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Ureaplasma urealyticum and *U. parvum* in sexually active women attending public health clinics in Brazil

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SUMMARY

Ureaplasma urealyticum and *U. parvum* have been associated with genital infections. The purpose of this study was to detect the presence of ureaplasmas and other sexually transmitted infections in sexually active women from Brazil and relate these data to demographic and sexual health, and cytokines IL-6 and IL-1β. Samples of cervical swab of 302 women were examined at the Family Health Units in Vitória da Conquista. The frequency of detection by conventional PCR was 76·2% for *Mollicutes*. In qPCR, the frequency found was 16·6% for *U. urealyticum* and 60·6% *U. parvum* and the bacterial load of these microorganisms was not significantly associated with signs and symptoms of genital infection. The frequency found for *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Gardnerella vaginalis* and *Chlamydia trachomatis* was 3·0%, 21·5%, 42·4% and 1·7%, respectively. Higher levels of IL-1β were associated with control women colonized by *U. urealyticum* and *U. parvum*. Increased levels of IL-6 were associated with women who exhibited *U. parvum*. Sexually active women, with more than one sexual partner in the last 3 months, living in a rural area were associated with increased odds of certain *U. parvum* serovar infection.

Key words: *Ureaplasma parvum*, *Ureaplasma urealyticum*, urogenital infection.

INTRODUCTION

Ureaplasma urealyticum and *U. parvum* are members of the class *Mollicutes*, commonly referred to as mycoplasmas. Both species are commensals of the human urogenital tract. Infection rates as high as 40–80% in women and 50% in men have been reported [1] and most of these infections are asymptomatic [2]. These *Mollicutes* are also responsible for a variety of diseases and events such as non-gonococcal urethritis, endometritis, pelvic inflammatory disease (PID), chorioamnionitis, spontaneous abortion, premature birth, stillbirth and neonatal bronchopulmonary dysplasia [3].

In the early days, *U. urealyticum* was divided into 14 serovars grouped into two biovars; biovar 1 composed of serovars 1, 3, 6, 14, while biovar 2 had 10 serovars 2, 4, 5, and 7–13, respectively. In 2002, biovars 1 and 2 were renamed *U. parvum* and *U. urealyticum,* respectively. This reclassification was based on

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differences based on genome size, 16S rRNA gene sequences, the 16S–23S rRNA intergenic region, enzyme polymorphisms, DNA–DNA hybridization, and differences in the multiple-banded antigen (*mba*) genes [4].

The main characteristic of *Ureaplasma* spp. is their ability to hydrolyze urea and release ammonia causing a local cytotoxic effect. In addition they also produce IgA protease, urease, phospholipases A and C, and hydrogen peroxide [5].

The opportunistic role of mollicutes includes some features of the innate immune response [6]. Ryckman *et al.* [7] observed that vaginal IL-1β, IL-6, and IL-8 levels in pregnant were significantly higher in women with any mycoplasma compared with those without. This may be related to bacterial surface molecules. Recognition of bacterial surface molecules by the innate immune system can trigger the production of various proinflammatory cytokines from manifold cells, a surface component that causes inflammation [8–10]. Therefore it is possible to conclude that the immune response to mollicutes is variable due the host and the antigenic mosaic and variation of mollicutes. This may explain in part the potential role of *Mollicutes* in mammals to be excellent opportunistic microorganisms that modulate the immune response differently [11].

When these *Mollicutes* are identified in clinical samples, *U. parvum* are observed to be more frequent than *U. urealyticum*. However, this distribution is controversial due the antigenic variation or identification methods of these *Mollicutes*. In this context, the aim of the present study was to detect the prevalence of *U. urealyticum*, *U. parvum*, *U. parvum* serovars, and other microorganisms of genital infections among sexually active women attending health clinics in Vitória da Conquista, Brazil.

