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Seroprevalence of *Chlamydia trachomatis* IgA, IgM and IgG Antibodies and Associated Risk Factors among Sexually Active Individuals at Saint Vincent de Paul Hospital in Dschang, West Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TDT, ILSN and RN conceived and designed the study, Authors RN, ACNS and VNM were responsible for data collection and biological analysis. Authors TDT, ILSN, VRN and MOK analyzed the data. Authors: RN, TDT, ILSN, ACNS and wrote the first draft of the manuscript. Authors VRN, MJPC, MOK, BLBME, FRNK, RHT and CTK provided critical reading of the first draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Chlamydia trachomatis* is the most common cause of sexually transmitted infections worldwide. Infections caused by this pathogen are usually symptomatic in men and asymptomatic in about 2/3 of women resulting in a variety of clinical complications.
Aim: To determine the seroprevalence of immunological markers and the risk factors associated with the seropositivity to *C. trachomatis* infection in sexually active individuals visiting the Saint Vincent de Paul Hospital in Dschang, West region Cameroon
Study Design: This was an analytical cross-sectional study conducted between July and September 2020 at Saint Vincent de Paul Hospital in Dschang, Cameroon.
Methods: A total of 154 participants were recruited during the data collection period. An indirect ELISA method was used to analyse participant's serum samples. Risk factors were assessed through univariate and multivariate logistic regression using SPSS 25.
Results: The seroprevalence of *C. trachomatis* infection was 38.3% (95%CI: 30.6 - 46.0). Only 39.6% (95%CI: 31.6 - 47.3) of the study population had never been in contact with the bacterium. The reinfection rate among participants was 8.4% (95%CI: 4.0 - 12.8). Results revealed that being Male is a protective factor against the infection [*aOR*: 0.12; *95% CI*: 0.03 - 0.56; *P* = .007]. The non-use of condom [*aOR*: 21.58*; 95% CI*: 3.53 – 132.06; *P* = .001] and having encountered three or more sexual partners [*aOR*: 9.90; *95%CI*: 1.07 – 91.60; *P* = .043] were the significant predictors of *Chlamydia* seropositivity.
Conclusion: The implementation of proactive strategies to curb down the spread of the infection is necessary in this locality. This can be done by providing NAATs to as many health facilities as possible, educating the population and standardizing treatment protocols.

Keywords: Chlamydia trachomatis; Antibodies; Seroprevalence; ELISA; risk factors.

1. INTRODUCTION

Chlamydia trachomatis is a strictly human pathogenic bacterium with an obligatory intracellular multiplication [1]. It is the most common cause of sexually transmitted infections (STIs) worldwide [1–3]. *C. trachomatis* infection is usually symptomatic in men and asymptomatic in about 2/3 of women [4]. Pelvic inflammatory diseases, spontaneous abortions, ectopic pregnancy, low birth weight babies, puerperal infections, conjunctivitis, non-gonococcal urethritis, infertility and epididymitis are among the commonly reported clinical complications [5–9].

Laboratory diagnosis of *C. trachomatis* infection by nucleic acid amplification techniques (NAATs) and cell culture is difficult to popularize due to their high cost [10,11]. In low-income regions of the world, the enzyme-linked immunosorbent assay (ELISA) for the detection of specific serum immunoglobulins remains the most accessible and widely used [12,13]. Joyee *et al.* [14] suggested the usefulness of serology to trace the aetiology of *Chlamydia* infection, especially in the upper genital tract infection, this to facilitate proper clinical management because having found a good correlation between serological analysis and positivity of the polymerase chain reaction (PCR). Similarly, Woodhall *et al.* [15] demonstrate that *C. trachomatis* serology is useful not only for studying the incidence of infection but also for identifying biomarkers of scarring sequelae, guiding treatment, and determining subjects requiring vaccination.

