



The Open Microbiology Journal

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RESEARCH ARTICLE

Low Prevalence of Common Sexually Transmitted Infections Contrasting with High Prevalence of Mycoplasma Asymptomatic Genital Carriage: A Community-Based Cross-Sectional Survey in Adult Women Living in N'Djamena, Chad

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Abstract:

Background:

We herein report a cross-sectional study which consecutively enrolled adult women from the community living in N'Djamena, Chad. The aim of the study was to estimate the burden of asymptomatic genital carriage of common curable sexually transmitted infections (STIs) (including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis*) and genital *Mycoplasma* spp., as well as to assess their possible associated risk factors.

Methods:

A total of 251 women were consecutively included and screened for common curable STIs as well as for genital mycoplasma carriage by multiplex real-time PCR.

Results:

Only seven (2.8%) women were found to be infected with at least one common STI by multiplex real-time PCR: *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* were recovered from 3 (1.2%), 1 (0.4%), 4 (1.6%) and 1 (0.4%) women, respectively. No sociodemographic and behavioral characteristics could be associated in multivariate analysis with the genital carriage of the four detected common curable STIs. In contrast, the prevalence of genital mycoplasmas was much higher (54.2%) with a predominance of *Ureaplasma parvum* (42.6% of the total population).

Conclusion:

Our study shows a low prevalence of common STIs in contrast with a high prevalence of mycoplasmas among asymptomatic adult women recruited on a community basis in Chad. These observations highlight the need for etiologic management of STIs relying on PCR-based techniques rather than a syndromic approach in resource-limited countries.

Keywords: Sexually transmitted infections, *Mycoplasma*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, Adult women, Sub-Saharan Africa, Chad.

Article History

Received: March 07, 2019

Revised: June 18, 2019

Accepted: June 21, 2019

1. INTRODUCTION

Worldwide Sexually Transmitted Infections (STIs) stand as a major global health concern with more than a million of STIs being acquired every day [1]. Common bacterial and parasitic STIs conveyed by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis* could easily be treated if properly detected. The World Health Organization (WHO) reports global numbers of 78, 131 and 142 million every year for new cases due to *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* infections, respectively [2]. These STIs are commonly associated with cervicitis and may lead to pelvic inflammatory disease [3, 4]. Furthermore, they may have serious consequences on pregnancy outcomes, such as preterm delivery, spontaneous abortion, low birth weight or postpartum endometritis [5].

According to WHO estimations, sub-Saharan Africa represents 40% of the global burden of STIs, covers 44% of the need for services and 30% of global control cost related to STIs [2]. When reviewing the prevalence rates of common curable STIs in Sub-Saharan Africa, Mullick and colleagues reported infection rates in pregnant women of 2-7%, 3-29% and 40% for *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis*, respectively [5]. In 2012, a systematic review performed on behalf of the WHO reported slightly lower rates in 15 - 49 year-old African women from the general population (*N. gonorrhoeae*, 1.7%; *C. trachomatis*, 3.7%; *T. vaginalis*, 11.5%) [6].

Chad is a central African country and Africa's fifth-largest nation, with a national population of 15 million inhabitants and many of them are young women [7, 8]. There is little information available about the STI prevalence rates in Chad. For HIV-1, a prevalence rate of 1.3% was previously reported in 2017 in the adult population [9], with high disparities between regions, and a higher prevalence in women. To our knowledge, there is currently no published information on the epidemiology of common bacterial STIs in Chad.

We herein report a cross-sectional study which consecutively enrolled adult women from the community living in N'Djamena, the capital city of Chad. The aim of the study was to estimate, by multiplex real-time PCR, the burden of asymptomatic genital carriage of common curable STIs (including *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis*) and genital *Mycoplasma* spp., as well as to assess their possible associated risk factors.

2. MATERIALS & METHODS

2.1. Study Design

The study was a descriptive, quantitative and cross-sectional survey conducted over one month among adult women recruited from the community in N'Djamena, Chad.

The cohort consisted of adult women who were contacted by peer educators in community-churches and mosques or women association networks from twenty-three sites in neighborhoods of 5 districts randomly selected out of the 10 districts of N'Djamena city. They were proposed a survey mainly focusing on prevention strategies against STIs and screening of cervical cancer. After oral consent, the selected women were invited, with paid transportation, to go to the "La Renaissance Plus" clinic, which is the city's main clinical center for medical and surgical gynecology and obstetrics, voluntary HIV-1 counselling, as well as testing, diagnosis and care of patients suffering from STIs.