METHODS

Endocervical samples

This analytical cross-sectional study analyzed clinical samples from Vitória da Conquista city, the third largest city of Bahia State, in the northeast of Brazil. The women included in the study attended a Brazilian public health clinic, known as the 'Unified Health System'. Cervical swabs were obtained from 302 women consecutively attending a health clinic in Vitória da Conquista, Brazil, from May to July 2011 and in January 2012. Women with and without symptoms of genital infection were studied, including 236 symptomatic women and 64 asymptomatic as a control group. Two women had missing observations related to symptoms of genital infection, but kept in the overall prevalence calculations. The following women were excluded: sexually inactive women, those using antibiotics in the last 3 months, pregnant and HIV-positive women. The cervix of selected women was accessed using a sterile speculum, and exudate was removed with a sterile swab. Two swabs were collected from each woman. One was then placed in 5 ml of sterile transport medium [12] and stored at −20 °C for molecular analysis. The other swab was then placed in a tube containing 1 ml phosphate-buffer saline solution and stored at −70 °C for cytokines analysis.

A questionnaire that surveyed demographic details, obstetric and gynecologic history and knowledge about sexual behavior was administered. The questionnaire and the study were approved by the ethics committee of the Institute of Biomedical Sciences, University of São Paulo, Brazil.

DNA extraction

Swabs were vigorously homogenized in a transport medium and 1 ml of medium was used for the extraction. The DNA was extracted following the manufacturer's instructions for Invitrogen Purelink Genomic DNA Kits (Invitrogen, São Paulo, SP, Brazil). *U. parvum* ATCC 27815 and *U. urealyticum* ATCC 33175 were used as standard strains. The standard strains of *Ureaplasma* were the positive control, and UltraPure™ DNase/RNase-Free Distilled Water (Life Technology, Brazil) was the negative control for the PCR and qPCR methodology. The DNA of clinical samples and reference strains were stored at −80 °C.

Conventional PCR and real-time TaqMan PCR

This PCR procedure was used for detecting *Mollicutes* (as a screening test), as described by van Kuppeveld *et al.* [13]. Positive samples for *Mollicutes* were analyzed for *Ureaplasma* species. Primers and probes for *U. urealyticum* (UUureF/UUureR/ProbeUU) and *U. parvum* (UPureF/UPureR/ProbeUP) were used for Real-time PCR, as described by Cao *et al.* [14]. All reactions were performed in duplicate in a StepOnePlus real-time PCR instrument (Applied Biosystems, Brazil). Quantification was performed using an absolute quantification technique, based on a predetermined standard curve ranging from 10^7 to 10 microorganisms/µl. The

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data were acquired during the annealing step and analyzed using the StepOne Software 2.1 (Applied Biosystems, Life Technologies Corporation). The Ct (threshold cycle) of the genome dilutions was plotted against the log number of genome copies and used as input to create the standard curve. Linear regression analyses were then applied to calculate the r^2 and slope values. Assuming 100% efficiency if the DNA template is doubled in each cycle, the PCR efficiency was calculated as $E = 10(-1/\text{slope}) - 1$, where E is PCR efficiency. The positive samples for *U. parvum* were then submitted to a specific conventional PCR to detect serovars 1, 3, 6, and 14 of *U. parvum* [15].

Other sexually transmitted microorganism detection

Cervical samples were also tested with conventional PCR methodology to detect the targeted DNA of *Chlamydia trachomatis* [16], *Neisseria gonorrhoeae* [16], *Trichomonas vaginalis* [17] and *Gardnerella vaginalis* [18].

Measurement of IL-6 and IL-1β

The cervical interleukin (IL)-6 and IL-1β levels were dosage in duplicate using a commercially available ELISA kit (eBioscience, San Diego, CA, USA). To compare the difference between IL-6 and IL-1β titers, the values were log-transformed before statistical analysis.

Statistical data analysis

Statistical analyses of the questionnaire results were conducted through software SPSS 16.0 (SPSS Inc., Chicago, IL, USA). To check association between variables, a Pearson a χ^2 Test or Fisher's exact Test were used, $P < 0{\cdot}05$ and 95% CI. The odds ratio (OR) was computed in Univariate Analysis to estimate the power of association between presence of vaginal infection, with risk factors such as demographics and women's sexual health. A Multivariate Analysis Logistic Regression was also used to determine which variables were independently associated with infection.

Mann–Whitney test was used to compare the titers of cervical IL-6 and IL-1β between individuals with *U. urealyticum* or *U. parvum* infections.

