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Serocarriage of *Ureaplasma Urealyticum* in Male of Various Age Ranges and Occupation in Nnewi, Nnewi North Local Government Area, Anambra State, Nigeria

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Abstract

Introduction: Awareness about *Ureaplasma urealyticum* is not too poor in this part of the country especially among male populace. Male infertility is increasing in this part of the country and has been proved to be up to 30% cause of infertility among couples. Lack of awareness on the causes of increase of prostatitis and other related diseases has paved a way to higher incidence of this infection. Due to the fact that this infection is mostly asymptomatic, it is therefore recommended that it should be tested at least once a year.

Aim: A study to determine the serocarriage of *Ureaplasma urealyticum* in male of various age ranges and profession in Nnewi was conducted in a total of 93 subjects.

Method: *Ureaplasma urealyticum* antigen was analysed using ELISA method with a commensally prepared kit (UUAB kit). Using internal built control. All method was as instructed in the user's manual.

Result: Results revealed that out of the 93 male in Nnewi (single and married) that were sampled 3(3.2%) showed a positive result to *Ureaplasma urealyticum*, while 90(96.8%) blood sample showed a negative result to *Ureaplasma urealyticum*. The highest prevalence of subject was within the age range of (18-24, 25-29 and 42-47) with a percentage of 33.3% respectively, there were no marked statistical differences. There were about seven (7) professions found within the area of study but the highest prevalence were traders with a percentage of 100%, there was no marked statistical difference. The highest prevalence of subject within the marital status are those married, with a percentage of 66.7% and there was no marked statistical difference. The subject with the number of children within the range of 2 and none were the highest serocarriage of this organism with a percentage of 66.7% and 1.1% respectively and there was a marked statistical difference of 0.02. Six states were

found within the area of study but the highest prevalence were Enugu and Anambra with the percentage of 66.7% and 33.3% with no statistical differences.

Conclusion: Serocarriage of *Ureaplasma urealyticum* can be said to be of low incidence probably because of the area of study and indiscriminate use of antibiotics in the study area.

Keywords: Serocarriage; *Ureaplasma urealyticum*; Male infertility; Nigeria

Introduction

Ureaplasma urealyticum is a bacterium that is found in the urogenital tracts of humans. It is a common commensal which can be harmless and symptom-free but is associated with inflammation, premature spontaneous delivery, septicemia, meningitis and pneumonia in newborn infants and is gaining recognition as an important opportunistic pathogen during pregnancy and human non-gonococcal urethritis [1,2]. Men with this infection often experience fertility problems and can even be rendered infertile. *Ureaplasma urealyticum* is also found in males, but is less common [3]. *Ureaplasma urealyticum* can be transmitted in various ways, including directly by sexual transmission through direct contact between couples, vertically from mother to offspring, through hospital-acquired infections from transplanted tissues, or through unprotected sexual contact with an infected person, whether vaginal, oral or anal [4]. *Ureaplasma urealyticum* is similar to the Mycoplasmas and shares in similar cell structure [5]. This may not seem like a big deal but because most antibiotics that are used to treat bacterial infections usually attack the cell wall of the bacteria, this can make many forms of antibiotics ineffective against Mycoplasma and *Ureaplasma*.

On male, the infection can firstly endanger the mucosae of urethral canal, and then secondarily cause prostatitis, vesiculitis, Orchitis and Epididymitis, and the like, of which the symptoms will manifest themselves less or more seriously [6]. Nevertheless, *Ureaplasma urealyticum* can adhere to the head

or tail of sperm, directly weaken the viability, lower the quantity, lead to abnormality at comparatively high proportion, of germs, or even cause necrospermia and male infertility [7]. Men would typically have burning or pain when they urinate, they may also experience frequent urination, slow urine stream or difficulty emptying the bladder. Originally recognised as tiny (T)-strain mycoplasmas due to their small colony size, ureaplasmas were first isolated from human genital samples in 1954 and assigned to a new genus and species [8].

Aim

The study was done to assess the Serocarriage of *Ureaplasma urealyticum* in Male of Various Age Ranges and Profession in Nnewi, Anambra State.

Materials and Methods

Study area

The place of study was at Nnewi, Nnewi North Local Government Area, Anambra State.