Inclusion criteria were as follows: being a volunteer older than 18 years, having given signed informed consent, being sexually active, not menstruating, have had no sexual intercourse for at least 48 hours and have completed the questionnaire. Exclusion criteria included age below 18, not willing to participate to the study, having genital troubles, having menstruations, have had recent sexual intercourse in less than 48 hours, and not willing to answer the face-to-face questionnaire for data collection.

After informed signed consent, the selected women benefited from free HIV-1, Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) testing, clinical services including gynecological examination, family planning counselling, STI diagnosis, and laboratory analysis when necessary. Appropriate treatment was given to those suffering from gynecologic disorders or from genital or HIV-1 infections.

2.2. Structured Questionnaire and Genital Sampling

Selected women were interviewed by experienced counsellors specifically trained for this purpose using a structured face-to-face questionnaire, which contained questions on age, marital status, social occupation, education level, age at first sexual intercourse, last unprotected sexual intercourse in the past week, history of STIs, genital hygiene practices, and use of any contraceptive method.

After placing the speculum (without lubricant prior to insertion), a cervical sample was collected by a properly trained nurse for each volunteer using a flocculated swab (Copan Diagnostic Inc., California, USA). Briefly, specimens were obtained by inserting the swab into the vaginal canal until the cervix mucosa, gently rotating 5 times and then removing and placing it into its container before freezing at -80°C. Any abnormal vaginal discharge at the time of sampling was recorded by the attending nurse.

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2.3. Molecular Microbiological Analysis

Genital swabs were transported in frozen ice packs to the Hôpital Européen Georges Pompidou, Paris, France, for molecular analyses. DNA was extracted from swab samples using the DNeasy Blood and Tissue extraction kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. After extraction, DNA was concentrated in 100 µL of the elution buffer provided in the extraction kit and stored at -80°C before STI DNA detection.

Six bacteria (*N. gonorrhoea*, *C. trachomatis*, *M. genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum* and *Ureaplasma urealyticum*) and one protozoan parasite (*T. vaginalis*) were detected by molecular biology using the CE IVD-marked multiplex real-time PCR Allplex™ STI Essential Assay (Seegene, Seoul, South Korea, distributed in France by Eurobio Laboratories, Courtaboeuf, France) [10]. The kit contains specific primers targeting each of the seven microorganisms' DNA, and is based on Seegene's proprietary DPO™ and MuDT™ technologies [11], which avoid mismatch priming and quantify each target in a single fluorescence channel, respectively. The multiplex PCR reaction was carried out on the CFX96™ real-time PCR instrument (Bio-Rad, Marnes-la-Coquette, France) using 5 µl of swab-extracted DNA and 15 µl of PCR mix. The test was applied to all samples according to the manufacturer's recommendations. The microbiology laboratory was accredited by the Comité Français d'Accréditation according to the ISO 15189 norma for molecular biology.

2.4. Screening for HIV-1, HBV and HCV Infections

HIV-1, HBV and HCV serostatus was determined on site by multiplex immunochromatographic rapid test (Biosynex, Strasbourg, France), as previously described [12].

2.5. Statistical Analysis

Prevalence of STIs was expressed as a percentage. Statistical analyses were conducted using IBM® SPSS® Statistics 20 software (IBM, SPSS Inc, Armonk, New York, USA). *P*-values were calculated using Pearson's χ^2 test or Fisher's exact test for categorical variables and the non-parametric Mann-Whitney U-test for quantitative variables. The association between each independent variable and infection was assessed by logistic regression analysis. All the variables statistically significant ($P < 0.05$) in univariate

analyses were computed into multivariate logistic regression models. Crude Odds Ratio (COR) and adjusted Odds Ratio (aOR) were calculated as appropriate along with their 95% Confidence Intervals (CI).

2.6. Ethical Approval

The study was formally approved by the Scientific Committee Faculty of Health Sciences of the University of N'Djamena, which constitutes the National Ethical Committee. All included women gave their informed signed consent to participate in the study. A record of consent from each woman was documented in each questionnaire. This consent procedure was formally approved by the Ethical Committee. All individual results of STI detection as well as of HIV, HBV and HCV serologies were given to each study participant, and women harboring clinical STIs were further cared for at the "La Renaissance Plus" clinic. Furthermore, the study results have been *in extenso* reported to the health authorities of Chad during the national congress of gynecologists and midwives, held from 13 to 17 of November 2018 in the Centre d'Etudes et de Formation pour le Développement (CEFOD), N'Djamena, Chad.

