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Original Article

Chlamydia activity in North East zone of Nigeria

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Abstract. *Chlamydia* includes organisms formally called Taiwanese Acute Respiratory (TWAR) and the Trachoma Inclusion Conjunctivitis (TRIC) and those responsible for genital tract infections with very serious sequelae. Information regarding relative frequencies of *Chlamydia* infections in Nigeria are sparse. A total of 300 blood samples were collected from males and females in North East zone of Nigeria and tested for *Chlamydia* complement fixing antibody (CCFA). All the positive subjects had either endocervical swabs, urethral swabs or throat swabs taken from them and cultured into the yolk sac of embryonated eggs before being tested using the Romanowsky-Giemsa staining technique which acted as a control to the complement fixation test. Statistical analysis was carried out in Microsoft excel and epi-info software. Only 211 samples were positive to CCFA (70.3%) while 201 (95.3%) of the positive samples were positive using the culture method as seen using the Romanowsky-Giemsa staining technique. Of the positive result using the culture method, 120 were positive to *Chlamydia pneumoniae* while only 81 were positive to *Chlamydia trachomatis.* From the total of 211 positive samples only 135 were females while 76 were males. The total number of positive symptomatic patients was 127 of which 92 were females and 35 males. The 84 non-symptomatic positive patients, only 20 were males while 64 were females. Age groups 31-35 years had the highest positive cases. There was no significant difference between the number of males and females and there was also no significant difference between the positive samples due to culture and CCFA. A high percentage of positive samples validated by Chi-square test shows that *Chlamydia* infections are endemic in the population and effort should be made to screen the pathogen early to avoid the serious sequelae posed by these organisms.

Key words: *Chlamydia trachomatis*, *Chlamydia pneumoniae* ,trachoma, pelvic inflammatory disease, urethritis

1. Introduction

The *Chlamydiae* were first recognized in the ancient times of Greece and Egypt. They were thought to be viruses and were referred to as large viruses. Three species were first recognized which includes *Chlamydia trachomatis, Chlamydia pneumoniae and Chlamydia psittaci* (1). A new addition to the family is called *Chlamydia pecorium.* They belong to the order *Chlamydiales* and family *Chlamydiaceae.* Chlamydiae were first detected with cytotoxic stains in the early 20th century in specimens from persons with trachoma and thereafter in infants with conjunctivitis and their parents (2). The widespread importance and frequencies of genital and infant chlamydial infection were first appreciated in the 1960's. *C. trachomatis* is composed of two biovars, the lymphogranuloma venereum agents and the ocular serotype which may be distinguished by antigens well described seroprevalent with distinctive outer membrane proteins (3). Though members of this family have a group distinctive antigen, *C. psittaci* infections are common among those handling poultry and psittacine birds while *C. pneumoniae* which is unique to humans causes upper and lower respiratory tract infections and conjunctivitis, along with pneumonia which may not be clinically distinguishable from those by *Mycoplasma pneumoniae* (4, 5).

Chlamydia lymphogranuloma venereum is a biotype of trachomatis causing a generalised infection transmitted venerally which is of worldwide distribution and has been described as 6th V.D. *C. trachomatis* causes