A real assessment of the severity of *C. trachomatis* infection and other STIs in the population is essential for the implementation of adequate palliative measures [16,17]. More than 4 million cases of *Chlamydia* infections are reported in the United States each year [7]. The number of people diagnosed with Chlamydia infection in France was estimated at 267,097 in 2016. The vast majority of cases are observed in women. Young women aged 15 to 24 are the most affected (that is 2271/100000) [18]. The incidence of infection in people under 25 in the United Kingdom (UK) has been reported to be 10.1% in women and 13.3% in men [19].

In Cameroon, Ngandjio et al. [20] reported in a study conducted in Yaoundé in 2003 a prevalence of 3.78% in a cohort of sexually active students. Other studies by Ngonde et al. [21] in Yaoundé in 2016 reported a prevalence of 22.52% obtained in sexually active women. In the same line, a study carried out in Douala in 2020 revealed a prevalence of 34.4% obtained by ELISA method in infertile women [22].

Young age and the number of sexual partners have been reported as a risk factor for the occurrence of *C. trachomatis* infection in Brazil [23]. In a study conducted in the United Kingdom, risk factors varied by sex. Thus, age (16 - 19 years), non-white ethnicity and sexual behaviours were associated with the occurrence of the infection in women while only age (20 - 24 years) and non-white ethnicity were associated with infection in men [19]. Factors that clearly explain the resurgence of this infection in Cameroon have not yet been clearly identified and in particular in the locality of Dschang where the extent of this pathology is poorly understood.

The study aimed to determine the seroprevalence of immunological markers and the risk factors associated with the seropositivity to *C. trachomatis* infection in sexually active individuals visiting the Saint Vincent de Paul Hospital in Dschang, Cameroon.

2. MATERIAL AND METHODS

2.1 Study Design and Duration

This was an analytical cross-sectional study, carried out over a period of two months, from July to September 2020.

2.2 Study Site and Participants

The study was conducted at Saint Vincent de Paul Hospital in Dschang (SVPHD), a secondary confessional Hospital located in the Dschang Health District, West Region of Cameroon. Were consecutively included in the study, all sexually active individuals attending Medical consultation at the SVPHD who gave their free and informed consent to participate in the study. A total of 154 participants were thus recruited during the data collection period.

2.3 Data Collection and Biological Analyses

A structured questionnaire was used to collect the demographic data of the participants as well as to evaluate the potential predictor of the occurrence of the infection. A 3 ml blood sample was taken aseptically from each participant in an additive-free test tube at the SVPH laboratory. Serum obtained by centrifugation was separated and stored in cryotubes at 2 - 8ºC for a maximum of three days before analyses. The indirect ELISA technique was used for the detection of anti-*C. trachomatis* isotypes A, M and G immunoglobulins using the ERBA reagent kit (Calbiotech, Inc. 1935 Cordel Ct., El Cajon, CA 92020 USA). Semi-quantitative results were obtained by calculating the ratio of the optical density (OD) of each sample to the OD of calibrator. According to the manufacturer's instructions, any ratio ≥ 1.1 was considered positive, the equivocal result was between 0.9 and 1.1 and a ratio < 0.9 was considered negative. Any patient with an equivocal result was sampled again two weeks later for a new analysis.

2.4 Data Analysis

The data collected were recorded in Microsoft Excel 2013 spreadsheet and then transferred to IBM SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA) for cleaning and analysis. The results are expressed in terms of numbers and proportions. The proportions specific to each characteristic calculated with their 95% confidence intervals (CI), the medians with their interquartile range (IR) and presented in the tables. The assessment of risk factors was first done with a univariate logistic regression and the potential predictor variables that retained significance were then introduced into a multivariate model. The significance level of 5% (p <0.05) was considered at each step.

3. RESULTS

3.1 Socio-Demographic Characteristics of the Study Population

The socio-demographic data of the study participants are shown in Table 1. A total of 154 participants were recruited during the study period. Their ages ranged from 17 to 66 years with a median of 27 years (IR: 23.0 - 35.3). People under the age of 27 were the most represented (51.3% of the total population). The majority of participants were Female and represented 75.3% of the study population. Roughly more than half of the participants (51.3%) were married people. Regarding occupation, students represented 51.3% of the study population. Participants with a University level of education represented 60.4% of the study population.