3. RESULTS

3.1. Study Population

In July 2017, 272 women from the 23 inclusion sites participated to the study [1st district (43, 15.8%): Farcha (n=15), Amsiné (n=13), Karkandjeri (n=15); 3rd district (55, 20.2%): Gardolé (n=12), Ardep Djoumal (n=19), Kabalaye (n=11), Sabangali (n=13); 6th district (47, 17.4%): Moursal (n=23), Paris-Congo (n=24); 7th district (61, 22.5%): Ambata (n=5), Amtoukouin (n=6), Atrone (n=9), Boutalbagara (n=5), Chagoua (n=7), Dembé (n=8), Gassi (n=9), Habena (n=9), Kilwiti (n=3); 9th district (66, 24.3%): Digangali (n=10), Gardolé (n=7), Ngueli (n=11), Toukra (n=12), Walia (n=26)].

Ten women were excluded because of genital troubles (genital bleeding: 5; recent sexual intercourse in less than 2 days: 5), and 11 because of incomplete questionnaire or biological sampling. Finally, a total of 251 women (mean age, 35.0 years; range, 20-65) referred to the "La Renaissance Plus" clinic were consecutively included in the study and effectively screened for STIs. Their socio-demographic characteristics, sexual behavior and hygiene practices are summarized in (Table 1).

Table 1. Socio-demographics, behavioural and hygiene characteristics of the 251 women living in N'Djamena, Chad, recruited from the community.

Variable / category	Study women (N = 251) n (%) [95% CI]*
Age (years)	
< 30	78 (31.1) [25.4–37.2]
≥ 30 < 40	66 (26.3) [21.0–32.2]
≥ 40 < 50	69 (27.5) [22.1–33.5]
≥ 50	38 (15.1) [10.9–20.2]
Marital status	
Married or living in a couple	203 (80.9) [75.5–85.6]

(Table 1) contd....

Variable / category	Study women (N = 251) n (%) [95% CI]*
Age (years)	
Single	48 (19.1) [14.4–24.5]
Occupation	
Unemployed	137 (54.6) [48.2–60.9]
Student	36 (14.3) [10.3–19.3]
Employed	78 (31.1) [25.4–37.2]
Education level	
Never schooled	47 (18.7) [14.1–24.1]
Elementary school	43 (17.1) [12.7–22.4]
High school	80 (31.9) [26.2–38.0]
University	81 (32.3) [26.5–38.4]
Age at first sexual intercourse (years)	
≤ 17	136 (54.2) [47.8–60.5]
≥ 18	110 (43.8) [37.6–50.2]
No response	5 (2.0) [0.6–4.6]
Number of sexual partners in everyday life	
One regular partner	201 (80.1) [74.6–84.8]
Several partners [1 to 5]	41 (16.3) [12.0–21.5]
Past history of STIs**	
Yes	11 (4.4) [2.2–7.7]
No	240 (95.6) [92.3–97.8]
HIV-1 status	
Positive	9 (3.6) [1.7–6.7]
Negative	242 (96.4) [93.3–98.3]
HBV status	
Positive	19 (7.6) [4.6–11.6]
Negative	232 (92.4) [88.4–95.4]
HCV status	
Positive	8 (3.2) [1.4–6.2]
Negative	243 (96.8) [93.8–98.6]
Vaginal tampon use	
Never***	215 (85.7) [80.7–89.7]
Sometimes***	14 (5.6) [3.1–9.2]
Often***	4 (1.6) [0.4–4.0]
Always	18 (7.2) [4.3–11.1]
Regular genital toilet	
Yes	201 (80.1) [74.6–84.8]
Water	110 (54.7) [47.6–61.7]
Water + soap	91 (45.3) [38.3–52.4]
No	50 (19.9) [15.2–25.4]
Postcoital genital toilet	
Yes	230 (91.6) [87.5–94.7]
Water	141 (61.3) [54.7–67.6]
Water + soap	89 (38.7) [32.4–45.3]
No	21 (8.4) [5.3–12.5]
Abnormal vaginal discharge on exam	
Yes	120 (47.8) [41.5–54.2]
No	131 (52.2) [45.8–58.5]
Use of contraceptives	
Yes	66 (26.3) [21.0–32.2]
Pill	17 (25.8) [15.8–38.0]
Intrauterine device	11 (16.7) [8.6–27.9]
Condom	7 (10.6) [4.4–20.6]

(Table 1) contd.....