Study population

The study population was consisting of males of age range of between 18-75 years living within Nnewi North as of time of study. Who may be married or singled.

Research design

This is a cross sectional area designed to determine the Serocarriage of *Ureaplasma urealyticum* In Males of Different Age Range and Profession in Nnewi, Anambra State.

Ethical consideration

The ethical approval for this research was being obtained from the faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus Ethical Committee.

Sampling technique

Random sample was being selected based on sex, age ranges and profession.

Sample collection

5 mL of blood sample will be aseptically collected based on methods described in Chesbrough [9]. Serum will be extracted and stored at -4°C and analyzed within 2 weeks.

Subject recruitment

A subject was being recruited based on age, sex and profession. Random selection method was being used for subject recruitment.

Sample analysis

Ureaplasma urealyticum antigen was being analysed using Elisa method with commensally prepared kit (UUAB kit) Internal built control was being used. All methods were being as instructed in the user's manual.

Specimen collection and preparation

- **Specimen collection:** No special patient's preparation required. Collected the specimen in accordance with the normal laboratory practice. Either fresh serum specimens was used with this assay. Blood collected by venipuncture was allowed to clot as early as possible as to avoid haemolysis of the RBC. Care was taken to ensure that the serum specimens are clear and not contained by microorganisms.
- Highly lipaemic, icteric, or haemolytic specimens was not used as they can give false results in the assay. Didn't heat inactive specimens. This can cause deterioration of the target analyte. Samples with visible microbial contaminations were never used.
- UU ELSIA was intended ONLY for testing of individual serum samples. Didn't use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
- Transportation and storage: Stored specimens at 2-8°C. Specimens not required for assaying within 3 days was stored frozen (-20°C or lower). Multiple freeze-thaw cycles was avoided. For shipment, samples was packaged and labelled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

Procedure

- All reagents was allowed to reach room temperature for 15 minutes before use
- The wash buffer was diluted at the rate of 1:40 dilution with distilled water before use.
- 2 drops (100µL) of sample diluents was add in the corresponding hole, 5µL Sample was added in the corresponding hole (Do not add in the blank hole). 1drop (50 µL) of the positive control and negative control was added to the positive control hole and negative control hole. The sample corresponding to the number of micro plate, each plate was provided with a negative control 2 holes, positive control 1 holes and blank control1 holes. Notes: A separate disposal pipette tip was used for specimen, Negative and Positive Control as to avoid cross-contamination
- Shaked gently to mix for 30s. Water bath with the sealing plate in 37°C for 20 minutes.
- At the end of the incubation, removed and discarded the plate cover. Took out, wash bufferwas added to each well for 20 seconds. Repeated 5 times and patted to dry. After the final washing cycle, turned the plate over onto blotting paper or clean towel and tapped it to remove any remainders.

- Respectively added HPR Conjugate 50µL (Didn't add in the blank hole)
- Water bathed with the sealing plate membrane sealing plate in 37°C for 10 minutes. Repeated the wash step for 5 times as in step 5
- Added substrate A (50µL) and substrate B 1 drop (30µL) (Didn't add in the blank hole). Water bathed with the sealing plate membrane sealing plate in 37°C for 10 minutes.
- Added 50 µL Stop Solution to each well (didn't add in the blank hole). Mixed gently by shaking, measure the results within 10 minutes. Microplate reader at 450nm wavelength is set at (suggested dual wavelength 450nm, each well was measured A value with blank well as zero).

Data analysis

Data was being analyzed using SPSS (Statistical package for Social Science Version 21). Simple Prevalence percentage and Chisquare analysis Correlation was being used and values set at 95% confidence interval, at (0.05) significant value.

Results

Table 1 shows the demographic characters of the study.

Table 1 showing the demographic characters of the study.

