inflammatory disease (PID) which affects the fallopian tubes and causes damage to the ovaries [12]. *Chlamydia trachomatis* have human as their only natural host primates may be susceptible to experimental infection[13]. *Chlamydia trachomatis* causes about 40 % non-gonococcal in men and occur concurrently with *Niesseria gonorrheae* in as many as 50 % of the later in women. *Chlamydia trachomatis* causes muco-purulent cervicitis, urethritis, endometritis, salpingitis, perihepatitis and later post partum endometritis. At least a third of infected females have no symptom[12]. Young children are particularly vulnerable to the infection. Transmission is usually by contact with formitis where it causes pharyngitis in children. Approximately 75 % of infants born by vaginal infected mothers become infected. The infection may remain latent for several months after birth [14]. Less commonly infants born with caesarian sections may also be infected. The anatomic sites most commonly infected in infants are the conjunctiva which often manifest as purulent conjunctivitis and nasopharynx. Serious manifestation of post-natal chlamydial infection is pneumonia, which may range in severity.

Chlamydia psittaci is a diverse species that has poorly been characterized. Strains that infect psittacine birds seem to differ from those that infect poultry. Several mammals and marsupials have species-specific strains[1]. Generally humans are not susceptible to infection with most mammalian strains of *Chlamydia psittaci*. A major problem with *Chlamydia psittaci* is that it is zoonotic in nature. In birds *Chlamydia psittaci* may present as an upper respiratory infection with nasal and ocular discharge, diarrhea or a combination of both. In some cases birds may be infected with no sign. These cases are

of importance because birds are carriers and shed the organism. Psittacosis in humans can result in mild and severe disease. In several cases, humans that are infected often have severe fever with night sweats leading to pneumonia. It is very important that pet birds´owners and handlers of poultry become aware of this disease in order to prevent outbreak [15].

The first *Chlamydia pneumoniae* case which was formally called Taiwanese acute respiratory (TWAR) agent strain was first cultured in 1960s in chick embryo sac but was thought to be a member of the species *Chlamydia psittaci*. *Chlamydia pneumoniae* as an important respiratory pathogen has led to the reappraisal of our concept chlamydia respiratory infections [16,17]. *Chlamydia pneumoniae* is mainly unique to man and in man infection may vary from mild to severe cases. Result of surveys indicated that sub clinical chlamydial infections occur, which often remains undiagnosed because of their similarity to other respiratory infections. The onset of pneumonia may be sudden with chills, fever, anorexia, sore throat, severe headache and photophobia or the disease may develop gradually. In severe cases, nausea, vomiting and diarrhea or constipation may be observed. The fever remains high in severe cases while it may fall to normal within a week in milder cases. Cyanoids and low blood pressure may be observed. Generally, *Chlamydia pneumoniae* causes pneumonia, bronchitis and pharyngitis in school children[18]. Some physicians have reported *Chlamydia pneumoniae* infection in patients with asthma.

Despite all the disease sequelae due to *Chlamydia*, reports about their relative frequencies in Nigeria is still sparse. This study tends to establish a prevalence of *Chlamydia* infections in a section of the country- North West zone.

MATERIALS AND METHODS

Study design

The study was carried out mainly on patients visiting clinics and hospitals, which will include gynaecological clinics and other clinics where *Chlamydia* infections are suspected. They may be symptomatic or asymptomatic and also those consenting individuals not visiting clinics but in the general population and may or may not be symptomatic. This study also screened infants and any infant whose either parent was screened and available for screening automatically qualified for inclusion in the study. Those for prenatal counseling include mainly women for infertility cases who were mainly asymptomatic. Most of their spouse who visited the clinics was also included in the study. The genus *Chlamydia* have a group reactive antigen which is detected by complement fixation test a method we used in this study, so in order not to report false positive result, we have to distinguish the two human species, by culture method.

Sample collection

Three hundred and thirty three (333) blood samples were collected from individuals attending clinics in various hospitals in the North West zone of Nigeria. The blood samples were aseptically collected into sterile vacutannier, centrifuged (Hetituch) at 3 000 rpm and serum collected into vials and stored at-20°C until used.