Variable / category	Study women (N = 251) n (%) [95% CI]*
Age (years)	
Other	31 (47.0) [34.6–59.7]
No	185 (73.7) [67.8–79.0]

* The frequency of each variable is presented with their 95% confidence interval in brackets; ** Including infections due to *N. gonorrhoeae*, *C. trachomatis*, and syphilis; *** Alternative of vaginal tampon used for feminine hygiene during menstruation was the use of sanitary napkins. HIV-1: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus

The population had a median age of 37.5 years (range, 20–65 years), and most women (57.4%) were young, aged between 20 to 40 years, in a relationship with a male partner (80.9%) and with a relatively high education level (31.9% and 32.3%, in high school and university, respectively), but most were unemployed (54.6%). The majority of the women (80.1%) reported having only one regular sexual partner in their life, while about 16% reported having up to 5 different sexual partners. Generally, the women included in this study began being sexually active before the age of 17 (54.2%). The vast majority of the women (73.7%) did not use any birth control method. Concerning feminine hygiene during menstruation, most women (85.7%) used sanitary napkins, while a minority (14.3%) used commercially available tampons. Genital (vulva or vagina) toilet was the rule (80.1%), including a post-coital toilet with water and finger in 91.6%. No past history of curable STIs was found for most of the patients (95.6%), and 3.6%, 7.6% and 3.2% were seropositive for HIV-1, HBV (positivity for HBs Ag) and HCV, respectively.

3.2. Genital Pathogens Evidenced by Multiplex Real-Time PCR

Seven (2.8%; 95% CI: 1.1–5.7) women were infected with at least one common STI. *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* were recovered from 3 (1.2%; 95% CI: 0.2–3.5), 1 (0.4%; 95% CI: 0.0–2.2), 4 (1.6%; 95% CI: 0.4–4.0) and 1 (0.4%; 95% CI: 0.0–2.2) participants, respectively. A combination of two pathogens was found in two patients (infected with *M. genitalium* plus *N. gonorrhoeae* and *M. genitalium* plus *C. trachomatis*, respectively).

The prevalence of *M. hominis*, *U. parvum* and *U. urealyticum* is reported in (Table 2). A total of 136 (54.2%) women carried at least one genital mycoplasma, and *U. parvum* was the most represented species and was present alone or associated with other species in 80.9% of positive participants. Combinations of two or three species were observed in 22 (8.8%; 95% CI: 5.6–13) and 2 (0.8%; 95% CI: 0.1–2.8) women, respectively. *M. hominis* associated with *U. parvum* was the most frequent association.

3.3. Risk Factors Associated with Common Curable STIs Due to *C. trachomatis*, *N. gonorrhoea*, *T. vaginalis* and *M. genitalium*.

We examined potential associations between general characteristics of the participants and the presence of any of the four common curable STIs. Most of the variables were not associated with STIs in univariate analysis (not shown). Only the systematic use of vaginal tampons was associated with a higher prevalence of STIs in univariate analysis (OR: 5.70,

95% CI: 1.02–31.72; $P=0.026$), but this was not confirmed in the multivariate analysis (aOR: 2.13, 95% CI: 0.18–25.77; $P=0.554$). In contrast, a lower prevalence of STIs was observed in women who never used vaginal tampons in univariate analysis (OR: 0.29, 95% CI: 0.05–0.97; $P=0.029$), but this was not confirmed in the multivariate analysis (aOR: 0.32, 95% CI: 0.03–3.05; $P=0.323$).

Table 2. Prevalence of genital mycoplasmas (apart from *Mycoplasma genitalium*) among the 251 living in N'Djamena, Chad, recruited from the community.

Species	No. (%) [95% CI]*
<i>M. hominis</i>	4 (1.6) [0.4–4]
<i>Ureaplasma spp.</i>	110 (43.8) [37.6–50.2]
<i>U. urealyticum</i>	19 (7.6) [4.6–11.6]
<i>U. parvum</i>	89 (35.5) [29.5–41.7]
<i>U. parvum</i> + <i>U. urealyticum</i>	2 (0.8) [0.1–2.8]
<i>M. hominis</i> + <i>Ureaplasma spp.</i>	22 (8.8) [5.6–13]
<i>M. hominis</i> + <i>U. parvum</i>	14 (5.6) [3.1–9.2]
<i>M. hominis</i> + <i>U. urealyticum</i>	6 (2.4) [0.9–5.1]
<i>M. hominis</i> + <i>U. parvum</i> + <i>U. urealyticum</i>	2 (0.8) [0.1–2.8]
Total	136 (54.2) [47.8–60.5]

* The frequency of each variable is presented with their 95% confidence interval in brackets.

STIs were not significantly associated with a report of abnormal vaginal discharge at the time of sampling (OR: 1.47, 95% CI: 0.32–6.71; $P=0.452$). History of STIs (including those due to *N. gonorrhoeae*, *C. trachomatis*, or syphilis) was infrequently reported in 4.4% of study participants (Table 1) and was not associated with current STIs ($P=0.728$).