RESULTS

The PCR methodology showed that 230 (76·2%) of the women studied were colonized by *Mollicutes*. The real-time PCR detected 50 positive samples for *U. urealyticum* and 183 for *U. parvum*. Co-infection of both species was observed in 14 samples. *U. parvum* serovars 6 and 3/14 were the most frequent isolates in 64 (40·8%) and 62 (39·5%), respectively, followed by serovar 1 in 37 samples (23·6%). There were 27 samples that were cervical positive for *U. parvum*; however, they were negative for all four known *U. parvum* serovars.

We observed a prevalence of 42·4% (128/302) of *G. vaginalis* infection, 21·5% (65/302) of *N. gonorrhoeae*, 3·0% (9/302) of *T. vaginalis* and 1·7% (5/302) of *C. trachomatis*. Among women with *U. urealyticum*, four were coinfected with *T. vaginalis*, nine with *N. gonorrhoeae*, 25 with *G. vaginalis* and one with *C. trachomatis*. However, among women with *U. parvum*, nine were co-infected with *T. vaginalis*, 40 with *N. gonorrhoeae*, 82 with *G. vaginalis* and four with *C. trachomatis*.

Among the organisms determined by real-time PCR, the number of *U. urealyticum* was 50 (16·6%) of the cervical samples (bacterial load ranging from 10 to 10^4 organisms per sample). *U. parvum* organisms were detected in 183 (60·6%) of the samples (ranging from 10^1 to 10^7 organisms per sample). The bacterial load of *U. urealyticum* was significantly higher ($P = 0{\cdot}033$, Mann–Whitney test) in the asymptomatic group than in the symptomatic group. In contrast, there was no significant difference between the bacterial of *U. parvum* in women with and without symptoms.

Table 1 shows the demographic, reproductive, and behavioral characteristics of the women screened for *U. urealyticum* and *U. parvum* infections detected by qPCR. Women living in rural areas (OR 2·85, 95% CI 1·50–5·41, $P = 0{\cdot}001$) and having more than one sexual partner in the last 3 months (OR 8·07, 95% CI 1·08–60·35, $P = 0{\cdot}016$) were significantly associated with the *U. urealyticum* infection. However, women with an active sex life (OR 3·25, 95% CI 1·39–7·57, $P = 0{\cdot}004$) and having more than one sexual partner in the last 3 months (OR 3·79, 95% CI 1·82–7·88, $P < 0{\cdot}001$) were associated with the *U. parvum* infection.

In the multivariate analyses, the following factors were found to be associated with *U. urealyticum* infection (Table 1): living in a rural area (adjusted odds ratio (AOR) 2·4, 95% CI 1·2–4·6), with five or more lifetime sexual partners (AOR 3·8, 95% CI 1·1–13·2) and having more than one sexual partner in the last 3 months (AOR 5·3, 95% CI 0·6–44·1) had a higher risk, and those not in a stable relationship (AOR 0·7, 95% CI 0·2–2·0) had a lower risk for *U. urealyticum* infection.

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Table 1. *Characteristics of study participants and association with* Ureaplasma urealyticum *and* U. parvum *infections in women from Vitória da Coquista, Brazil*

