

Ureaplasma Parvum and Urealyticum Prevalence in Symptomatic and Asymptomatic Reproductive aged Gambian Women Seeking Primary Health Care: A Case - Control Study

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Submitted: 03 September 2024 Accepted: 09 September 2024 Published: 16 September 2024

doi <https://doi.org/10.63620/MKJSHAR.2024.1009>

Citation: Bah, H., Ceesay, F., Bojang, K., Banja, F., Mboob, K. S., Barrow, E., & Kimmitt, P. T. (2024). Ureaplasma Parvum and Urealyticum Prevalence in Symptomatic and Asymptomatic Reproductive aged Gambian Women Seeking Primary Health Care: A Case - Control Study. *Journal of Sexual heal and Aids Res*, 1(5), 01-09.

Abstract

Background: Sexually transmitted infections (STIs) are a major public health concern, as most of these infections are asymptomatic in women, potentially leading to adverse reproductive health. The aim of this study was to determine the prevalence of urogenital *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma genitalium*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Neisseria gonorrhoeae* in reproductive-aged Gambian women attending Edward Francis Small Teaching Hospital (EFSTH), the main referral hospital for The Gambia.

Method: A total of 232 reproductive women aged 20 – 49 years were recruited in a case-control study (115 symptomatic and 117 asymptomatic). Both vaginal and endocervical swabs were collected. Nucleic acid was extracted and tested by real-time PCR for *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma genitalium*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*. Microbiological analysis was carried out for *Trichomonas vaginalis* and *Neisseria gonorrhoea*. A questionnaire was administered to assess risk factors that may be associated with *Ureaplasma* infection.

Results: The overall prevalence of *Ureaplasma* was 46%. The distribution in symptomatic and asymptomatic participants was as follows: *Ureaplasma parvum* (38% symptomatic, 52% asymptomatic); *Ureaplasma urealyticum* (4% symptomatic, 14% asymptomatic); *Neisseria gonorrhoeae* (6% symptomatic, 4% asymptomatic); and *Trichomonas vaginalis* (4% symptomatic, 3% asymptomatic). Eighty percent of participants' partners had never used a condom during sexual intercourse. Early sexual debut and new sexual partners were found to be statistically associated with *Ureaplasma* infection ($p \leq 0.05$).

Conclusion: This is the first study to highlight the burden of *Ureaplasma* infection in Gambian women of reproductive age. The high prevalence observed in this pilot study requires further investigation to determine its association with adverse reproductive outcomes and the need for enhanced surveillance for *Ureaplasma* in countries where there is limited data on prevalence.

Keywords: Ureaplasma Parvum, Ureaplasma Urealyticum, Infertility, Risk Factors, STI, Trichomonas Vaginalis, Chlamydia, Female Genital Mutilation, Neisseria Gonorrhoeae.

Background

Sexually transmitted infection (STI) is one of the most important public health challenges, with approximately one million new cases reported every day. Globally [1]. The burden of STIs is important in developing countries, where access to primary screening is a challenge. Infants born to mothers with untreated STIs can be infected through vertical transmission, resulting in potentially serious neonatal health complications such as neonatal sepsis, low birth weight, pneumonia, prematurity, congenital deformities, and neonatal conjunctivitis [2, 3].

Ureaplasma species and Mycoplasma genitalium belong to the class Mollicutes and are known to be sexually transmitted in males and females. During infection, these attach to the mucosal surfaces of the urogenital tracts, where colonisation occurs, resulting in ascending infections. Mycoplasma genitalium has been identified as a 'true' STI pathogen and is associated with urethritis in males. In females, infection with Mycoplasma genitalium can cause infertility, salpingitis, and an increased risk of pelvic inflammatory diseases (PIDs) [4, 5]. However, Ureaplasma is found in the urogenital tracts of 40-80% of asymptomatic individuals, which may later result in ascending symptomatic infections [6]. Ureaplasma colonisation has been associated with increased sexual activity, younger age, multiple sexual partners, and the use of hormone contraceptives [7].

Although colonisation with Ureaplasma is common, there is evidence of an association with a variety of clinical outcomes, such as PID, cervicitis, infertility and chorioamnionitis, in women [7, 8]. Two Ureaplasma species have been shown to be associated with STIs, Ureaplasma parvum (U. parvum) and Ureaplasma urealyticum (U. urealyticum) [9]. Ureaplasma parvum includes serotypes (1, 3, 6 and 14), while U. urealyticum includes serotypes [2, 4, 7-13].