Procedure

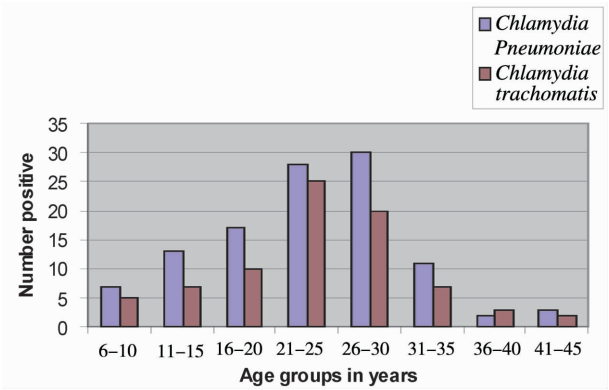
Complement fixation test was carried out as described by Krivoshein[19]. The antigen used was obtained by inoculating embryonic egg with materials obtained from *Chlamydia trachomatis* positive patients and later titerated to the required concentration. The sheep red blood cell used was obtained by bleeding the jugular vein of a sheep aseptically. The blood was washed and stored until used in a 4°C refrigerator as described by Krivoshein[19]. The complement and haemolysin used to sensitize the sheep red blood cells were obtained commercially (Wellcome, Middlesex, U. K). Patients whose samples were positive to complement fixation test had their endocervical swab for females and Urethral discharge or scrapings for males collected with sterile swab stick with the help of clinicians. Those patients with respiratory diseases had their throat washings, sputum or nasal swab collected for *Chlamydia pneumoniae* analysis. The swabs were emulsified with sterile phosphate buffered saline and then inoculated into the yolk sac of embryonic eggs as described by Krivoshein[19] and Romanowsky-Giemsa staining technique was then carried out on smears prepared from materials obtained from the harvested yolk sac of embryonic eggs. Observing for species-specific inclusion bodies under the oil immersion objective did identification of specific species. Epi-info epidemiological and statistical package version 3. 5. 1 was used to validate the results obtained.

RESULTS

From the samples ($n=333$) collected from subjects visiting the clinics in North West zone of Nigeria, only 287 (86 %) were positive (Table 1) while 215 (75 %) of the positive samples were *Chlamydia trachomatis*, 135 (47 %) were *Chlamydia pneumoniae*. Figure 1 shows the distribution of *Chlamydia* spp according to age groups. The distribution of symptomatic and asymptomatic individuals shows that more females ($n=71$) were symptomatic as compared with males ($n=31$) who were symptomatic (Table 2). Of the total of 104 asymptomatic females were 67 women of child bearing age attending anti-natal clinics in North West zone of Nigeria, while from the 92 asymptomatic males were 45 spouses of asymptomatic women attending anti-natal clinics. Of the 31 symptomatic males were 22 positive to *Chlamydia pneumoniae* and the symptoms being that of respiratory syndrome while 9 had difficulty urinating. All the symptomatic women had symptoms resembling that of the pelvic inflammatory disease (PID) and vaginal discharge. Age group distribution to *Chlamydia* shows that age group 31-35 had the highest number of patients screened as well as the total number positive while the extreme ages of 6-10 and 41-45 had the lowest positive individuals. The extreme ages were more positive to *Chlamydia pneumpniae* as compared to *Chlamydia trachomatis*. And more males were positive to *Chlamydia pneumoniae* than to *Chlamydia trachomatis*.

Table 1 Age and sex distribution of *Chlamydia* in North West zone of Nigeria.

Age groups	Number Tested	Number Positive	
		Male(%)	Female(%)
6-10	20	7(35)	9(45)
11-15	34	13(38)	15(44)
16-20	40	13(33)	23(58)
21-25	52	20(39)	29(56)
26-30	65	21(32)	32(49)
31-35	73	25(34)	40(55)
36-40	34	14(41)	15(44)
41-45	15	8(53)	3(20)
Total	333	121(36)	166(50)



Figture Distribution of *Chlamydia* spp according to age groups

Table 2 Distribution of symptomatic and asymptomatic individuals according to sex and age groups.