Variables	*Ureaplasma urealyticum* (positive) ($N = 50$)			*Ureaplasma parvum* (positive) ($N = 183$)		
	n (%)*	OR (95% CI)	AOR (95% CI)	*n* (%)*	OR (95% CI)	AOR (95% CI)
Demographic data						
Area						
Rural	21 (42·0)	2·85 (1·50–5·41)[a]	2·4 (1·2–4·6)	48 (26·2)	1·41 (0·81–2·45)	–‡
Urban	29 (58·0)			135 (73·8)		
Reason for visit[5]						
Symptomatic	17 (34·7)	1·39 (0·72–2·66)	–‡	53 (29·4)	1·07 (0·64–1·80)	–‡
Routine	32 (65·3)			127 (70·6)		
Age						
<25 years	11 (22·0)	1·45 (0·68–3·07)	–‡	34 (18·6)	1·28 (0·69–2·39)	–‡
⩾25 years	39 (78·0)			149 (81·4)		
Race						
Black/Indigenous	41 (82·0)	1·65 (0·76–3·58)	–‡	140 (76·5)	1·25 (0·74–2·12)	–‡
Caucasian/Asian	9 (18·0)			43 (23·5)		
Education						
<High school	38 (79·2)	1·64 (0·77–3·47)	–‡	128 (73·6)	1·30 (0·77–2·19)	–‡
⩾High school	10 (20·8)			46 (26·4)		
Stable relationship						
No	6 (12·0)	0·47 (0·19–1·15)	0·7 (0·2–2·0)	36 (19·7)	0·83 (0·47–1·46)	–‡
Yes	44 (88·0)			147 (80·3)		
Sexual health						
Menarche						
<15 years	41 (82·0)	1·27 (0·58–2·78)	–‡	143 (78·1)	0·90 (0·51–1·60)	–‡
⩾15 years	9 (18·0)			40 (21·9)		
Age of first sexual intercourse						
<15 years	15 (30·0)	1·47 (0·75–2·87)	–‡	42 (23·0)	0·88 (0·52–1·51)	–‡
>15 years	35 (70·0)			141 (77·0)		
Sex life[1]						
Active	49 (98·0)	5·42 (0·72–40·96)	–‡	174 (95·1)	3·25 (1·39–7·57)[b]	1·8 (0·5–5·9)
Inactive	1 (2·0)			9 (4·9)		
Libido						
Decreased	19 (38·0)	0·71 (0·38–1·33)	–‡	81 (44·8)	0·99 (0·62–1·58)	–‡
Maintained	31 (62·0)			100 (55·2)		
Lifetime sexual partners						
⩾5	7 (14·0)	1·88 (0·75–4·72)	–‡	19 (10·4)	1·59 (0·67–3·77)	–‡
<5	43 (86·0)			164 (89·6)		
Sexual partner in last 3 months§						
⩾1	48 (98·0)	8·07 (1·08–60·35)[a]	5·3 (0·6–44·1)	169 (93·4)	3·79 (1·82–7·88)[a]	2·6 (0·9–6·8)
None	1 (2·0)			12 (6·6)		

Table 1 (*cont.*)

Variables	*Ureaplasma urealyticum* (positive) (*N* = 50)			*Ureaplasma parvum* (positive) (*N* = 183)		
	n (%)*	OR (95% CI)	AOR (95% CI)	*n* (%)*	OR (95% CI)	AOR (95% CI)
Condom use						
Rare/occasional	36 (72·0)	0·58 (0·29–1·17)	–‡	138 (78·0)	0·74 (0·40–1·34)	–‡
Always	14 (28·0)			39 (22·0)		
Hormonal contraception use						
Yes	30 (60·0)	0·86 (0·48–1·60)	–‡	112 (62·6)	0·96 (0·59–1·55)	–‡
No	20 (40·0)			67 (37·4)		
Dyspareunia[2]						
Yes	18 (36·0)	1·28 (0·67–2·42)	–‡	61 (34·1)	1·37 (0·82–2·29)	–‡
No	32 (64·0)			118 (65·9)		
Postcoital bleeding[3]						
Yes	5 (10·0)	1·17 (0·42–3·28)	–‡	16 (8·9)	1·00 (0·44–2·99)	–‡
No	45 (90·0)			164 (91·1)		
Dysuria						
Yes	11 (22·0)	1·06 (0·51–2·21)	–‡	42 (23·0)	1·31 (0·74–2·34)	–‡
No	39 (78·0)			141 (77·0)		
Pelvic pain						
Yes	24 (48·0)	1·17 (0·64–2·15)	–‡	87 (47·5)	1·34 (0·84–2·14)	–‡
No	26 (52·0)			96 (52·5)		
Itch						
Yes	18 (36·0)	1·41 (0·74–2·66)	–‡	61 (33·3)	1·55 (0·92–2·61)	1·5 (0·8–2·6)
No	32 (64·0)			122 (66·7)		
Discharge[4]						
Yes	24 (48·0)	1·18 (0·64–2·17)	–‡	89 (48·9)	1·57 (0·98–2·52)	1·3 [0·7–2·3]
No	26 (52·0)			93 (51·1)		

AOR, adjusted odds ratio; OR, odds ratio.