3.2 Assay of IgG, IgM and IgA Antibodies anti-*Chlamydia trachomatis*

Table 2 shows the positivity rates for anti-*C. trachomatis* immunoglobulin isotypes. The results of the enzyme-linked immunosorbent assay of these antibodies reveal that 13.6%, 27.3% and 34.4% of participants respectively possessed type A, M and G anti-*C trachomatis* antibodies.

3.3 Serological Profile of the Infection in the Study Population

According to Table 3 presenting the serological profiles of participants, an overall seroprevalence of *C. trachomatis* infection of 38.3% (95% CI: 30.6 - 46.0) was determined. In addition, only 39.6% (95% CI: 31.6 - 47.3) of the study population had never been in contact with the bacterium. We also note a reinfection rate of 8.4% (95% CI: 4.0 - 12.8).

Table 1. Demographic data of the study population

CHARACTERISTICS	*n* (%)
Age ranges (years)	
Median (IR)	27.0 (23.0 – 35.25)
< 27	79 (51.3)
28 – 37	41 (26.6)
38 – 47	14 (9.1)
> 48	20 (13.0)
Gender	
Female	116 (75.3)
Male	38 (24.7)
Marital Status	
Single	69 (44.8)
Married	79 (51.3)
Free union	6 (3.9)
Profession	
Students	79 (51.3)
Formal sector	25 (16.2)
Informal sector	28 (18.2)
Housewife	22 (14.3)
Level of education	
Unschooled	4 (2.6)
Primary	6 (3.9)
Secondary	51 (33.1)
University	93 (60.4)
Total	**154 (100)**

n = Frequency; % = Percentage

Table 2. Positivity rates of immunoglobulins anti-*C. trachomatis* isotypes

	IgA *n* (%)	IgM *n* (%)	IgG *n* (%)
Positive	21 (13.6)	46 (27.3)	53 (34.4)
Negative	133 (86.4)	108 (70.1)	101 (65.58)
Total	154	154	154

Table 3. Serological profile of *C. trachomatis* infection

SEROLOGICAL PROFILE	*n* (%)	*95% CI*
Seronegative (IgA-/IgM-/IgG-)	61 (39.6)	31.8 – 47.3
Scarring sequelae (IgA-/IgM-/IgG+)	34 (22.1)	15.5 – 28.6
Ongoing acute Infection[1] (IgA+/IgM+/IgG-)	8 (5.2)	1.7 – 8.7
Reinfection[2] (IgA+/IgM-/IgG+)	13 (8.4)	4.0 – 12.8
Recent Infection[3] (IgA-/IgM+/IgG+)	6 (3.9)	0.8 – 6.9
Primo-infection[4] (IgA-/IgM+/IgG)	32 (20.8)	14.4 – 27.2
Total	154 (100)	
Global Seroprevalence (1+2+3+4)	*59 (38.3)*	*30.6 – 46.0*

CI: Confidence interval

3.4 Serological Profile of the Infection in the Study Population

Table 4 present the logistic regression of potential risk factors of seropositivity to *C. trachomatis* infection in the study population. Univariate logistic regression showed a statistically significant association between *Chlamydia trachomatis* seropositivity and age less than 27 years old (*P* = .000), Male gender (*P*= .000), unmarried status (*P* = .002), student occupation (*P*= .001), No knowledge of the modes of contamination (*P*= .013), the non-use of condom during sexual intercourse (*P*= .036), the age of the first sexual intercourse less than 20 years old (*P* = .033) and having encountered three or more sexual partners (*P* = .003). Predictors that retained significance in the multivariate model were the Male gender [*aOR*: 0.12; *95% CI*: 0.03 - 0.56; *P* = .007], the non-use of condom [*aOR*: 21.58*; 95% CI*: 3.53 – 132.06; *P* = .001] and having encountered three or more sexual partners [*aOR*: 9.90; *95%CI*: 1.07 – 91.60; *P* = .043].