In the Gambia, there is limited laboratory-based evidence of the overall prevalence of STIs. Recently, a study to determine the prevalence of STIs among pregnant women attending antenatal care (ANC) clinics in the West Coast Region of The Gambia showed a prevalence of 53.6%. The most common pathogenic agents isolated were Candida albicans (31.8%), Streptococcus agalactiae (15.0%), Treponema pallidum (6.8%), HIV (5.7%), Trichomonas vaginalis (3.9%), Neisseria gonorrhoea (1.8%) and Chlamydia trachomatis (0.7%) [10].

Suspected STI cases are usually treated using the syndromic management approach. This approach can result in overtreatment and missed treatment, as the majority of STIs are asymptomatic [11]. Furthermore, there is no laboratory based STI surveillance evidence in place. Therefore, it is important to characterise risk factors that are associated with STIs at a national level for improved and more effective intervention programmes.

The characterisation of Ureaplasma species in reproductive-aged Gambian women and the risk factors for infection may help in the management of women with STIs and infertility.

Material and Methods

Study Location, Population, and Design

This pilot study was nested on an HPV case-control study conducted at the EFSTH. Reproductive aged women (20 – 49 years

old) seeking primary health care who met the inclusion criteria were recruited consecutively by trained health care providers. Women with symptoms of STIs were recruited into the case group. Asymptomatic women attending the contraceptive (family planning) clinic were recruited as a control group. The minimum sample size was calculated using a simple Daniel formula [12]:

$$n = \frac{Z^2 P (1 - P)}{d^2}$$

Inclusion Criteria

The inclusion criteria for cases included the presence of one or more of the following: vaginitis/cervicitis, dysuria (painful and burning urination), lower abdominal pain, bleeding between periods, dyspareunia (pain during sexual intercourse), and vaginal itching/discharge with a fishy or strong odour. The inclusion criteria for controls were asymptomatic.

Consent to Participate in the Study

Informed consent was obtained from all participants who met the inclusion criteria, and a participant information sheet was provided for them.

Exclusion Criteria

Participants were excluded from the study if they lived outside the study area, were pregnant or were on their menstrual cycle at the time of sampling, had reached menopause or were above 49 years old.

Ethical Statement

The study was reviewed and approved by The Gambian Government and Medical Research Council Joint Ethics Committee, The Gambia and the University of Westminster Research Ethics Committee, London.

Sample Collection

A total of two hundred and thirty-two (232) women were recruited consecutively (115 symptomatic and 117 asymptomatic). Endocervical and high vaginal swabs were collected from each participant prior to treatment using the syndromic management treatment guidelines.

The swabs for PCR amplification for Ureaplasma, Mycoplasma genitalium, Chlamydia trachomatis and Neisseria gonorrhoeae were placed immediately into specimen transport media (M4RT, Microtest, Oxoid, Basingstoke, UK). A questionnaire was administered to capture behavioural risk factors that might be associated with STIs in reproductive-aged Gambian women.

Methods

Bacterial Vaginosis and Trichomonas Vaginalis

Detection of BV and T. vaginalis was carried out at the clinic's site laboratory. High vaginal swabs were used for direct wet mount microscopy, detection of fishy amine odour ('Whiff' test) when mixed with 10% (w/v) potassium hydroxide (KOH) preparation and vaginal pH determination (range 4.0 – 7.0). The wet preparation was viewed microscopically for the presence of 'clue cells', yeast cells, and motile T. vaginalis. Bacterial vaginosis was diagnosed using Amsel's clinical criteria [13].

Neisseria Gonorrhoeae Isolation and Antibiotic susceptibility Testing

Routine microbiological detection of *Neisseria gonorrhoeae* was performed in the Department of Medical Microbiology, EFSTH, using standard operating procedures. Endocervical swabs were cultured on chocolate agar (Oxoid, Basingstoke, UK) and incubated overnight at 37°C in 5% carbon dioxide atmospheric conditions. Colonies of interest were subcultured and incubated overnight to generate pure colonies. These were selected for Gram staining and biochemical identification with the oxidase test for presumptive diagnosis of *Neisseria gonorrhoeae*. Suspected oxidase-positive *Neisseria gonorrhoeae* isolates were further confirmed using the analytical profile index test API NH (Biomerieux, Basingstoke, UK). *Neisseria gonorrhoeae* isolates were tested against the following selection of antibiotics: ciprofloxacin (CIP, 5 µg), cefotaxime (CTX, 30 µg), tetracycline (TE, 30 µg), penicillin (P, 10 units), and ceftriaxone (30 µg) using the disc diffusion method. Antibiotic susceptibility testing was interpreted using the European Committee on Antimicrobial Susceptibility Testing and British Society for Antimicrobial Chemotherapy guidelines [14, 15].