Missing observations:

[1] Sex life: loss of 1 (*n* = 301).

[2] Dyspareunia: loss of 10 and excluded women in inactive sex life (*n* = 292).

[3] Postcoital bleeding: loss of nine and excluded women in inactive sex life (*n* = 293).

[4] Discharge: loss of 1 (*n* = 301).

[5] Reason for visit: loss of 4 (*n* = 298).

* Percentage of total study population with characteristic that tested positive for *U. urealyticum* and *U. parvum* by qPCR.

§ Sexual partners in the last 3 months.

‡ Variables with a p value >0·10 were not included in the multivariate analysis.

[a] $P < 0{\cdot}001$.

[b] $P < 0{\cdot}05$.

Table 2. *Multivariate logistic-regression analysis for assessment of independent risk factors for Serovars* Ureaplasma parvum *infections in women from Vitória da Conquista, Brazil*

	U. parvum Serovar 1		*U. parvum* Serovar 3/14	
Independent factor	AOR	95% CI	AOR	95% CI
Rural area	3·4	(1·6–7·3)	–	–
Education (<high school)	1·6	(0·6–4·2)	–	–
Menarche (<15 years)	0·6	(0·2–1·5)	–	–
Sexual partner in last 3 months (⩾1)*	–	–	4·6	(1·1–19·8)
Dysuria	1·9	(0·8–4·4)	–	–
Pelvic pain	1·3	(0·6–2·8)	–	–
Discharge	–	–	1·8	(0·9–3·4)

* Sexual partners in the last 3 months.
AOR, adjusted odds ratio; CI, confidence interval.

Sexually active women (AOR 1·8, 95% CI 0·5–5·9), with more than one sexual partner in the last 3 months (AOR 2·6, 95% CI 0·9–6·8) and presenting itch (AOR 1·5, 95% CI 0·8–2·6) and vaginal discharge (AOR 1·3, 95% CI 0·7–2·3) were associated with an increase in odds for *U. parvum* infection (Table 1).

Multivariate analyses of the risk factors associated with *U. parvum* serovar infections are shown in Table 2. Women living in rural areas (AOR 3·4, 95% CI 1·6–7·3), with an education level of less than high school (AOR 1·6, 95% CI 0·6–4·2), dysuria (AOR 1·9, 95% CI 0·8–4·4) and pelvic pain (AOR 1·3, 95% CI 0·6–2·8) were independently associated with serovar 1 *U. parvum*. Menarche before 15 years had a lower risk for infection of serovar 1 *U. parvum*. Women with a higher number of sexual partners in the last 3 months (AOR 4·6, 95% CI 1·1–19·8) and vaginal discharge (AOR 1·8, 95% CI 0·9–3·4) presented a higher risk to be infected with *U. parvum* serovars 3/14.

The reason for the medical care visit (AOR 1·6, 95% CI 0·8–2·8), age below 25 years (AOR 2·3, 95% CI 1·1–4·4), being sexually active (AOR 1·9, 95% CI 0·6–6·3), age at first sexual intercourse until 15 years (AOR 1·6, 95% CI 0·8–2·9), and dyspareunia (AOR 1·5, 95% CI 0·8–2·7) were associated with an increase for the odds of *G. vaginalis* infection (Table 3). A negative association was correlated with living in a rural area (AOR 0·6, 95% CI 0·3–1·1) and an education level of less than high school (AOR 0·7, 95% CI 0·4–1·2). Postcoital bleeding (AOR 3·6, 95% CI 1·4–9·2) was associated with an increase in the odds for *N. gonorrhoeae* infection, and living in a rural area (AOR 0·1, 95% CI 0·02–0·3) were associated with decreased odds of *N. gonorrhoeae* infection.

Significant differences were observed between IL-6 and IL-1β concentrations in cervical samples among symptomatic and non-symptomatic women (Fig. 1A and B). The association between IL-6 levels did not present a significant difference with the *U. urealyticum* infections (Fig. 1D). However, higher IL-1β levels were observed in women with *U. urealyticum* infection (Fig. 1C). The relationships between cervical IL-1β and IL-6 levels and *U. parvum* infection were significant. Higher IL-1β and IL-6 levels were detected in women with *U. parvum* infection (Fig. 1F and G).