3.4. Risk Factors Associated with the Carriage of Genital Mycoplasmas (Apart from *Mycoplasma Genitalium*).

By univariate analysis, genital mycoplasma shedding was positively associated with the variables “unemployment” (OR = 1.83, 95% CI: 1.11–3.03; $P=0.018$), “regular vaginal cleaning” (OR=2.0, 95% CI: 1.07–3.77; $P=0.029$) and “occasional vaginal tampon use” (OR = 5.56, 95% CI: 1.22–25.39; $P=0.014$), and negatively associated with the variable “employment” (OR=0.47, 95% CI: 0.27–0.81; $P=0.006$). By multivariate analysis, the variable “regular vaginal cleaning” (aOR=2.26, 95% CI: 1.17–4.36; $P=0.016$) and “occasional vaginal tampon use” (aOR = 7.71, 95% CI: 1.59–37.44; $P=0.011$), and “employment” (aOR=0.43, 95% CI: 0.19–0.99; $P=0.046$) remained associated with mycoplasma shedding. All other variables, including “age” and “abnormal vaginal discharge” were not associated with mycoplasma genital shedding by multivariate analysis (not shown).

4. DISCUSSION

This cross-sectional study was undertaken among community-recruited asymptomatic adult women living in N'Djamena, to assess by molecular biology the prevalence of common curable STIs (*N. gonorrhoea*, *C. trachomatis*, *M. genitalium*, and *T. vaginalis*) as well as genital mycoplasma (*M. hominis*, *U. urealyticum*, and *U. parvum*) colonization. The results showed a low incidence of common curable STIs, as only seven women (2.8%) were found to be infected. The prevalence of genital mycoplasmas (apart from *M. genitalium*) was much higher (54.2%) with a predominance of *U. parvum* (42.6% of the total population).

Epidemiological data on the prevalence of common bacterial STIs in Chad has been poorly reported until now. In a cohort of 311 HIV-1-seropositive women, Mortier and colleagues found a prevalence of 1% for *T. vaginalis* [13]. To our knowledge, our observations are the first ones for *N. gonorrhoea*, *C. trachomatis*, *M. genitalium* as well as for genital mycoplasmas in adult women living in Chad. In neighboring countries, such as Nigeria, Cameroon, Central African Republic and Sudan, high prevalence rates of female genital recovery of *C. trachomatis* (0.7 - 31%), *N. gonorrhoeae* (0 - 3.1%) and *T. vaginalis* (0.4 - 17.6%) have been reported [14 - 21]. This high heterogeneity could likely be explained by the wide variations in study settings and populations, and laboratory methods used for genital sampling and STI screening. In our study, none of the sociodemographic and behavioral variables could be associated in multivariate analysis with the genital carriage of the four common bacterial STIs, likely because of the low prevalence encountered for these pathogens and the resulting lack of analysis power which would have necessitated a larger population size.

Although infections due to *N. gonorrhoeae* and *C. trachomatis* may present clinical symptoms such as cervicitis and vaginal discharge, most STIs are asymptomatic and can only be diagnosed by systematic screening, especially among women [22]. In our study population, the seven women presenting with common STIs did not show any general or genital clinical symptom, and vaginal discharge during sampling was not associated with the presence of STIs. Moreover, no sociodemographic factor, sexual behavior, or hygiene practice could be associated with the presence of STIs, which did not facilitate the diagnosis, as was previously suggested [23]. Furthermore, although age and education level have previously been reported to be associated with cervical infections due to *N. gonorrhoea* and *C. trachomatis* [23], no such tendency was observed in our study. Asymptomatic individuals may, therefore, provide a reservoir for infection of the general population, as they remain untreated for prolonged periods and may contaminate their sexual partner(s). The syndromic approach, which is currently recommended by the WHO and spares the need for costly laboratory testing in resource-limited settings [5] has been suspected to lacking sensitivity and specificity in diagnosing STIs [24], which was confirmed in this study.

The high rate of carriage of genital mycoplasmas (54.2%) in our study population is reminiscent of previous reports in women of reproductive age from neighboring countries, with