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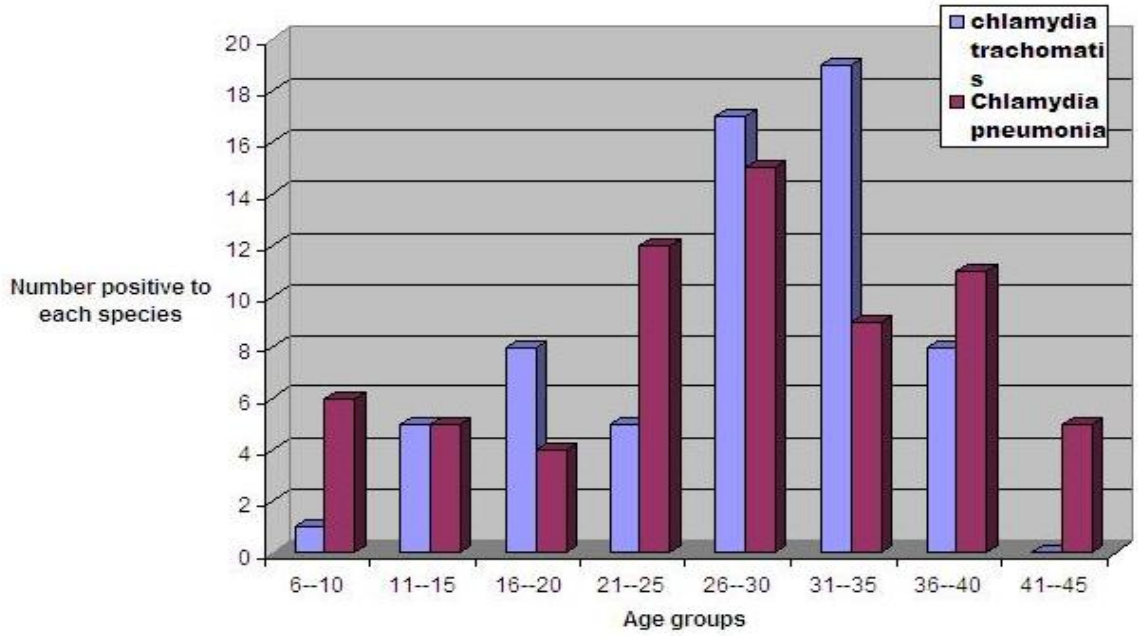

Fig. 3.1: Species distrbution of Chlamydia across the different age groups in N. Eastern Nigeria

mucopurulent cervicitis, urethritis, endometritis, salpingitis, perihepatitis (Fitz-High-Curtis syndrome), and latter post partum endometritis. At least one third of infected females have no symptoms (6). Young children are particularly vulnerable to infection. Transmission is usually by contact with formitis in an unhygienic environment (7). *C. trachomatis* could also cause pharyngitis in children and adults. And disease due to *C. trachomatis* is more common in the tropics. Evidence are accumulating that most infections in infants occur during birth from infected genital passages. *C. trachomatis* similar serotype has been isolated from the eyes of a baby, vagina of the mother and urethra of the father (7). *C. trachomatis* are common in pregnant women in developed and developing countries and the prevalence varies with age (6). Approximately 75% of infants born to vaginal infected women become infected. And the infection may remain latent several months after birth (2). Less commonly infants born by caesarean sections may also be infected. The anatomic sites most commonly infected in infants is the conjunctiva, which often manifest as purulent conjunctivitis and the nasopharynx, often manifesting as chronic congestion.

Table 1. Age group distribution of positive samples to CCFA from North Eastern Nigeria

Age group Year	Number for positive		Total
	Male	Female	
6 – 10	5	9	14
11 – 15	3	7	10
16 – 20	5	15	20
21 – 25	19	17	36
26 – 30	18	30	48
31 – 35	18	39	57
36 – 40	9	15	24
41 – 45	2	3	5
Total	76	135	211

(t=2.312; CI=99%)

The most serious manifestation of perinatal chlamydial infection is pneumonia, which may range in severity from mild to fatal if untreated (6). In many cases *C. trachomatis* is the most common cause of purulent conjunctivitis in the

first few months of life and of a febrile pneumonia in the first 3 months of life (6).

The first *C. pneumoniae* which was formally called Taiwanese Acute Respiratory Agent (TWAR) strain was first obtained in 1960's in chick embryo sac, but was thought to be a member of the species *C. psittaci*. *C. pneumoniae* as an important respiratory pathogen has led to a reappraisal of our concept of Chlamydial respiratory infections (8,9).