DNA Extraction

DNA was extracted from the endocervical and high vaginal swabs using the QI Aamp DNA mini extraction kit (Qiagen, Crawley, UK) following the manufacturer's instructions. Both endocervical and high vaginal swabs were used for *Ureaplasma parvum/urealyticum* and *Mycoplasma genitalium* PCR, while only endocervical swabs were used for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Real-time PCR and PCR

All real-time PCRs were performed with Rotorgene-Q (Qiagen, Crawley, UK) in a 20 µl reaction volume containing 0.25 µM Rotorgene probe mastermix kit (Qiagen, Crawley, UK), 1 µM of each primer (unless otherwise stated) and 50 ng -100 ng of DNA template. Two channel wavelengths of carboxyfluorescein (FAM) and 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE) were used to identify the specific species in a duplex real-time PCR. Black hole quencher-1 (BHQ-1) was used for the probes. All samples were tested in duplicate within one run and were considered positive if the cycle threshold (Ct) value was ≤ 35. All samples were tested in duplicate, and those that were not in accordance were repeated.

PCR amplifications were performed in a 25 µl volume containing 5 µM of each primer, 1x Taq PCR master mix containing 2.5 units of Taq DNA polymerase, 0.2 mM deoxynucleotide triphosphates and 1.5 mM MgCl₂ (Qiagen, Crawley, UK) and 50 – 200 ng of DNA template. Extraction control, negative control (molecular grade water) and positive controls (Genekam Biotechnology, Duisburg, Germany) were included in each run.

Detection of Ureaplasma Parvum, Ureaplasma Urealyticum, and Mycoplasma Genitalium

Real-time PCR was carried out to distinguish between the two *Ureaplasma* species, *U. parvum* (parvo) and *U. urealyticum* (T960). A common primer for the two species and two species-specific probes (Table S1) were used to amplify 90 base pairs (bp) of the urease gene. The 78 (bp) conserved region of the *M. genitalium* adhesion MgPa gene was targeted using a specific set of primers (Table S1). Thermal cycling and detection of fluorescent signals upon amplification of the specific target sequence were performed as previously described [16, 17].

PCR for the *M. genitalium* 16S rRNA gene was also carried out using the MG16-45 F and MG16-447 R primers (Table S1), which are in the V1 and V3 hypervariable regions, respectively. The amplification reaction consisted of 40 cycles and was carried out as follows: denaturation at 95°C for 30 seconds, annealing at 60°C for 60 seconds, elongation at 72°C for 30 seconds and a final extension step at 72°C for 5 minutes. Amplified products were resolved by gel electrophoresis using 2% (w/v) agarose gel (Sigma Aldrich, Haverhill, UK).

Detection of Chlamydia Trachomatis and Neisseria Gonorrhoeae

A duplex real-time PCR was carried out for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using dual-labelled probes (Table S1). In addition to the mastermix, 10 µmol/L of each primer and 10 µmol/L of each probe were added. Both thermal cycling and detection of fluorescent signals were performed as previously described [18].

Primers and Probes Used for Real-time PCR and PCR for the Detection of Sexually Transmitted Pathogens (Supplementary Table 1)

Key: UU-Ureaplasma, MG-Mycoplasma genitalium, CT-Chlamydia trachomatis, NG-Neisseria gonorrhoea, F-Forward primer, R-Reverse primer.

Supplementary Table 1

Primer and Probe name	Sequence	Amplicon size (bp)
Real time – PCR		90
UU – 1613 F	AAG GTC AAG GTA TGG AAG ATC CAA	
UU – 1524 R	TTC CTG TTG CCC CTC AGT CT	
UU-T960	JOE –ACC ACA AGC ACC TGC TAC GAT TTG TTC – BHQ1	
UU – Parvo	FAM – TCC ACA AGC TCC AGC AGC AAT TTG – BHQ1	78
MgPa – 355T	GAG AAA TAC CTT GAT GGT CAG CAA	
MgPa – 432R	GTT AAT ATC ATA TAA AGC TCT ACC GTT ATC	
MgPa – 380	FAM – ACT TTG CAA TCA GAA GGT – BHQ1	144
NG – F	AAC TGC CGG GCG TTT ATA TCG	