Variables	Frequency	Percentage (%)
Sex		
Male	93	100
Age		
18-23	53	57
24-29	10	10.8
30-35	17	18.3
36-41	9	9.7
42-47	4	4.3
Profession		
Farmer	1	1.1
Trader	45	48.4
Okada	42	45.2
Student	2	2.2
Security	2	2.2
Pastor	1	1.1
Marital status		
Single	70	75.3
Married	22	23.7
Polygamy	1	1.1
Number of children		

2	8	8.6
4	6	6.5
1	4	4.3
3	1	1.1
7	1	1.1
None	73	78.5
State of origin		
Anambra	66	71
Ebonyi	3	3.2
Enugu	16	17.2
Imo	4	4.3
Abia	3	3.2
Other	1	1.1

Age ranges 18-24, 25-29 and 42-47 recorded 33.3% each of those who tested positive. Pearson chi-square revealed that there was no significant relationship between age and *Ureaplasma urealyticum* test result ($p \geq 0.05$) (**Table 2**). This could be as a result of their lifestyle in the study area sampled. Their lifestyle may be at the pick of their youth age. The age ranges of those that are negative are 30-35, 36-41, whose percentage are (0.0% and 0.0%) respectively. The absence of positive status does not have any focus.

Table 2 Association of age with *Ureaplasma urealyticum* test result.

Age (Years)	<i>Ureaplasma urealyticum</i>		Total F%	χ^2	P-value
	Negative F%	Positive F%			
18-24	52 (57.8)	1 (33.3)	53 (57.0)	8.716	0.069
25-29	9 (10.0)	1 (33.3)	10 (10.8)		
30-35	17 (18.9)	0 (0.0)	17 (18.3)		
36-41	9 (10.0)	0 (0.0)	9 (9.7)		
42-47	3 (3.3)	1 (33.3)	4 (4.3)		
Total	90 (100)	3 (100)	93 (100)	-	-

100% of those who tested positive to the test are traders. Pearson chi-square revealed that there was no significant relationship between profession and *Ureaplasma urealyticum* test result ($p \geq 0.05$). This could be as a result that traders in their profession are exposed to travelling and meeting people daily and they are susceptible to being tempted. The lowest prevalence of negative which include farmers, students, security and pastors, then does not mean that they are not

susceptible due to the area sample were mainly traders (Table 3).

Table 3 Association of profession with *Ureaplasma urealyticum* test result.

Profession	<i>Ureaplasma urealyticum</i>			χ^2	P-value
	Negative F%	Positive F%	Total F%		
Farmer	1 (1.1)	0 (0.0)	1 (1.1)	3.307	0.653
Trader	42 (46.7)	3 (100)	45 (48.4)		
Okada	42 (46.7)	0 (0.0)	42 (45.2)		
Student	2 (2.2)	0 (0.0)	2 (2.2)		
Security	2 (2.2)	0 (0.0)	2 (2.2)		
Pastor	1 (1.1)	0 (0.0)	1 (1.1)		
Total	90 (100)	3 (100)	93 (100)		

66.7% of correspondent who tested positive are married while 33.3% are single. Pearson chi-square revealed that there was no significant relationship between marital status and *Ureaplasma urealyticum* test result ($p \geq 0.05$). This could be as a result that married people are more susceptible because marital status has always been known to be a factor due to the high of chance of sexual intercourse among couple and the possibility of promiscuity among couple and because infertility is higher. The prevalence of those single are (33.3%). From polygamous the frequency of sampling is very low (Table 4).

Table 4 Association of marital status with *Ureaplasma urealyticum* test result.

Marital status	<i>Ureaplasma urealyticum</i>			χ^2	P-value
	Negative F%	Positive F%	Total F%		
Single	69 (76.7)	1 (33.3)	70 (75.3)	3.182	0.204
Married	20 (22.2)	2 (66.7)	22 (23.7)		
Polygamy	1 (1.1)	0 (0.0)	1 (1.1)		
Total	90 (100)	3 (100)	93 (100)		

Pearson chi-square revealed that there was significant relationship between number of children and *Ureaplasma urealyticum* test result ($p \geq 0.05$). The number of children that are positive to *Ureaplasma urealyticum* are 3 with the percentage of (66.7 %). Number of children does not follow the rule that the higher the number of children, the higher the chance. The higher incidence in the number of children was a marked significant difference among them (Table 5).

Pearson chi-square revealed that there was no significant relationship between state of origin and *Ureaplasma*

urealyticum test result ($p \geq 0.05$). There was high prevalence of *Ureaplasma urealyticum* in subject from Enugu state with the percentage of 33.3%. This can be as a result of the fact that diseases has been known to have genetic predisposition in relation to tribe despite the fact that Anambra has the higher sample size but the higher prevalence of positive was from Enugu due to genetic factors. That is, there may be a tendency of having the infection (Table 6).