prevalence rates of 65% in Cameroon [25] and 36% in Nigeria [26]. Genital mycoplasmas apart from *M. genitalium* are generally considered as commensals of the female urogenital tract, and may be found at rates up to 80% in sexually active women [27]. However, their pathogenic role in urogenital infections has been suggested, and there is evidence for their implication in perinatal and neonatal infections [27]. Genital mycoplasmas, especially *M. hominis*, have also been associated with bacterial vaginosis [28]. In our study population, the variables "regular vaginal cleaning" and "occasional vaginal tampon use" were positively associated with mycoplasma genital shedding by multivariate analysis, whereas the variable "employment" was negatively associated. These observations indicate that genital manipulation during the genital toilet or hygiene practice during menses may favor the carriage of genital mycoplasmas, likely by modifying the cervicovaginal ecology. The higher economic status of employed women may also negatively influence the genital shedding of mycoplasma, *a priori* indirectly by multiple risk behaviors strongly associated with socio-economic status. Although this is beyond the scope of this study, further studies are needed to determine the specific role of genital mycoplasmas in infertility and adverse pregnancy outcomes in low-income countries. Such studies would be mandatory before formulating any recommendation concerning an eventual antimicrobial treatment for asymptomatic women carriers, especially when considering the high prevalence rates found in women living in Chad and more generally in sub-Saharan Africa. To date, evidence of the deleterious role of genital mycoplasmas (apart from *M. genitalium*) in asymptomatic women (including asymptomatic pregnant women) is not strong enough to advocate systematic screening and eradication. From a public health perspective, extensive testing and treatment of genital mycoplasmas in low-resource countries would lead to substantial economic costs as well as high antibiotic consumption with an increased risk of resistance selection, including resistance to common bacterial STIs. Taken together, the benefit of antibiotic treatment of genital mycoplasmas appears unclear. Health policies on bacterial STIs in low-income countries should therefore primarily focus on the systematic screening and eradication of well-known pathogens such as *N. gonorrhoea*, *C. trachomatis* and *M. genitalium*.

Our study had several strengths. Firstly, common curable STIs may remain undiagnosed by conventional methods, while multiplex PCR as we used could be successfully employed in low-income countries including Central Africa [29]. Secondly, we tried to limit the selection bias of the study population, as the women were referred to the "La Renaissance Plus" clinic, N'Djamena, although they were not patients from the clinic, thus avoiding the obvious bias of recruitment by health care facilities. Furthermore, 9 (3.5%), 19 (7.5%) and 8 (3.2%) study participants were HIV-, HBsAg- and HCV-specific antibody-positive, respectively, in accordance with the high endemicity of these three viral infections in Chad [9]. Thirdly, we screened a large sample of community-recruited women who represent a high-risk population.

However, our study had certain limitations. First, although 251 women were enrolled in the study, the insufficient size of the study population may have introduced bias, especially in

risk factor analyzes. The fact that women participated on a voluntary basis, and were recruited from community-churches and associative networks, may also have led to recruitment bias. Secondly, we had to rely on a face-to-face questionnaire to collect sociodemographic data and information on sexual behavior, a method which may be prone to reporting bias for sensitive and intimate questions [30]. Third, genital swabs were stored at -80°C before being transported in frozen ice packs for PCR testing in France. Detection rates of genital pathogens may be inferior to rates before storage, as it has been previously reported for *M. genitalium* [31].

CONCLUSION

In conclusion, this study provides a first insight into the prevalence of common STIs among asymptomatic adult women living in Chad. It further confirms the need for implementing an etiologic management of STIs relying on PCR-based techniques rather than on a syndromic approach, even in resource-limited countries. It also shows a high prevalence of genital mycoplasmas apart from *M. genitalium* in this population, which needs further investigation to assess its clinical consequences and potential adverse pregnancy outcomes.

AUTHORS' CONTRIBUTIONS

FC, ZAN, DS, CA, DK and LB have conceived and designed the research; DS and AMM dealt with the ethical issues; RSMB carried out the experiments; RSMB and STW performed statistical analyses; FC, ZAN, DS, AHM, RSMB and IP analyzed the results; FC, ZAN, DS, FC HP and LB drafted the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was formally approved by the Scientific Committee Faculty of Health Sciences of the University of N'Djamena, which constitutes the National Ethical Committee.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Written informed consent was obtained from all patients.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Zita Aleyo Nodjikoambaye is a PhD student from the Ecole Doctorale en Infectiologie Tropicale, Franceville,

Gabon. Ralph-Sydney Mboumba Bourassa is a PhD student from the Ecole Doctorale en Infectiologie Tropicale, Franceville, Gabon, benefiting from a scholarship of the Gabonese Government and is the holder of merit from the Agence Universitaire de la Francophonie. The authors are grateful to Bernard Chaffringeon, V-Veil-Up Pharma Ltd., Nicosia, Cyprus, for the multiplex PCR kits for the study. We are greatly appreciative to all women who participated in our study. We also thank our native English-speaking colleague Mrs Zainab Edoe from our lab, for her assistance in the English editing of the whole paper.

