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Prevalence of Human Genital *Ureaplasma* sp. in a Cohort of Subjects in Southern and Northern, Nigeria.

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Abstract

Ureaplasma sp. can be found on the mucosal surfaces of the cervix or vagina of 40 to 80% of sexually matured women and implicated in several complications from urethritis to miscarriages. This study was thus aimed at ascertaining the prevalence of genital *Ureaplasma* sp. in Southern and Northern, Nigeria. Qualitative case controlled study was carried out in two different cities in Nigeria, while convenience sampling and a closed ended questionnaire where used to obtain data from 824 subjects. Triplicate urogenital swabs were taken from participants and transferred into transport and culture media. Specimens were inoculated onto A7 agar incubated at 37^{o}C, for 5 days in 5% CO_2. Cultures were examined microscopically daily for 5 days for the appearance of typical mycoplasma colonies. Results showed that *Ureaplasma* sp. was isolated from 22 of 70 males (31.4%) at the Northern Nigerian location, and from 19 of 70 males (27.1%) at the Southern Nigerian location. In females, 83 of 156 (53.2%) swabs were positive at JUTH, and 50 of 104 (48%) at LUTH. At the two locations, a total of 324 asymptomatic participants were sampled, and 28(8.6%) were positive. The prevalence rate of *Ureaplasma* sp. among the sexually transmitted disease (STD) patients was 24% while a value of 3.9% was recorded for the STD controls. Risk factors associated with genital *Ureaplasma* sp infection among the subjects include subjects with STI (p value=0.030), those with multiple number of sexual partners (p value=0.040), lack of the use of condoms (p value=0.014), age of sexual debut <18 years of age (p value=0.023) subjects with low socioeconomic status in occupation (p value=0.020), and level of education (p value=0.025). The association of genital mycoplasmas infections was strongest in participants <40 years of age (p value=0.059).

Key words: Sexually transmitted infections, Demographic information, Ureaplasma, Risk factors

Introduction

Mycoplasma and Ureaplasma species have been reported to be important etiological agents in urogenital ureaplasmosis (CDC, 2002). The three species that have been frequently isolated from human genitourinary tracts include *Ureaplasma* biovars *urealyticum* and *parvum*, *Mycoplasma hominis*, *Mycoplasma genitalium* (Casin *et al.*, 2002; Kiche *et al.*, 2004). These species have been confirmed to have a nexus with several complications in infected individuals including non-gonococcal urethritis in men, miscarriages, still birth and fetal defects in pregnant women as well as infertility in men and women. Urogenital ureaplasmosis have been identified in both sexually and non-sexually active individuals (Chukwuka *et al.*, 2013). The epidemiology and pathogenic roles of genital mycoplasmas in perinatal and neonatal infections in humans are fairly studied and understood globally; but it has been established that *Ureaplasma* sp. can be found on the mucosal surfaces of the

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cervix or vagina of 40 to 80% and *M. hominis* may occur in 21 to 53% of sexually matured women (Taylor-Robinson *et al*., 2005). Sexually matured persons with no history of sexual contact are infrequently colonized with genital mycoplasmas, even among those who are sexually experienced, colonization increases with increase in the number of different sexual partners. It is interesting to note that colonization increases more rapidly with increasing sexual experience in women than in men, suggesting that women are more susceptible to colonization with these organisms (Taylor-Robinson *et al.*, 2003).

In Nigeria several risk factors including age, occupation, gender, number of sexual partners, sexual frequency, level of education, the use of barrier cautions, such as condom and intrauterine devices (IUDs) have been associated with sexually transmitted diseases (STD) (Olorunshola *et al.*, 2007), but to the best of our knowledge, it appears no study has specifically addressed the prevalence and the risk factors associated with the transmission of human urogenital mycoplasmas which could form an epidemiological data for several locations in Nigeria. With the recent adoption of molecular characterization, the sensitivity and specificity in the identification of these species have increased.