NG – R	GAC CTT CGA GCA GAC ATC ACG	
NG probe	JOE – ACC GAA GCC GCC AGA ATA GAG CAA CA – BHQ1	111
CT – F	GCC AAT CTT CTT TGA AGC GTT GTG	
CT – R	CTA GGG TGC TCA GAC TCC GAC	
CT probe	FAM –TG AAGGTCACAGGACACGCAACCCCA – BHQ1	
PCR Primers		
MG16 – 45 F	TAC ATG CAA GTC GAT CGG AAG TAG	427
MG16 – 447 R	AAA CTC CAG CCA TTG CCT GCT AG	

DNA Sequencing

PCR amplicons were purified using a PCR purification kit (Sigma Aldrich, Haverhill, UK) and then sequenced using the Sanger chain termination method. An NCBI BLAST [<http://www.ncbi.nlm.nih.gov/BLAST/>] search was performed for each sequenced product.

Statistical Analysis

Data analysis was performed using Epi Info version 7.0 [<https://www.cdc.gov/epiinfo>]. Descriptive analyses, including frequencies, percentages, and averages, were used when appropriate to describe the study population. A chi-square test was used to assess the association between exposures and outcomes expressed as categorical variables. A confidence interval of 95% (CI) and p value of ≤ 0.05 were used to determine statistical significance. The strength of risk factors associated with STIs as an outcome was measured using the odds ratio (OR). The ORs of the risk characteristics were interpreted as increased odds (>1.0) or decreased odds (<1.0).

Results

Prevalence of Ureaplasma Parvum and Urealyticum amongst Symptomatic and Asymptomatic Women

Urogenital Ureaplasma was detected in 46% (107/232) of participants; 41% (47/115) were symptomatic (case group), and 51% (60/117) were asymptomatic (control group). Of the 47 symptomatic participants, 89% (42/47) reported vaginitis/cervicitis and vaginal discharge, 63% (30/47) reported lower abdominal pain, 53% (25/47) reported dysuria and 51% (24/47) reported dyspareunia.

Ureaplasma parvum was identified in 79 participants, 48% (38/79) of whom were symptomatic (case group) and 52% (41/79) of whom were asymptomatic (control group) (Table 1). Ureaplasma urealyticum was detected in 25 participants, with 68% (17/25) identified from the asymptomatic group, while three participants were infected with both species (Table 1).

No statistically significant difference was observed between the incidence of Ureaplasma distribution among the symptomatic and asymptomatic groups ($p > 0.05$).

Table 1: Distribution of Urogenital Ureaplasma in Symptomatic and Asymptomatic Women Attending Clinics at Edward Francis Small Teaching Hospital, Gambia

Pathogen	Symptomatic n = 115		Asymptomatic n = 117		Overall Distribution (n= 232)	
	N	%	N	%	N	%
U. parvum	38	33.0	41	35.0	79	34.1
U. urealyticum	8	7.0	17	14.5	25	10.8
U. parvum & U. urealyticum	1	0.9	2	1.7	3	1.3
Total	47	40.9	60	51.2	107	46.2

Distribution of Ureaplasma Serotypes in Study Participants

Ureaplasma parvum serotype 3 was the most frequently detected type in both endocervical and high vaginal samples, followed by serotype 1 (Table 2). In addition, eight (8) participants were coinfecting with different U. parvum serotypes.

In contrast, all the samples positive for U. urealyticum were identified as serotype 10. Furthermore, 61% (65/107) were identified to harbour Ureaplasma species in both the vagina and cervix, 28% (30/107) had vaginal colonisation, and 11% (12/107) had cervical infection (Table 2).

Table 2: Total number of participants infected or colonised with Ureaplasma either in the cervix, vagina, or both.

Anatomical sites	1	3	1/3/6/14	1/6/14	10
Cervix	2	5	1	1	3
Vagina	5	17	1	1	6
Both*	9	36	1	3	16
Total participants	16	58	3	5	25

Prevalence of Ureaplasma Parvum and Urealyticum among the Different Age Groups

The distribution of Ureaplasma parvum among the different age groups showed that it was more frequent in the age range of 26 - 35 years in both study groups (Table 3). However, Ureaplasma urealyticum was more prevalent (35%) in the 21- to 25-year-old

group in the asymptomatic group than in the symptomatic group, which showed a prevalence of 25% in the 21- to 25-year-old and older age groups (Table 3). A pairwise statistical comparison showed no statistically significant differences among the age groups ($p > 0.05$) with Ureaplasma infection.