REFERENCES

- [1] World Health Organization. WHO data and statistics 2018 [https://www.who.int/en/news-room/fact-sheets/detail/sexually-transmitted-infections-\(stis\)2019](https://www.who.int/en/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis)2019).
- [2] World Health Organization. Global Health sector strategy on sexually transmitted infections 2016-2021: Towards ending STIs http://apps.who.int/gb/ebwha/pdf_files/WHA69/A69_33-en.pdf?ua=12019.
- [3] Lusk MJ, Konecny P. Cervicitis: A review. *Curr Opin Infect Dis* 2008; 21(1): 49-55. [PMID: 18192786]
- [4] Reekie J, Donovan B, Guy R, *et al.* Chlamydia and reproductive health outcome investigators. Chlamydia and reproductive health outcome investigators. Risk of pelvic inflammatory disease in relation to Chlamydia and Gonorrhea testing, repeat testing, and positivity: A population-based cohort study. *Clin Infect Dis* 2018; 66(3): 437-43. [<http://dx.doi.org/10.1093/cid/cix769>] [PMID: 29136127]
- [5] Mullick S, Watson-Jones D, Beksinska M, Mabey D. Sexually transmitted infections in pregnancy: Prevalence, impact on pregnancy outcomes, and approach to treatment in developing countries. *Sex Transm Infect* 2005; 81(4): 294-302. [<http://dx.doi.org/10.1136/sti.2002.004077>] [PMID: 16061534]
- [6] Newman L, Rowley J, Vander Hoorn S, *et al.* Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PLoS One* 2015; 10(12):e0143304 [<http://dx.doi.org/10.1371/journal.pone.0143304>] [PMID: 26646541]
- [7] Institut National de la Statistique. des Etudes Economiques et Démographiques (INSEED), Ministère de L'Économie et de la Planification du Développement République du Tchad TCHAD-POPULATION 2018. <http://www.inseed-td.net/index.php/thematiques/statistique-demo-graphique/population>
- [8] Institut National de la Statistique. des Études Économiques et Démographiques (INSEED), Ministère de la Santé Publique (MSP) et ICF International, 2014-2015 Enquête Démographique et de Santé et à Indicateurs Multiples (EDS-MICS 2014-2015) Rockville, Maryland, USA : INSEED Mai 2016, MSP et ICF International 2018. <https://dhsprogram.com/pubs/pdf/fr317/fr317.pdf>
- [9] UNAIDS. UNAIDS HIV and AIDS Estimates 2017. <http://www.unaids.org/fr/regionscountries/countries/chad>
- [10] Choe HS, Lee DS, Lee SJ, *et al.* Performance of Anyplex™ II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: Comparison with currently available methods. *Int J Infect Dis* 2013; 17(12): e1134-40. [<http://dx.doi.org/10.1016/j.ijid.2013.07.011>] [PMID: 24095619]
- [11] Lee YJ, Kim D, Lee K, Chun JY. Single-channel multiplexing without melting curve analysis in real-time PCR. *Sci Rep* 2014; 4: 7439. [<http://dx.doi.org/10.1038/srep07439>] [PMID: 25501038]
- [12] Robin L, Mboumba Bouassa RS, Nodjikoambaye ZA, *et al.* Analytical performances of simultaneous detection of HIV-1, HIV-2 and hepatitis C- specific antibodies and hepatitis B surface antigen (HBsAg) by multiplex immunochromatographic rapid test with serum samples: A cross-sectional study. *J Virol Methods* 2018; 253: 1-4. [<http://dx.doi.org/10.1016/j.jviromet.2017.12.001>] [PMID: 29208530]
- [13] Mortier E, Doudéadoum N, Némian F, Gaulier A, Kemian M. Feasibility of cervical smear in HIV-positive women living in Chad. *Bull Soc Pathol Exot* 2016; 109(3): 180-4. [<http://dx.doi.org/10.1007/s13149-016-0496-z>] [PMID: 27299910]
- [14] Amin JD, Zaria LT, el-Nafaty AU, Mai AM. Genital *Chlamydia trachomatis* infection in women in a Nigerian hospital. *Genitourin*