The aim of this research was to create an epidemiological data for urogenital ureaplasmosis across selected locations in Nigeria through a survey in major STD centres within the country. We also evaluated the possible risk factors associated with the ureaplasmosis through the use of structured questionnaires.

Materials and methods

Study design

Study design employed was a descriptive epidemiological study involving 5 study centers (3 in Lagos University Teaching Hospital (LUTH) and 2 in Jos University Teaching Hospital (JUTH). LUTH site involved Departments of Obstetrics, Gynecology and Surgery (Cytology and Urology units) as well as Microbiology diagnostic unit; while Special Treatment Clinics and Microbiology diagnostic unit were involved at JUTH site. The two teaching hospitals serve as the Federal Government's Reference Centers for HIV/AIDS /STD in the Southern and Northern parts of Nigeria, respectively.

Study population

The study involved STI patients who presented with discharge, itching, ulcerations and sores in genital parts, those who had abortion, as well as patients with various degrees of infertilities attending STD clinics at Lagos University Teaching Hospital (LUTH) and Jos University Teaching Hospital (JUTH). The asymptomatic patients (controls) were subjects accompanying STD patients attending antenatal, prenatal, or postnatal clinics, as well as individuals referred by employers for medical tests or religious leaders for HIV screening recommended during marriage counseling.

Samples size and sampling method

The qualitative case controlled study as described by Schlesselman (1982) was employed in the two metropolitan cities' hospitals in the Northern and Southern Nigeria. The study aimed at investigating the relationship among the studied genital mycoplasmas and reproductive maladies such as urethritis, cervicitis, pelvic inflammatory diseases, spontaneous abortion and various degrees of infertility among male and female patients attending STI clinics in Nigeria. A non-probability sampling method, known as convenience sampling method, was used. The sample size was determined using the formula recommended by WHO (2001a). LUTH Site's breakdown included 70 males with STI symptoms, 180 females with STI symptoms, 70 asymptomatic males and 92 asymptomatic females amounting to 412 participants. JUTH Site's Breakdown included, 70 males with STI symptoms, 180 females with STI symptoms, 70 asymptomatic males, 92 asymptomatic females amounting 412 participants all together.

Culture Media Preparation

Triplicate urogenital swabs were taken from participants referred to LUTH and JUTH. Samples were collected from patients with age ranging from 15 to 65 years. In order to maximize the growth of mycoplasmas both transport and culture media were prepared from PPLO broth supplemented with horse serum (20%), 10ml yeast extract (25%), 2ml phenol red (0.2%), penicillin (500u/ml), in 3 separate tubes containing 20ml urea (10%), for *Ureaplasma* sp.; 20ml L-arginine (10%) for *M. hominis* and *M. genitalium*; and 20ml glucose (10%) for *M. fermentance* and the pH of the substrates were adjusted to 6.0, 6.5 and 7.5

respectively. Urogenital swabs were transported in 5ml 2SP medium (Shepard and Luceford, 1976) on ice from the clinics to our laboratory. Specimens were inoculated onto A7 agar (Becton Dickinson, Cockeysville, Md21030), incubated at 37˚C, for 5 days in 5% CO_2. Cultures were examined microscopically daily for 5 days for the appearance of typical mycoplasma colonies. A7 agar incorporates a direct test for urease that allows the differentiation of Ureaplasma from other Mycoplasma (Shepard and Luceford, 1976).

Results

Subjects attending the study centers representing 30 out of the 37 states in Nigeria participated in the completion of the questionnaires at JUTH and LUTH centers. A total of 824 samples were collected from participants with age ranging from 11 to over 50 years. Table 1 shows the geographical distribution of the subjects. The percentage of younger participants (11-20yrs) recorded in the north was 54.2% as opposed the south which experienced participation from older subjects (31-40yrs) at 43.9% (Table 1).