Although it was isolated from the conjunctiva of a child in Taiwan in 1965 and in Iran in 1967, it was in 1986 that it was confirmed as a respiratory pathogen when it was isolated from the throat swabs of students with acute respiratory symptoms at the University of Washington Seattle (10). In 1989 it was classified as the third species of the genus *Chlamydia* (5). Since then seroepidemiological studies have suggested that *C. pneumoniae* is the commonest Chlamydial infection in humans worldwide with antibodies to this organism found in 59% of adults in Pittsburgh, 75% in Taiwan, 63% in Spain and 15% in Solomon's Island (5). Furthermore, seroepidemiological studies showed that 75% of the adult populations worldwide have

immunoglobulin G (IgG) antibody against the organism. Serological evidence has also implicated an association of *C. pneumoniae* with other diseases including coronary heart disease, myocardial infraction and sercoidosis (8) but association with seropositivity with smoking, diabetes mellitus and hypertension (11).

C. pneumoniae is mainly unique to man and in man infection may vary from mild to severe. Results of surveys indicate that sub- clinical chlamydial infections occur, which often remains undiagnosed because of their clinical 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 diarrhoea or constipation may be observed. The fever remains high in severe cases, while it may fall to normal within a week in milder cases. Cyanosis and low blood pressure may be observed. Myocardial involvement may be indicated in electro cardiographs and may persist during convalescent period. Generally *C. pneumoniae* causes pneumonia, bronchitis, and pharygitis in school aged children (12). Recently it has become clear that several chronic illnesses may in part be caused by chronic bacterial infection. For example, infection with *Helicobacter pylori* bacteria has been implicated in gastric and duodenal ulcer disease. Some physicians have already reported the onset of asthma symptoms in patients who have had a *C. pneumoniae* infection; as well as an improvement in asthma symptoms in adults and children treated for this infection (13). In Nigeria literature still remain scanty when relating to the scourge of this "silent" organism worldwide. The pathogen has been given little or no attention and they have been allowed to cause damages unnoticed and/or unchallenged. Hence this study looked at the scourge of this infection in North East zone of Nigeria. It mainly focuses on involvement of *Chlamydia* in various infections plaguing the locality and generally the prevalence of *Chlamydia* in the population especially where it may be responsible for infection.

2. Materials and methods

2. 1. Sample collection

Blood samples were collected from consenting individuals attending clinics in North East zone of Nigeria. Other individuals who were not in the hospital settings but agreed to take part in the study were also sampled. Blood samples were collected by venal puncture and stored in venoject vacutaneers and allowed to clot. The sera were separated by spinning the blood in a centrifuge (Hitutich Universal) at 3,000 rpm. The sera were now separated and stored away in a -20 ^{0}C refrigerator till used. Paired serum samples were also collected. Endocervical swabs, urethral swabs and throat swabs were collected with the help of a clinician. All swabs were put in phosphate buffered saline and kept in the -20 ^{o}C refrigerator.

2. 2. Procedure

All the sera obtained were subjected to complement test. Materials used for complement fixation test included sheep red blood cells, veronal buffer diluents which were commercially obtained as CFT tablet (Oxoid), hemolysin which was titrated to reach the required dilution and complement which was commercially obtained as preserved guinea pig serum (Welcome Research Laboratory, England). The antigen used was that of *C. trachomatis* and was originally isolated from a patient using embryonated eggs. Complement fixation test as adapted by microtiter

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Table 2. Antibody titre of samples positive to CCFA from North Eastern Nigeria

Age groups	Antibody titres								
	1/8	1/16	1/32	1/64	1/128	1/156	1/512	1/1024	Total
6 – 10	7	3	1	–	2	1	–	–	14
11 – 15	–	4	2	2	–	1	1		10
16 – 20	8	2	2	–	3	2	2	–	20
21 – 25	10	3	7	2	2	1	1	1	27
26 – 30	16	5	8	4	5	3	3	4	48
31 – 35	2	8	4	27	8	–	7	1	57
36 – 40	5	–	9	5	5	–	–	–	24
41 – 45	–	–	–	2	1	1	1	–	5
Total	75	24	33	15	26	7	14	6	205

Table 3. Distribution of symptomatic and asymptomatic individuals according to sex and age groups

Age groups	Symptomatic		Asymptomatic	
	Males	Females	Males	Females
6-10	2	9	3	-
11-15	2	5	1	2
16-20	3	7	2	8
21-25	7	12	12	5
26-30	6	23	10	7
31-35	11	28	7	10
36-40	2	6	7	9
41-45	2	2	-	1
Total	35	92	44	43

plate was performed as described by Krivoshein (14). Antibody titration was also carried out on all the positive samples to CCFA. Swabs were collected into sterile phosphate buffer saline and cultured into yolk sac of 10 days old embryonated after candling the eggs using the method of Krivoshein (14). The eggs were harvested after 14 days of incubation (Gallenkamp). Upon harvest of the eggs for *Chlamydia* the specimens were freeze-thawed and Romanowsky-Giemsa staining techniques carried out on the specimens using the method of Krivoshein (14).