- Med 1997; 73(2): 146-7.
[http://dx.doi.org/10.1136/sti.73.2.146-b] [PMID: 9215104]
- [15] Blankhart D, Müller O, Gresenguet G, Weis P. Sexually transmitted infections in young pregnant women in Bangui, Central African Republic. *Int J STD AIDS* 1999; 10(9): 609-14.
[http://dx.doi.org/10.1258/0956462991914753] [PMID: 10492429]
- [16] Buvé A, Weiss HA, Laga M, *et al.* Study Group on Heterogeneity of HIV Epidemics in African Cities. The epidemiology of gonorrhoea, chlamydial infection and syphilis in four African cities. *AIDS* 2001; 15(Suppl. 4): S79-88.
[http://dx.doi.org/10.1097/00002030-200108004-00009] [PMID: 116 86469]
- [17] Buvé A, Weiss HA, Laga M, *et al.* Study Group on Heterogeneity of HIV Epidemics in African Cities. The epidemiology of trichomoniasis in women in four African cities. *AIDS* 2001; 15(Suppl. 4): S89-96.
[http://dx.doi.org/10.1097/00002030-200108004-00010] [PMID: 116 86470]
- [18] Ngandjio A, Clerc M, Fonkoua MC, *et al.* Screening of volunteer students in Yaounde (Cameroon, Central Africa) for *Chlamydia trachomatis* infection and genotyping of isolated *C. trachomatis* strains. *J Clin Microbiol* 2003; 41(9): 4404-7.
[http://dx.doi.org/10.1128/JCM.41.9.4404-4407.2003] [PMID: 12958 277]
- [19] Adeoye GO, Akande AH. Epidemiology of *Trichomonas vaginalis* among women in Lagos metropolis, Nigeria. *Pak J Biol Sci* 2007; 10(13): 2198-201.
[http://dx.doi.org/10.3923/pjbs.2007.2198.2201] [PMID: 19070181]
- [20] Abdelaziz ZA, Ibrahim ME, Bilal NE, Hamid ME. Vaginal infections among pregnant women at Omdurman Maternity Hospital in Khartoum, Sudan. *J Infect Dev Ctries* 2014; 8(4): 490-7.
[http://dx.doi.org/10.3855/jidc.3197] [PMID: 24727516]
- [21] Abdelrahim NA, Ahmed HI, Fadl-Elmula IM, Bayoumi MA, Homeida MM. Sexually transmitted infections other than HIV/AIDS among women of low socio-economic class attending antenatal clinics in Khartoum, Sudan. *Int J STD AIDS* 2017; 28(8): 781-7.
[http://dx.doi.org/10.1177/0956462416668080] [PMID: 27582306]
- [22] Farley TA, Cohen DA, Elkins W. Asymptomatic sexually transmitted diseases: The case for screening. *Prev Med* 2003; 36(4): 502-9.
[http://dx.doi.org/10.1016/S0091-7435(02)00058-0] [PMID: 12649 059]
- [23] Romoren M, Sundby J, Velauthapillai M, Rahman M, Klouman E, Hjortdahl P. Chlamydia and gonorrhoea in pregnant Batswana women: Time to discard the syndromic approach? *BMC Infect Dis* 2007; 7: 27.
[http://dx.doi.org/10.1186/1471-2334-7-27] [PMID: 17437632]
- [24] Pettifor A, Walsh J, Wilkins V, Raghunathan P. How effective is syndromic management of STDs?: A review of current studies. *Sex Transm Dis* 2000; 27(7): 371-85.
[http://dx.doi.org/10.1097/00007435-200008000-00002] [PMID: 109 49428]
- [25] Njunda AL, Nsagha DS, Assob JC, *et al.* Genital mycoplasmas in women attending the Yaoundé University Teaching Hospital in Cameroon. *J Public Health Africa* 2011; 2(1)e16
[http://dx.doi.org/10.4081/jphia.2011.e16] [PMID: 28299057]
- [26] Agbakoba NR, Adetosoye AI, Adewole IF. Presence of mycoplasma and ureaplasma species in the vagina of women of reproductive age. *West Afr J Med* 2007; 26(1): 28-31.
[http://dx.doi.org/10.4314/wajm.v26i1.28299] [PMID: 17595988]
- [27] Waites KB, Katz B, Schelonka RL. Mycoplasmas and ureaplasmas as neonatal pathogens. *Clin Microbiol Rev* 2005; 18(4): 757-89.
[http://dx.doi.org/10.1128/CMR.18.4.757-789.2005] [PMID: 16223 956]
- [28] Onderdonk AB, Delaney ML, Fichorova RN. The human microbiome during bacterial vaginosis. *Clin Microbiol Rev* 2016; 29(2): 223-38.
[http://dx.doi.org/10.1128/CMR.00075-15] [PMID: 26864580]
- [29] Gueye SB, Diop-Ndiaye H, Gningue A, *et al.* Performance of the Abbott Real Time CT/NG assay in urines and cervico-vaginal samples from Senegal. *J Infect Dev Ctries* 2014; 8(7): 898-903.
[http://dx.doi.org/10.3855/jidc.4026] [PMID: 25022301]
- [30] Teunis N. Same-sex sexuality in Africa: A case study from Senegal. *AIDS Behav* 2001; 5(2): 173-82.
[http://dx.doi.org/10.1023/A:1011335129358]
- [31] Carlsen KH, Jensen JS. *Mycoplasma genitalium* PCR: Does freezing of specimens affect sensitivity? *J Clin Microbiol* 2010; 48(10): 3624-7.
[http://dx.doi.org/10.1128/JCM.00232-10] [PMID: 20720022]