Table 3: Prevalence of Ureaplasma parvum and urealyticum in the Different Age Groups of Symptomatic (case group) and Asymptomatic (control group) Participants

	Symptomatic group	Asymptomatic group
Ureaplasma species	n (%)	n (%)
U. parvum (n=79)		
Total positive	38(48.1)	41(51.9)
Age group		
20 years	1 (2.6)	0 (0.0)
21 – 25	6 (15.8)	7 (17.1)
26 – 30	10 (26.3)	12 (29.3)
31 – 35	10 (26.3)	9 (21.9)
36 – 40	2 (23.7)	8 (19.5)
41 – 49	9 (23.7)	5 (12.2)
U. urealyticum (n=25)		
Total positive	8(32.0)	17(68.0)
Age group		
20years	1 12.5	1 (5.9)
21 – 25	2 (25.0)	6 (35.3)
26 – 30	1 (12.5)	3 (17.6)
31 – 35	2 (25.0)	4 (23.5)
36 – 40	2 (25.0)	2 (11.8)
41 – 49	0 (0.0)	1 (5.9)

Distribution of other sexually Transmitted Pathogens amongst Symptomatic and Asymptomatic Participants

Neisseria gonorrhoeae was detected in 6% (7/115) of symptomatic (case) and 4% (5/117 asymptomatic (control) participants. Three cervical samples from asymptomatic participants were N. gonorrhoeae culture-negative but PCR-positive. Ciprofloxacin resistance was observed in 44% (4/9) of culture-positive N. gonorrhoeae isolates. Two isolates (2/9, 22%) of N. gonorrhoeae were found to be resistant to both ceftriaxone and cefotaxime but sensitive to ciprofloxacin. Nine (9) participants (5 symptomatic) and 4 (asymptomatic) were coinfectd with Trichomonas

vaginalis, and 23 (16 symptomatic) and 7 (asymptomatic) tested positive for bacterial vaginosis. Chlamydia trachomatis and Mycoplasma genitalium were not detected in any of the samples from either study group.

Behavioural Risk Characteristics of Participants

Respondents identified risk variables that could enhance their risks of contracting and transmitting STIs. Although a majority of respondents (61%, 143/232) case group and (72%. 167/232) control group had their first sexual encounter at the age of 18 years or older, a small number, (8.7%, 10/115) of the case group

Table 2 footnote: Three participants were identified to harbour both serotypes 3 and 10 in the cervix and vagina. Twenty-four participants were colonised with U. parvum in the vagina. Multiple colonisations with different U. parvum serotypes were also observed in eight participants. Six participants were colonised with U. urealyticum, serotype 10. The U. parvum serotypes detected were serotypes 1, 3, 1/3/6/14 and 1/6/14. Legend: Both*= cervix and vagina

and (5.1%, 6/117) of the control group reported having their first sexual encounter below 14 years of age due to early marriage. One respondent in the case group stated that they had their first sexual encounter at the age of 9 years due to sexual abuse (Table 4). Furthermore, 78% of respondents reported having greater than two lifetime sexual partners, with 17% claiming to have had one or more new sexual partners in the previous 12 months. Sixty percent (60%) of women who had only one sexual partner in their lives reported that their partners had other sexual partners.

The data on respondents' attitudes toward safer sex practices showed that more than 80% (189/229) of their partners had

never worn a condom during sexual intercourse in the previous 12 months. Female genital mutilation (FGM), which is a known traditional practice in the Gambia and other parts of Africa, was carried out on 73% (84/115) of women from the case group and 52% (61/117) from the control group (Table 4).

A bivariate analysis on possible risk factors that may be associated with Ureaplasma infection was carried out on ages 26-35 years, as Ureaplasma was more prevalent in this age range. Only early sexual encounter (< 18 years) and a new sexual partner in the previous 12 months were found to be statistically associated with Ureaplasma infection ($p \leq 0.05$) (Table 5).

Table 4: Behavioural Characteristics of Participants

Variables	Case n (%)	Control n (%)
Age at first sexual encounter**		
<18	43 (38.39)	32 (27.35)
≥18	69 (61.61)	85 (72.65)
Number of lifetime sexual partner (s)		
1	60 (53.57)	78 (66.67)
≥2	52 (46.43)	39 (33.33)
Partner (s) had sex with others*		
Yes	36 (26.67)	48 (60.00)
No	8 (13.33)	20 (25.00)
Don't know/not sure	16 (33.33)	12 (15.00)
Sexual intercourse in the previous 12 months		
Yes	95 (84.81)	116 (99.15)
No	17 (15.18)	1 (0.85)
New Sexual partner (s) in the previous 12 months		
Yes	21 (22.10)	18 (15.65)
No	74 (77.89)	97 (84.35)
Condom use in the previous 12 months.		
Yes	15 (15.79)	7 (6.03)
No	80 (84.21)	109 (93.97)
Female genital mutilation		
Yes	84 (73.04)	61 (52.14)
No	31 (26.96)	56 (47.86)
Smoking		
Yes/Quit	3 (2.61)	
No	112 (97.39)	117 (100.00)
Hormone contraceptive use		
Yes	53 (46.09)	117 (100.00)
No	47 (40.87)	
Yes, but stopped using them	15 (13.04)	
History of Pregnancy		
Yes	93 (80.87)	114 (97.44)
No	22 (19.13)	3 (2.56)