Identification of species was done based on the criteria of Prescott et al. (15) after observation under the oil immersion of binoculars microscope. Though the culture method was used to speciate *Chlamydia* it serves as a quality control for the CFT.

3. Results

Only 211 of the 300 samples were positive to CCFA (70.3%) (Table 1) while 201 (95.3%) of the positive samples were positive using the culture method as seen using the Romanowsky-Giemsa staining technique. Of the positive result using the culture method, 120 were positive to *C. pneumoniae* while only 81 were positive to *C. trachomatis* (Fig. 1). From the total of 211 positive samples only 135 were females while 76 were males (Table 1). The total number of positive symptomatic patients was 127 of which 92 were females and 35 males (Table 2). The 84 non-symptomatic positive patients, only 20 were

males while 64 were females. The symptoms in females range from pelvic inflammatory disease and related diseases.

Of the total number of 92 symptomatic females, 58 were women of child bearing age attending gynaecological clinics in North East zone of Nigeria. In males symptoms include urethral discharge to difficulty in urination. All the 29 symptomatic males were spouses of the symptomatic females. Those patients positive to *C. pneumoniae* had symptoms which range from cough, cold, catttarh and symptoms relating to pneumonia. Age groups 31-35 had the highest positive cases while 41-45 had the lowest positive cases. Although these age groups also had the highest and the lowest number of samples respectively. The extreme ages proved to be more positive to *C. pneumoniae* than to *C. trachomatis.* There was no significant difference between the number of males positive to both *C. trachomatis* and *C. pneumoniae* ($\chi=^2 2.012$; CI=99%). There was no significant difference between the number of males and females (t=2.312; CI=99%) and there was also no significant difference between the positive samples due to culture and CCFA ($\chi=^2 1.057$; CI=99%). Antibody titeration shows that most of the positive samples had antibody titre above 1:16.

4. Discussion

Chlamydia spp. have been known to cause serious sequelae in infected individuals especially if left untreated for a long time. It has previously been reported that relative frequencies of *Chlamydia* infections in developing countries like Nigeria are sparse (16) and that infection could be higher in developing countries than expected. This study is likely to be the first kind in this locality. The high percentage of positive result reported in this study, is evidence of endemicity in the study population. This may be due to the fact that *Chlamydia* infections present with no clear cut symptoms and usually left untreated of are mistaken for other infections, thereby leading to serious sequelae.

Females seems to be more vulnerable to the infection than there male counterparts as shown in the result obtained since females have higher percentage of positivity compared to males. A reason that could be adduced to this is that the infection in females easily passes unnoticed and could remain in the system for a long time without being treated unlike in males where one might easily notice difficulty in urination as well as urethral discharge which is not easily detected in females. In this study however, more females presented themselves for screening than males especially in the age group 31-35 which represented the child bearing age group who will usually visit the hospitals either for infertility cases or ante-natal diagnosis since *C. trachomatis* has been reported to result in such sequelae. This also made sampling easier but most of their spouses refused to present themselves for sampling. The nature of female urinogenital organ may also be responsible for the reason for females testing more positive to *C. trachomatis* than males since their urinogenital organs harbour more organisms (17). This is more so when more males were positive to *C. pneumoniae* than females and increased male economic activities outside the homes may be attributed for the increased infection. Since males are usually involve in out- door activities than females in this population, they are more prone to come in contact with *C. pneumoniae* than females. Despite all these there was no significant difference betweenthe number of males and females sampled (t=2.312; CI=99%).