The number of respondents was relatively higher in females at both sites. In females, 58.3% and 66.0% were recorded while in males, 41.7% and 34.0% for LUTH and JUTH, respectively (Table 1). More than 50% of the participants had no children per household at both sites, while 9.5 - 40.3% had 1 or more children. Subjects from the northern geographical zone had higher number of children per household compared to the southern zone (Table 1). Over 50% of the participants were unemployed; the number of the employed respondents was the lowest in the occupation category. In Table 2, a higher percentage of the respondents- 45.5% and 55.6% (JUTH and LUTH, respectively) had their first sexual contact at 16 – 20 years age bracket. Over 75% of the participants were also married while approximately 20% were single by being unmarried, widowed or divorced. About 85% of the participants at both sites reported mono-sexual partners, also a higher percentage responded to having one sexual partner in the last three months. In JUTH, 87% of subjects admitted previous diagnosis of STI which is considerably higher than 59.8% seen in LUTH. The practice of safe sex (use of condom) in the southern region was more pronounced (57.3%) compared to the northern region (38.3%). Both sites however, showed a considerably low rate of IUD use for birth control (Table 2).

Based on the residents and geographical location of the participants in this study, the prevalence rate of genital ureaplasmosis is presented in Figure 1. Of the 824 overall participants, 512 Subjects came from the northern region, while 312 were from the southern region; total number of patients was 500 while total number of control was 324. The distributions of participants covered 6 geopolitical zones of Nigeria (North East, North West, North Central, South West, South East and South-south). The highest prevalence among the patients was recorded in the North Central zone with a frequency of 144 out of 400 (36%); while the least prevalence was observed in the North West zone (6.25%). Also for the controls, the highest prevalence was recorded in the North Central zone with a frequency of 158 out of 324 (48.8%).

Table 3 presents the **s**ummary of the results of the primary isolation of *Ureaplasma* sp. from Southern (LUTH) and Northern (JUTH) Nigeria. *Ureaplasma* sp. was isolated from 22 of 70 males (31.4%) at the JUTH location, and from 19 of 70 males (27.1%) at the LUTH location. In females, 83 of 156 (53.2%) swabs were positive at JUTH, and 50 of 104 (48%) at LUTH. At the two locations, a total of 324 asymptomatic participants were sampled, and 28(8.6%) were positive. The prevalence rate of *Ureaplasma* sp. among the STD patients was 24% while a value of 3.9% was recorded for the STD controls. Overall, 27.9% (202 of 724) of the total participants were positive for *Ureaplasma* sp. colonization/ infection.

. **Table 1. Distribution of correspondence by Zone**

		Patients		**Controls**		**Total**	
s/no	**Zones**	**Frequency**	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**
1	North East Zone	38	7.6	22	6.8	60	7.3
2	North West Zone	25	5.0	25	7.7	50	6.0
3	North Central Zone	244	48.8	158	48.8	402	48.8
	Sub total	**307**	**61.4**	**205**	**63.3**	**512**	**62.1**
4	South West Zone	59	11.8	33	10.2	92	11.2

5	South East Zone	92	18.4	50	15.4	142	17.2
6	South South Zone	42	8.4	36	11.1	78	9.5
	Sub total	**193**	**38.6**	**119**	**36.7**	**312**	**37.9**
	Gross total	500	100.0	324	100.0	824	100.0

Table 2: Demographic characteristics of the respondents by Teaching Hospitals

Characteristics	**Percentage of respondents**		
	JUTH	**LUTH**	**P value**
Age groups of the respondents			P = 0.059
11—20	54.2	35.9	
21—30	35.7	32.9	
31—40	39.0	43.9	
41--50+	12.3	23.2	
Sex			
Female	66.0	58.3	
Male	34.0	41.7	
No of children per family			
0	59.7	90.5	
1 and above	40.3	9.5	
Occupation			0.020
Unemployed	59.7	50.0	
Self-employed/Residual Category/Artisan	25.3	22.0	
Employed	15.0	28.0	
Level of education			0.025
Tertiary	37.0	42.7	
Secondary	33.8	45.1	
Primary	29.2	12.2	
Source of referral			
Counselor/Friend/self	34.4	14.2	
Dr.	42.9	81.0	
Employer	22.7	4.8	