Table 5: Selected Risk Variables in 26 – 35-year-old Participants Infected with UREAPLASMA (n = 52)

Variables	Ureaplasma n (%)	95% CI	P - value
Hormone contraceptive used	39 (75)	1.5 (0.8,2.8)	0.2
≥5 years pregnancies	17 (32)	1.3 (0.5, 3.0)	0.6
First sexual encounter (<18 years old)	26 (50)	0.5 (0.3, 0.9)	0.05*
New sexual partners (previous 12 months)	9 (17)	0.3 (0.1, 1.0)	0.05*
Condom used (previous 12 months)	7 (13)	1.3 (0.5, 3.3)	0.6
FGM	37 (71)	0.8 (0.5, 1.4)	0.7

Discussion

Difficulties in screening for STIs mean there is limited information on the burden of *Ureaplasma* as an STI, especially in resource-limited countries. This is the first observational study of *Ureaplasma* in reproductive-aged Gambian women. A previous study using culture did not detect *Ureaplasma* [19]. This indicates a previously hidden burden of *Ureaplasma* and highlights the importance of PCR-based assays in the detection of this pathogen and the importance of ongoing surveillance for pathogens associated with STIs, including *Ureaplasma*. *Ureaplasma parvum* serotype 3 was the most prevalent serotype in both study groups (Table 3). This is consistent with other studies carried out in reproductive-aged women in both developed and developing countries [19-21]. The high burden of serotype 3 in both endocervical and high vaginal swabs observed in this study is a concern as habitual and spontaneous abortions have been associated with this serotype [22].

An important public health concern found in this study is the high cervical colonisation of *Ureaplasma* species observed in asymptomatic participants, which without laboratory screening may put them at risk of developing ascending infections that can result in infertility [23]. In this study, 72% (77/107) of women infected with *Ureaplasma* were identified to be using hormone contraceptives, which increased the odds of being infected with the organism (OR = 1.5; $p=0.2$) (Table 5). Moreover, 51% of the younger age group (21 – 35 years old) reported using contraceptives, which may increase the vaginal epithelial glycogen content due to the high oestrogen level. This can enhance lactic acid production and reduce the vaginal pH, therefore promoting *Ureaplasma* vaginal colonisation [24, 25].

A higher *Ureaplasma* prevalence was observed within the ages 26 - 35 years, and a prevalence decrease was seen in the 36-year-old and above participants (Table 3). In contrast to this finding, *Ureaplasma* has been reported to be more prevalent in the 21 - 25 years age group [20, 23]. The differences noted could be attributed to the fact that Gambian women may be more sexually active between the ages of 26 - 35 years of age compared to other countries, where sexual activity is reported more frequently in the under 26 age group [24, 25]. A decrease in the prevalence of *Ureaplasma* between the ages of 36 and 49 years observed in this study could possibly be due to either reduced sexual activity in the older age groups, as the frequency of sexual activities has been reported to be associated with *Urea plasma* colonisation [7].

Chlamydia trachomatis and *Mycoplasma genitalium* were not detected in any of the screened samples. This finding is inconsistent with Demba et al. (2005), who found an overall prevalence

of chlamydial infection of 15% (34/227) in symptomatic Gambian women. Both assays used targeted the chlamydial cryptic plasmid gene; therefore, the inconsistency seen in the two studies needs further investigation [19]. However, a recent Gambian study carried out in antenatal showed chlamydia prevalence to be 0.7% indicating that Chlamydial infection might not be an issue in the Gambia compared to England where it is reported to have increased to almost 25% from 2021 to 2022 from all ages [26].

Forty-four percent (44%) and 22% of *N. gonorrhoeae* isolates were found to be resistant to ciprofloxacin and cefotaxime & ceftriaxone, respectively. This further strengthens the need for a laboratory-based investigation in STI management and antibiotic stewardship to limit antibiotic resistance and treatment failure.