DISCUSSION

Here we studied a group of women who live in an arid region of Brazil and compare their age, sexual activity and relationship status with the molecular detection of *Ureaplasma* and other STD agents in cervical swabs. Data regarding the relative frequencies of ureaplasmas in the Brazilian population are scant [19]. Moreover, as the separation of human ureaplasmas in two species is recent, the Brazilians studies do not provide this distinction, making it difficult to compare data. In the present study, *U. parvum* was detected more frequently than *U. urealyticum*. Other studies have also shown a higher prevalence of *U. parvum* in samples of the female urogenital tract than the *U. urealyticum* [20–22]. The molecular diagnosis was able to detect ureaplasmal DNA, but the results do not show viable microorganisms as the qPCR results do. But both methodologies are fast, and the quantification of DNA in a clinical sample is a more accurate indicator of infection. In the present study, the bacterial load of *U. urealyticum* was significantly higher in the

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Table 3. *Multivariate logistic-regression analysis for assessment of independent risk factors for* Gardnerella vaginalis *and* Neisseria gonorrhoeae *infections in women from Vitória da Conquista, Brazil*

	G. vaginalis		*N. gonorrhoeae*	
Independent factor	AOR	95% CI	AOR	95% CI
Rural area	0·6	(0·3–1·1)	0·1	(0·02–0·3)
Reason for visit	1·6	(0·8–2·8)	–	–
Age (<25 years)	2·3	(1·1–4·4)	–	–
Education (<high school)	0·7	(0·4–1·2)	–	–
Sex life (active)	1·9	(0·6–6·3)	–	–
Age of first sexual intercourse (⩽15 years)	1·6	(0·8–2·9)	–	–
Dyspareunia	1·5	(0·8–2·7)	–	–
Postcoital bleeding	–	–	3·6	(1·4–9·2)

AOR, adjusted odds ratio; CI, confidence interval.

asymptomatic group than in the symptomatic group. An explanation for this may be related to the commensal characteristic of these microorganisms. Despite the higher bacterial load, other factors related to the host could be related to clinical manifestations [1].

In the present study, *U. parvum* serovars 6 and 3/14 were the most frequent, followed by serovar 1. Multiple *U. parvum* serovars were detected in 18 cervical samples, while one was not typeable. Horizontal gene transfer (HGT) has been observed among *Ureaplasma* species, and the serovars can generate chimeric isolates with markers of two or more serovars [23]. The HGT was also observed between *U. parvum* and *Mycoplasma hominis* [24]. Xiao *et al.* [23] mentioned the failure to separate multiple serovars from some clinical isolates, suggesting the occurrence of hybrid isolates and explaining the detection of more than one serovar-specific in PCR assay. Antibody based or PCR methods have reported a certain number of non-typeable isolates [25, 26].

U. urealyticum infection was positively associated with the living in a rural area and having more than one sexual partner in the last 3 months, which was inversely associated with women not in a stable relationship. Tibaldi *et al.* [27] found a similar association with women between 14 and 25 years, without a stable sexual partner, history of abortion, IUD (intrauterine device) use, more than one sexual partner in the last 6 months and more than two sexual partners during their lifetime. *U. parvum* infection was more frequently associated with sexually active women than women with more than one sexual partner in the last 3 months with itching and vaginal discharge. Other studies have found an association with this *Ureaplasma* and PID, premature birth and bronchopulmonary dysplasia in neonates [20–22]. However, Jones *et al.* [28] detected inconsistent association of *U. parvum* infection and clinical signs.

In our study, the prevalence of *C. trachomatis* was estimated at 1·7%. Yamazaki *et al.* [29] in Sapporo, Japan found *C. trachomatis* in 14·3% of samples. In Brazil, several studies found this agent ranging from 5·0% to 19·6% among youth attending outpatient clinics and gynecological clinics, and attending the Family Health program [30, 31].