4. DISCUSSION

4.1 Immunologic Markers of *C. trachomatis* Infection

Results show that 13.6%, 27.3% and 34.4% of participants respectively possessed type A, M and G anti-*C. trachromatis* immunoglobulins. These frequencies are more considerable than the 11.1% ± 8.0, 2% ± 2 and 18.4% ± 6.9 obtained respectively for IgA, IgM and IgG by Bas et al. [24] in a study conducted in Switzerland. Also, Claude et al. [25] obtained lower prevalence of 7.8% for IgA and 18.8% for IgG in infertile people in Rwanda. This inconsistency can be explained by differences in the populations studied and the study period. We however notice that over time the seroprevalence tends to increase.

4.2 *Chlamydia Trachomatis* Seropositivity

The seroprevalence of *C. trachromatis* infection in the study population was 38.3% (95% CI: 30.6 - 46.0). Prevalences of 12.5% and 34.4% were reported by Nguefack *et al.* [22] respectively among fertile and infertile women in Douala. A prevalence of 19.6% was also obtained in a cohort of adolescent women in Brazil [23]. These prevalences are quite lower than that obtained in the present study. This discrepancy may arise from the fact that these studies were all performed in female participants. This would have contributed to neglecting the positivity of this most often asymptomatic infection in male subjects. Also, participants of this study are a mixed up of people with different ages and professions including young students in whom sexual activity is more recurrent and poorly controlled.

The reinfection rate of 8.4% (95% CI: 4.0 - 12.8) is greater than the 4.94% reported by Ngondé *et al.* [21] among pregnant women in Yaoundé. This difference would be due to the fact that pregnant women are generally tested for such STIs at the

Table 4. Univariate and multivariate logistic regression analyses for associations with *C. trachomatis* infection

Variables	Categories	Univariate analysis		Multivariate analysis	
		***OR* (95% CI)**	***P* value**	***aOR* (95% CI)**	***P* value**
Age ranges (in Years)	< 27	0.41 (0.25 – 0.67)	.000*	0.22 (0.03 – 1.57)	.130
	28 – 37	0.64 (0.342 – 1.20)	.163	0.37 (0.06 – 2.45)	.301
	38 – 47	0.40 (0.13 – 1.28)	.121	0.15 (0.02 – 1.39)	.095
	> 48	1.00	-	1.00	-
Gender	Male	0.47 (0.32 – 0.69)	.000*	0.12 (0.03 – 0.56)	**.007***
	Female	1.00	-	1.00	-
Marital Status	Single	0.44 (0.26 – 0.73)	.002*	0.531 (0.07 – 4.26)	.596
	Married	0.84 (0.54 – 1.30)	.432	0.56 (0.06 – 5.05)	.602
	Free union	1.00	-	1.00	-
Profession	Students	0.46 (0.29 – 0.74)	.001*	4.86 (0.952 – 24.86)	.057
	Formal sector	0.67 (0.25 – 1.48)	.321	3.29 (0.64 – 17.08)	.156
	Informal sector	1.80 (0.83 – 3.90)	.136	3.07 (0.49 – 19.21)	.230
	House wives	1.00	-	1.00	-
Knowledge of modes of contamination	No	0.25 (0.08 – 0.75)	.013*	1.024 (0.20 – 5.38)	.978
	Few	0.70 (0.354 – 1.39)	.306	2.226 (0.69 – 17.08)	.182
	Average	1.13 (0.65 – 1.95)	.675	1.47 (0.44 – 4.84)	.531
	High	1.00	-		
Condom use	No	0.60 (0.37 – 0.97)	.036*	21.58 (3.57 – 132.06)	**.001***
	At times	1.83 (0.91 – 3.70)	.091	3.36 (0.88 – 12.84)	.077
	Always	1.00	-	1.00	-
Age of first intercourse (years old)	< 20	0.43 (0.20 – 0.94)	.033*	3094457654	.999
	20 – 25	0.69 (0.48 – 1.00)	.048	1453399921	.999
	> 25	1.00	-	1.00	-
Number of sexual partners already met	1	1.64 (0.77 – 3.47)	.198	4.74 (0.33 – 69.03)	.255
	2	0.38 (0.10 – 1.41)	.147	5.33 (0.53 – 53.58)	.155
	3	0.55 (0.36 – 0.82)	.003*	9.898 (1.07 – 91.60)	**.043***
	> 3	1.00	-	1.00	-

*1.00: Reference Group; *: Significant Association; OR: Odd Ratio, aOR: Adjusted Odd Ratio*

beginning of each pregnancy and positive cases immediately treated with together with their spouses. On the other hand, our study included sexually active men and women whose infectious status with respect to this mostly asymptomatic infection was until then unknown.