Conclusion

The 51% prevalence of asymptomatic *Ureaplasma* and the lack of a significant difference observed in the distribution of *Ureaplasma* between the two study groups make it difficult to associate significant clinical outcomes with *Ureaplasma* infection. A further study to characterise this organism in investigating the cause of infertility and adverse reproductive health, such as spontaneous or habitual abortions in Gambian women, is recommended. This study shows a hidden burden of *Ureaplasma* in Gambian women and the need to have an STI surveillance in place to monitor the STI pathogens and new emerging organisms in the country.

Study Limitations

The study was a hospital-based study carried out in only one region of The Gambia, and therefore, the results may not be representative for the entire country. However, the strength of this study lies in the fact that it serves as a source of current data on *Ureaplasma* and the need for a multicentre study to further explore issues of STI in the general population.

Declaration

Human Ethics Approval and Consent to Participate

Ethical approval of the study was granted by The Gambian Government and Medical Research Council Joint Ethics Committee, Gambia and the University of Westminster Research Ethics Committee, London. R015 002v3 and VRE 1415 – 0262, respectively. The objectives and procedures of the study were carefully explained to all potential participants seeking primary health care at the EFSTH polyclinic in their preferred language to seek their informed consent to participate. Participants' identities and records were anonymized prior to analysis. The results were made available to the participants for further management by the attending health care provider.

Consent for Publication

Not Applicable

Conflict of Interest

The authors declare no competing interest.

Availability of Data and Materials

Datasets generated during this study and for this manuscript are available from the corresponding author upon request.

Funding

The study was funded by The Gambian Government.

Authors' Contributions

HB, PTK, and KB designed the study; FB, FC, EB, and KSM collected the data; FC and HB produced the draft manuscript. KB and PTK supervised the work and review and edited the first draft. All authors critically reviewed the manuscript and made significant input. All the authors have read and approved the final version.

Acknowledgement

We would like to thank the staff of the reproductive health clinic, EFSTH, Department of Laboratory Medicine and the women who participated in this research.