Table 3: Risk factors of human genital Ureaplasma amongst the respondents

Characteristics	**Percentage of respondents (%)**		
	JUTH	**LUTH**	**P value**
Age of first sexual contact			0.023
11—15	16.0	6.1	
16—20	45.5	55.6	
21—25	20.2	25.4	
26—30 and above	18.3	12.9	
Marital status			
Married	84.4	79.3	
Single	15.6	20.7	
No. of sex partner			

1	83.8	83.3	
2 and above	16.2	16.7	
Sexual partner in the past 3 months			0.040
0	17.5	39.0	
1	72.1	58.5	
2 and above	10.4	2.4	
History of STI			0.030
No	13.0	40.2	
Yes	87.0	59.8	
Use of condom			0.014
No	61.7	42.7	
Yes	38.3	57.3	
Use of IUD			
No	78.6	79.3	
Yes	21.4	20.7	

Table 3: Prevalence of *Ureaplasma* sp. among the subjects

Prevalence	**JUTH**				**LUTH**			
	Control		**Patient**		**Control**		**Patient**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
No. examined	70	116	70	156	60	78	70	104
No. of positive	5	8	22	83	9	6	19	50
Rate (%)	7.1	6.9	31.4	53.2	15	7.7	27.1	48

Discussion

From this study, epidemiological data on the incidence and prevalence of genital mycoplasma in the northern and southern regions of Nigeria were obtained. The predisposing factors to colonization or infection by genital mycoplasma were also assessed in the work. Generally, more females participated in the study at both study cente

rs which probably could be attributed to the increased susceptibility of females to STIs. In the study center representing the northern region (JUTH), it was discovered that a higher percentage (54.2%) of the participants from the study sites were relatively adolescents and young adults. Despite the young age bracket, 84.4% of the respondents are married and sexually active. This is in contrast to the respondents at LUTH, where the larger number belonged to 31 - 40 years of age with 79.3% being married. The higher number observed in JUTH could be as a result of the cultural and religious ethics of early marriage that is peculiar to the northern region.

Most of the subjects used in this study (from both study sites) also had their first sexual contacts between the age of 16 and 20 years. It was observed that over 80% of the participants at both study sites had just one sexual partner. The low number of multiple sexual partners could be significant in reducing the spread of STIs or mitigate the chances of contracting it. However, despite the high number of mono sexual partners, there was a relatively high report of previous cases of STI in this research. From the northern study center, 87% of the participants had at one time been diagnosed of STI while 59.8% followed suit at the southern study site. The use of condoms for safe sex practice as well as the use of IUD for birth control was low at JUTH; more participants used condom for safe sex practice at LUTH but the use of IUD was also relatively low. The high percentage of STI history especially in the northern region could be attributed to the low practice of safe sex which increases chances of contracting sexually transmitted infections.

The overall prevalence of genital mycoplasma is 27.9% (11.6% for LUTH; 16.3% for JUTH). It was

also observed that mycoplasma infection was more pronounced in females than in males in both study centers. Prevalence of genital mycoplasma was higher in females (20.3%) than in males (7.6%). This suggests that females are more susceptible to colonization or infection by mycoplasma or they are more symptomatic than males. However, there have been reports of higher prevalence from Ibadan (35.7%) (Agbakoba *et al.,* 2008) and northern Nigeria (32.5%) (Jombo *et al.,* 2009). The lower prevalence recorded in this study could be as a result of the population used could be as a result of single sexual partners which was highly reported by the respondents. %). The rate of isolation of *Ureaplasma* sp. was shown to be higher in patients (43.5%) with identified sexual risk behaviors than in asymptomatic controls (8.6%). This current study has also confirmed the presence of genital mycoplasma in Nigerians suffering from obstetrics and gynecological problems, utilizing the facilities at JUTH and LUTH, Nigeria. Our overall findings demonstrated a strong association between certain risk factors and detection of mycoplasmas, suggesting that infections could become endemic if control measures are not put in place.

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