4.3 Risk Factors

Women aged under 27 years were significantly more infected by than others according to the univariate logistic regression analysis (*P* = .000), nevertheless of age was not an independent predictor in the multivariate model. Claude et al. [25] found in the study conducted in Rwanda that young individuals (< 25 years) were more infected. The same observation was made by LaMontagne et al. [19] in the United Kingdom, where the highest rate of infection was found in participants aged between 16 and 24 years. This calls for more awareness among young people and also for the use of therapeutic protocols that will ensure healing. Canro et al. [26] pointed out that inadequate treatments leads to microbiological failures in therapy causing persistence of the disease over several years in poorly treated subjects and a fatal risk for reproductive health.

In the univariate analysis, students were significantly more infected (*OR* = 0.46; *95%CI*: 0.29 - 0.744; *P* = .001). Despite the non-significance in a multivariate analysis, this should however be of concern to us. Possible explanations include puberty and a certain degree of ignorance about the disease and its effects in a sexually active population of young people. Thus, the implementation of an effective

strategy to fight against STIs, in particular *Chlamydia* infections, as advocated by the World Health Organization [27] will require deep awareness and education in schools and universities environments.

Being Male is a protective factor againts the infection (*aOR*: 0.121; *95%CI:* 0.03 - 0.56; *P* = .007). A contradicting finding was made in the UK where 13.3% of Men were infected against a positivity of 10.0% observed in women [19]. Sayred et al. [28] also found higher positivity in Men than in Women of 19.5% and 11.5% respectively.

The none-use of condom during sexual intercourse was the major risk factor associated with the *Chlamydia* seropositivity (*aOR*: 21.58; *95% CI*: 3.53 – 132.06; *P* = .001). A study in South East England found that infection was more common in people with irresponsible sexual behaviour, the association was not statistically significant (*P* = .733) [19]. Whatever the case, this observation calls on sexually active people to adopt responsible attitudes in order to protect their self and the partner as well.

The multiplicity of sexual partners significantly increases the risk of acquiring *C. trachromatis* infection (*aOR*: 9.90; *95% CI*: 1.07 – 91.60; *P* = .043). Araujo et al. [23] have shown that having met 4 or more sexual partners during a lifetime doubled the risk of acquiring C. trachomatis infection. Syred *et al.* [28] also found that people with more than two sexual partners in the past 3 months had a higher prevalence of infection (21.3%) than those with less than 2. A biological examination and management of sexual partners before any sexual contact and fidelity to a single partner could help curb down the spread of this infection.

5. CONCLUSION

The study aimed to determine the seroprevalence of immunological markers and risk factors associated with the *C. trachomatis* seropositivity in sexually active individuals visiting the Saint Vincent de Paul Hospital in Dschang, West Cameroon. We can conclude that the rate of seropositivity obtained in this study is among the highest already recorded in the main cities of Cameroon and the moderated reinfection rate should not be neglected. While Being Male is a protective factor against the infection, the non-use of condom during intercourse and having three or more sexual partners are the significant risk factors for the spread of this STI in Dschang. The implementation of a proactive strategy to tackle this infection is necessary in this locality and in Cameroon in general. This can be done by making NAATs available to as much health facilities as possible, educating the population and standardizing treatment protocols.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

The study protocol was reviewed and approved by the administrative Authorities of SVPH in Dschang. The free and informed consent of each participant was sought before their inclusion in the study. The study was conducted in compliance with the Declaration of Helsinki on research involving humans [24].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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