Reference

1. World Health Organization. (2016). Global Health Sector Strategy on Sexually Transmitted Infections (2016-2021). Retrieved from <http://www.who.int/reproductivehealth/publications/rtis/ghss-stis/en/>
2. World Health Organization. (2019). Sexually Transmitted Infections (STI). Retrieved from [[https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-\(stis\)](https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis))]([https://www.google.com/search?q=https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-\(stis\)](https://www.google.com/search?q=https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis)))
3. National Institutes of Health. (2017). How do sexually transmitted diseases and sexually transmitted infections (STDs/STIs) affect pregnancy? Retrieved from <https://www.nichd.nih.gov/health/topics/stds/conditioninfo/infant>
4. Unemo, M., & Jensen, J. S. (2017). Antimicrobial-resistant sexually transmitted infections: gonorrhoea and Mycoplasma genitalium. *Nature Reviews Urology*, 14(3), 139-152.
5. Kim, S. J., Lee, D. S., & Lee, S. J. (2011). The prevalence and clinical significance of urethritis and cervicitis in asymptomatic people by use of multiplex polymerase chain reaction. *Korean Journal of Urology*, 52(10), 703-708.
6. Povlsen, K., Bjornelius, E., Lidbrink, P., & Lind, I. (2002). Relationship of *Ureaplasma urealyticum* biovar 2 to non-gonococcal urethritis. *European Journal of Clinical Microbiology and Infectious Diseases*, 21(2), 97-101.
7. Waites, K. B., Katz, B., & Schelonka, R. L. (2005). Mycoplasmas and Ureaplasmas as Neonatal pathogens. *Clinical Microbiology Reviews*, 18(4), 757-789.
8. Yamazaki, T., Matsumoto, M., Matsuo, J., Arakawa, K., Minami, K., & Suto, Y. (2012). Frequency of Chlamydia trachomatis in *Ureaplasma*-positive healthy women attending their first prenatal visit in a community hospital in Sapporo, Japan. *BMC Infectious Diseases*, 12(1), 82.
9. Paralanov, V., Lu, J., Duffy, L. B., Crabb, D. M., Shrivastava, S., & др. (2012). Comparative genome analysis of 19 *Ureaplasma urealyticum* and *Ureaplasma parvum* strains. *BMC Microbiology*, 12(1), 147.
10. Isara, A., & Baldeh, A. K. (2021). Prevalence of sexually transmitted infections among pregnant women attending antenatal clinics in West Coast Region of The Gambia. *African Health Sciences*, 21(2), 585-592.
11. Maina, A. N., Mureithi, M. W., Ndemi, J. K., & Revathi, G. (2021). Diagnostic accuracy of the syndromic management of four STIs among individuals seeking treatment at a health centre in Nairobi, Kenya: a cross-sectional study. *Pan African Medical Journal*, 40(1), 138.
12. Daniel, W. W. (1978). *Biostatistics: a foundation for analysis in the health sciences*. Wiley.
13. Amsel, R., Totten, P. A., Spiegel, C. A., Eschenbach, D., & Holmes, K. K. (1983). Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *American Journal of Medicine*, 74(1), 14-22.
14. The European Committee on Antimicrobial Susceptibility Testing. (2015). Breakpoint tables for interpretation of MICs and zone diameters (Version 5.0). Retrieved from <http://www.eucast.org>
15. British Society for Antimicrobial Chemotherapy. (2015). BSAC Methods for Antimicrobial Susceptibility Testing. Retrieved from <http://bsac.org.uk/wp-content/uploads/2012/02/BSAC-disc-susceptibility-testing-method-Jan-2015.pdf>
16. Yi, J., Yoon, B. H., & Kim, E. C. (2005). Detection and biovar discrimination of *Ureaplasma urealyticum* by real-time PCR. *Molecular and Cellular Probes*, 19(4), 255-260.
17. Jensen, J. S., Björnelius, E., Dohn, B., & Lidbrink, P. (2004). Use of TaqMan 5' nuclease real-time PCR for quantitative detection of *Mycoplasma genitalium* DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. *Journal of Clinical Microbiology*, 42(2), 683-692.
18. Kimmitt, P. T., Tabrizi, S. N., Crosatti, M., & Lidbrink, P. (2010). Pilot study of the utility and acceptability of tampon sampling for the diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections by duplex real-time polymerase chain reaction in United Kingdom sex workers. *International Journal of STD & AIDS*, 21(4), 279-282.
19. Domingues, D., Távora, L. T., Duarte, A., Sanca, A., Prieto, E., Gomes, V., & Azevedo, J. (2002). *Ureaplasma urealyticum* biovar determination in women attending a family planning clinic in Guiné-Bissau, Using Polymerase Chain Reaction of the Multiple-Banded Antigen Gene. *Journal of Clinical Laboratory Analysis*, 16(2), 71-75.
20. Domingues, D., Távora, L. T., Duarte, A., Sanca, A., Prieto, E., Gomes, V., & Azevedo, J. (2022). *Ureaplasma urealyticum* biovar determination in women attending a family planning clinic in Guiné-Bissau, Using Polymerase Chain Reaction of the Multiple-Banded Antigen Gene. *Journal of Clinical Laboratory Analysis*, 16(2), 71-75. Note: This appears to be a duplicate of reference 19. Please check if this is intentional.
21. Choi, S. J., Park, S. D., Jang, H., Uh, Y., & Lee, A. (2012). The Prevalence of Vaginal Microorganisms in Pregnant Women with Preterm Labour and Preterm Birth. *Annals of Laboratory Medicine*, 32(3), 194-200.

22. Hunjak, B., Sabol, I., Vojnovic, G., Fistonici, I., Babić Erceg, A., & Grce, M. (2014). *Ureaplasma urealyticum* and *Ureaplasma parvum* in women of reproductive age. *Archives of Gynecology and Obstetrics*, 289(2), 407-412.
23. De Francesco, M. A., Negrini, R., Pinsi, G., Peroni, L., & Manca, N. (2009). Detection of *Ureaplasma* biovars and polymerase chain reaction-based subtyping of *Ureaplasma parvum* in women with or without symptoms of genital infections. *European Journal of Clinical Microbiology and Infectious Diseases*, 28(6), 641-646.
24. Gupta, K., Hillier, S. L., Hooton, T. M., Roberts, P. L., & Stamm, W. E. (2000). Effects of contraceptive method on the vaginal microbial flora: a prospective evaluation. *The Journal of Infectious Diseases*, 181(2), 595-601.
25. Baka, S., Tsirmpa, I., & Tsouma, I. (2012). Increased quinolone-resistant *Ureaplasma urealyticum* and *Mycoplasma hominis* in reproductive-age women with vulvovaginitis [Conference presentation]. 22nd European Congress of Clinical Microbiology and Infectious Diseases. ECCMID London.
26. GOV.UK. (2023, October 25). Sexually transmitted infections and screening for chlamydia in England: 2022 report. Retrieved from <https://www.gov.uk/government/statistics/sexually-transmitted-infections-stis-annual-data-tables/sexually-transmitted-infections-and-screening-for-chlamydia-in-england-2022-report>