The result from the CCFA and culture gave no significant difference ($\chi=1.057$; CI=99%) since *Chlamydia* contains a group reactive antigen which could be detected by CFT and this justifies why the CFT was first used to detect *Chlamydia* from blood sample before proceeding to detect individual species with the culture method which is more specific for species detection. The difference in result from both methods is due to the fact that serology will detect both life and dead cells and dead cells will not grow in the culture method of diagnosis even, *Chlamydia* reticulate body might not be detected by culture method. Hence antibody titration was carried out to evaluate the CFT and the high antibody titres confirm the high positive results in the culture method. It has been reported that a titre of 1:16 and above where paired serum sampling is absent is diagnostic and therefore a pointer to recent infection. It should also be noted that the CFT result depends on the time of infection and CFT also detects antibody during convalescence stage of infection, a stage where the organism would have faded away. The culture method could also have results positive where CFT reads negative since in the acute stage where actively multiplying organisms might be involved, complement fixing antibodies may not have been formed, hence results were validated statistically. It has earlier been reported that the culture method has been the gold standard long before the advent of nucleic acids amplification methods

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(18). Hence the culture method could also be said to act as the quality control to the CFT as well as speciating the pathogens.

Age group 31-35 years of age had the highest number of positive individuals to CCFA and culture method though due to the fact that individuals in that age group presented themselves for screening and that is the child-bearing age group and most of them were pre and ante-natal patients. Most of their spouses did not present themselves for screening which account for why more of the positive samples were from females even if the females have been reported to harbour the organisms more than males. These results were however limited to *C. trachomatis* as the result reverted with *C. pneumoniae.* There is also the possibilities of the females acquiring the infection during their adolescent age and would have carried the organism to their child-bearing age. The adolescence age has been reported to be the main sexually active age group and responsible for the transfer of most *C. trachomatis* infection (19). This was seen in the result trend where the percentage of positive result was high for the adolescent age group. It could then be affirmed from this study that the infection is an infection of high sexual activity. Okoror et al. (20) reported the high possibility of neonatal *C. trachomatis* infection, a factor which is suggested to be the reason why children of age group 6-10 still harbour CCFA since antibody acquire from maternal source could still persist. Infection due to *C. trachomatis* acquired from maternal sources during birth could persist to a certain age in life. This account for infants in this study having high positive result.

A further differentiation of *Chlamydia* into species shows that age group 6-10 is very vulnerable to the infection and may account for the high positive result in that age group. This also confirms earlier study by Storz (21) who suggested that infants could acquire *C. pneumoniae* infection in nursery homes. Old age group of 41-45 years had very high percentage of positive cases which could be due to the fact that immunity may be reduced due to old age as well as in infants where immunity may not have been fully developed. Another reason that could be adduced to high positive percentage in older age group may be that they are more frequently exposed especially when they leave the home for external economics activities. *C. pneumoniae* has sometimes be referred to as an infection of the extreme ages (22). The result also shows that more of the males were positive than females because in this part of the world males are more involved in out-door economic activities thereby been more exposed.

C. pneumoniae were more visible in symptomatic infections than *C. trachomatis* which then suggests that *C. trachomatis* were more involved in latent infection than *C. pneumoniae.* The asymptomatic infection are more common in females as confirmed by these results because it has earlier been reported that because of the female genital tract they could harbour the infection for a very long time without visible symptoms and where symptoms exist they resemble those of other infections and hence *C. trachomatis* are not treated, hence leading to such sequelae such as pelvic inflammatory disease, endometritis, salpingitis and even infertility (23). Males had less asymptomatic infection because it is easy to notice symptoms like urethral discharges and difficulty during urination in males but infection can persist due to wrong diagnosis and the wrong infection is treated leading to sequelae which could be very serious.

It is obvious that *Chlamydia* is responsible for a wide range of infections in the population leading to serious sequelae. This study therefore suggests that *Chlamydia* should be screened for in cases of similar symptoms rather than empirical diagnosis which could lead to wrong diagnosis and wrong treatment. Women especially those preparing for conception should periodically be screened for *Chlamydia* infection to reduce the risk of transmission from mother to child during child birth.

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