Table 5 Association of number of children with *Ureaplasma urealyticum* test result.

Number of children	<i>Ureaplasma urealyticum</i>			χ^2	P-value
	Negative F%	Positive F%	Total F%		
2	6 (6.7)	2 (66.7)	8 (8.6)	13.355	0.02
4	6 (6.7)	0 (0.0)	6 (6.5)		
1	4 (4.4)	0 (0.0)	4 (4.3)		
3	1 (1.1)	0 (0.0)	1 (1.1)		
7	1 (1.1)	0 (0.0)	1 (1.1)		
None	72 (80)	1(1.1)	73 (78.5)		
Total	90 (100)	3 (100)	93 (100)		

Table 6 Association of state of origin with *Ureaplasma urealyticum* test result.

State of origin	<i>Ureaplasma urealyticum</i>			χ^2	P-value
	Negative F%	Positive F%	Total F%		
Anambra	65 (72.2)	1 (33.3)	66 (71.0)	5.394	0.37
Ebonyi	3 (3.3)	0 (0)	3 (3.2)		
Enugu	14 (15.6)	2 (66.7)	16 (17.2)		
Imo	4 (4.4)	0 (0.0)	4 (4.3)		
Abia	3 (3.3)	0 (0.0)	3 (3.2)		
Other	1 (1.1)	0 (0.0)	1 (1.1)		
Total	90 (100)	3 (100)	93 (100)	-	-

Discussion

The study showed that the highest age range positive to *Ureaplasma urealyticum* were 18-24, 25-29, 42-47 years with the percentage of 33.3%, 33.3%, 33.2% respectively. This could be as a result of their lifestyle in the study area sampled. Their lifestyle may be at the pick of their youth age. The age ranges of those that are negative are 30-35, 36-41, whose percentage are (0.0% and 0.0%) respectively. The absence of positive status does not have any focus. That is it cannot be explained, because all age ranges have been implicated in the possibility of being infected and susceptible to this infection which is related to similar work done by [10], and there was no marked statistical differences ($p > 0.05$)

It also showed that the highest prevalence of positive was obtained in traders whose percentage was (100%). This could be as a result that traders in their profession are exposed to travelling and meeting people daily and they are susceptible to being tempted. The lowest prevalence of negative which include farmers, students, security and pastors, then does not mean that they are not susceptible due to the area of sampling were mainly traders opposite to the similar work done by Shepard et al. [7]. And there was no marked statistical difference ($p>0.05$).

The prevalence of positive in marital status are those married with the percentage of (66.7%), which could be as a result that married people are more susceptible because marital status has always been known to be a factor due to the high of chance of sexual intercourse among couple and the possibility of promiscuity among couple and because infertility is higher which is opposite to a similar work done by [11]. The prevalence of those single are (33.3%). From polygamous the frequency of sampling is very low. And there was no statistical difference ($p>0.05$).

The numbers of children that are positive to *Ureaplasma urealyticum* were 3 with the percentage of (66.7 %). Number of children does not follow the rule that the higher the number of children, the higher the chance. The higher incidence in the number of children was a marked significant difference among them. There was a marked statistical difference ($p>0.05$).

There was a high prevalence of *Ureaplasma urealyticum* in subject from Enugu state with the percentage of 33.3%. This can be as a result of the fact that diseases has been known to have genetic predisposition in relation to tribe despite the fact that Anambra has the higher sample size but the higher prevalence of positive was from Enugu due to genetic factors. That is, there may be a tendency of having the infection in related to similar work done by [12] and there was no marked statistical difference ($p>0.05$).

Conclusion

Conclusively, it was discovered that the serocarriage of *Ureaplasma urealyticum* can be said to be of low incidence probably because of the area of study and indiscriminate use of antibiotics in the area of study and those that are sexually active are not in high tendency of having the infection. *Ureaplasma* can be effectively treated and cured with a course

of antibiotics. If one is suffering any symptoms, it is important to provide a urine sample for testing by a pathology laboratory.

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