Age groups	Symptomatic		Asymptomatic	
	Male	Female	Male	Female
6-10	2	-	7	9
11-15	5	5	8	10
16-20	2	10	11	13
21-25	-	13	20	16
26-30	5	12	16	20
31-35	12	20	13	20
36-40	2	8	12	7
41-45	3	3	5	-
Total	31	71	92	104

DISCUSSION

Chlamydia spp has been reported to be responsible for a wide range of mild and chronic infections in humans. It was previously been reported that information on relative frequencies of *Chlamydia* infections in developing countries are sparse[9] and that infection in developing countries could be higher has been reported which justify more researches in developing countries like Nigeria. The lack of information on relative frequencies of *Chlamydia* infections have led to the non-screening for the infection in routine hospital laboratory and the infection continue to thrive unchecked. This is however, a main reason for the high positive result in this study and similar to earli-

er result obtained by Okoror et. al.[9,10]. Another major reason for lack of diagnosis of *Chlamydia* infection is that the organism is found of latency with a no clear-cut symptom and could remain in the system for a long time unnoticed and hence untreated. *Chlamydia* infections are then endemic in the population.

Sex distribution shows that more females were positive for *Chlamydia* Complement Fixing Antibody (CCFA) which may not be unconnected with fact that more females showed up in the hospitals for screening since *Chlamydia trachomatis* infection is more visible in females than in males with chronic infections such as pelvic inflammatory diseases (PID). Since complement fixation test will only detect the group reacting antigen and does not speciate the organisms, it could then be said that *Chlamydia trachomatis* is responsible for the high number of females testing positive. Another reason that could be adduced to this is that more females presented themselves for screening than the males since symptoms are usually not seen in males. Even when symptoms are there sample collection is usually difficult in males except the infection presents with urethral discharge which is a source of bias in this study, this was however, taken care of by statistical analysis which shows that there was no significant difference in the number of males and females tested. There were more males testing positive to *Chlamydia pneumoniae* than females which could be traced to the fact that the males were more engaged in outdoor activities and mix properly with other people in the population than the females who are always religiously restricted to the homes. The males during economic activities stand a chance of contacting the infection. They could also transmit the infection to their female counterparts which could be a reason for the high positive results to *Chlamydia pneumoniae* in females though more males were positive.

A comparison of the result of the culture and that of the complement fixation test (CFT) shows that both results were not significantly different or exactly the same. This not undermining that CFT detects only a group reactive antigen common to both organisms. This could hence be said that both tests (culture and CFT) were both sensitive to screening *Chlamydia*. Though CFT detects antibody in both acute and convalescence stage of infection, it could then be said that the subjects screened were still shedding the organism at the time of sampling and since most of the individuals were those visiting the clinics. There was no need for collection of a paired serum sample to measure a fourfold rise in titre since the culture method was used to ascertain and confirm the infection in both species.

The age group with the highest number of infected individuals been 31-35 may be due to the fact that it is the age group where symptoms becomes more visible and not necessarily due to recent infection. There is the possibility that the females of this age group may have contacted the infection due to *Chlamydia trachomatis* while at the more sexually active ages. The occurrence of symptoms at this age group is suggested to be due to a possibility of hormonal changes due to pregnancy and other adult activities. This does not completely rule out new or reinfection. Age group 26-30 also high positive individuals an age group of sexually active individuals and more of the positive individuals were due to *Chlamydia trachomatis*. This age group is also close to another sexually active age of 21-25 which confirm earlier study that *Chlamdia trachomatis* is a disease of the sexually active ages[20]. It is possible that most of the individuals that were positive in age group 6-10 and 11-15 may have contacted the infection neonatally or during birth as *Chlamydia trachomatis* could enter opening in a child via the birth canals[21] and the infection could persist to early childhood. Children of school age could also contact *Chlamydia pneumoniae* in heavily populated nurseries[22]. Transmission could also take place via formities on toys and other play tools in schools. It is also possible for children to contact *Chlamydia pneumonia* infection from their unhygienic mothers. There were a high percentage of individuals at the older age group of 41-45 which suggest that there could be reduction in immunity at these ages. There have been reports that *Chlamydia pneumoniae* is an infection of extreme ages and immune compromised patients[14] which also suggests why a high percentage of positive

individuals were seen in age group 6-10 where antibody developing cells have not yet matured. From the foregoing, all *Chlamydia* infection are said to be endemic in the population.

REFERENCES

1 **Strickland TG.** Trachoma. In: *Hunters Tropical Medicine*. 7th ed. USA: Mc-Grawhill, 1988. 1001-1003.

2 **Prescott LM**, Harley JP, Aklein D. Human diseases caused by other bacteria (Chlamydia, Mycoplasma, Rickettsia), dental and nosocomial infections. In: *Microbiology*. 4th ed. USA: WBC/Mc-Grawhill companies, 1999. 8002.

3 **Hay PE**, Thomas BJ, Horner PJ, Macleod E, Renton AM, Taylor-Robinson D. *Chlamydia trachomatis* in women: the more you seek, the more you find. *Genitourin Med* 1994; 70: 97-100.

4 **Gerald HC**, Branigan PJ, Hudson AP. Expression of *Chlamydia trachomatis* gene required for DNA synthesis and cell division in active versus persistence infection. *Mol Microbiol* 2001; 44: 731-741.

5 **Miyuashita N**, Kanamoto Y, Matsumoto A. The morphology of *Chlamydia pneumoniae*. *J Med Microbiol* 1993; 38: 418-425.

6 **Abram AJ**. Lymphogranuloma venereum. *J Am Med Associ* 1982; 205: 59.

7 **Moulder JW**. The relation of psittacosis group (Chlamydiae) to bacteria and viruses. *Ann Review Microbiol* 1966; 20: 107-130.

8 **Lin JS**, Jones WE, Yan L, Wirthwein KA, Flaherty EE, Haivanis RM, et al. Under diagnosis of *Chlamydia trachomatis* infection. Diagnostic limitations in patients with low - level infection. *Sex Transm Dis* 1992; 19:259-265.

9 **Okoror LE**, Omilabu SA, Orue PO, Ajayi G. Seroepidemiological survey of *Chlamydia trachomatis* in patients attending pre and post natal clinics in Lagos Nigeria. *The Open Trop Med J* 2008; 1: 83-86.

10 **Okoror LE**, Agbonlahor DE, Esumeh FI, Umolu PI. Prevalence of *Chlamydia* in patients attending gynaecological clinics in south eastern Nigeria. *Afri Health Sci* 2007; 7 (1):18-24.

11 **Johnson J**, Neas B, Parker DE, Forteberry JD, Cowan LD. Screening for urethral infection in adolescent and young adult males. *J Adolesc Health* 1994; 14: 233-237.

12 **Hollows FC**. Community based action for the control of trachoma. *Review Infect Dis* 1985; 7: 777.

13 **Orienston E**. Chlamydiae . In: *Medical Microbiology*. 21st ed. USA: Appleton and Lange Publishers, 1998. 310-318.

14 **Schachter J**, Dawson CR. *Human Chlamydia infections*. PPG Publishing Co. Littleton Mass. 1978. 215.

15 **McPhee SJ**, Harrington W. Psittacosis. *West Afri J Med* 1987; 196: 285-309.

16 **Grayston JT**, Campbell LA, Kuo CC. A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR K. *Infect Dis* 1990; 161: 618-625.

17 **Grayston JT**. Infections caused by *Chlamydia pneumoniae* strain TWAR. *Clin Infect Dis* 1992; 15: 757-763.

18 **Karvonen M**, Tuonilento J, Naukkarinem A, Saikku P. The regional distribution of antibodies against *Chlamydia pneumoniae* (strain TWAR) in Finland in 1958. *Intern J Epidemiol* 1992; 21: 391-397.

19 **Krivoshein YS**. *Handbook on Microbiology Laboratory Diagnosis of Infectious Diseases*. Moscow: Mir Publishers, 1989. 319.

20 **Delpiano M**, Magliano EM, Latino MA, Nicosia R, Pustorine R, Sanitino I, et al. Epidemiology of urinogenital infection caused by *Chlamydia trachomatis* and outline characteristic features of the patients at risk. *J Med Microbiol* 1994; 41: 168-172.

21 **Kuo CC**, Wang SP, Wentworth BB, Grayston JT. Primary isolation of TRIC organism in HeLa 229cells treated with DEAE-dextran. *J Infectious Dis* 1972; 121: 665-668.

22 **Stray A**. Public health aspects with emphasis on European Perspective. In: *Proceedings of European Society for the Chlamydia Research Asculapio*. 1992. 275-277.