Three per cent of the studied women were positive for *T. vaginalis*. Similarly, a study in the USA found the prevalence was 3·1% among women aged between 14 and 49 years [32]. Other studies found a higher prevalence for this microorganism [33, 34]. Here we found no association between *C. trachomatis* and *T. vaginalis* with symptoms of urogenital infection. Gaydos *et al.* found an association between Chlamydia and young women, and the number of sexual partners. The *T. vaginalis* association was found with age at first intercourse, previous sexually transmitted infections (STIs) and presence of vaginal discharge, and with black women, the number of sexual partners in the year preceding the study, bisexual relationships and occasional condom use [35].

N. gonorrhoeae was detected in 21·5% of samples and was significantly associated with the symptom of postcoital bleeding. Other studies showed lower prevalence of *N. gonorrhoeae* infection [36, 37]. According to Mgone *et al.* [16] the prevalence of *N. gonorrhoeae* was highest in young women between 20 and 24 years. *G. vaginalis* was detected in 42·4% of vaginal samples, and was associated with symptomatic women, age <25 years, sexually active, age at first intercourse and symptom dyspareunia. A study

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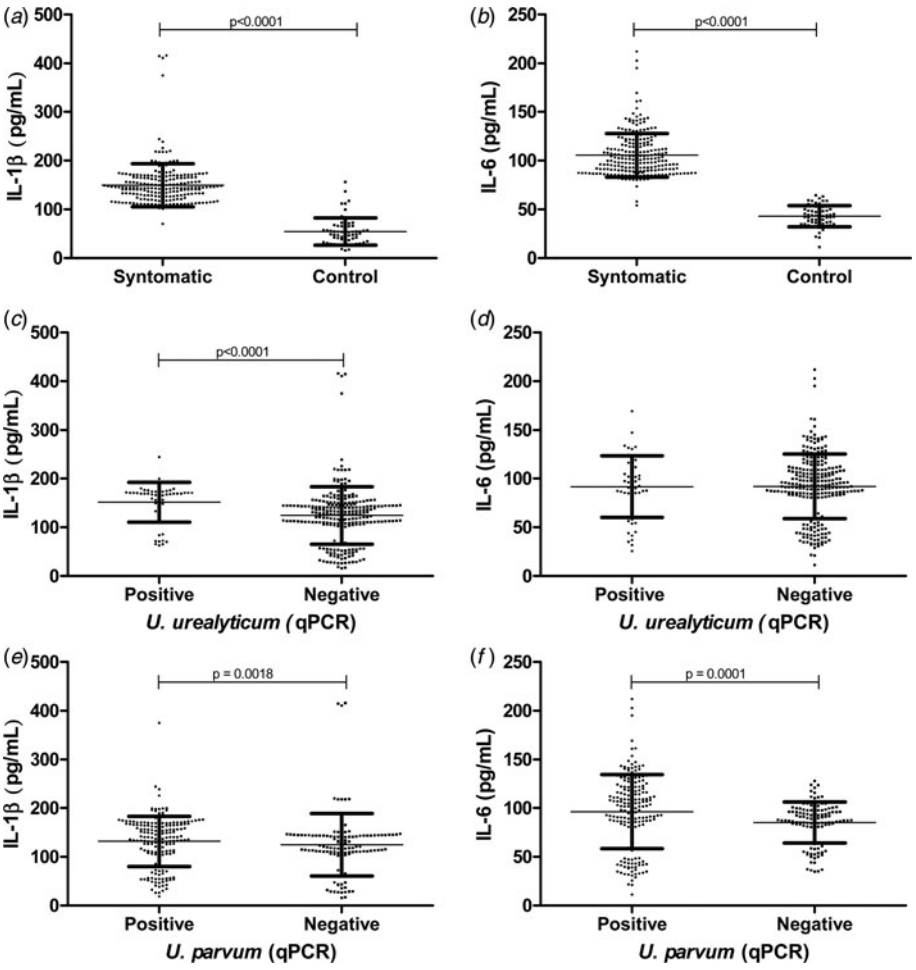

Fig. 1. Relationship between vaginal infection by *U. urealyticum*, and the concentrations of IL-1β and IL-6 in cervical samples using ELISA among women attending health units in Vitória da Conquista, Brazil, 2013. (A) Concentration of IL-1β (pg/mL) in cervical samples of women and symptomatic and controls. (B) Concentration of IL-6 (pg/mL) in cervical samples of symptomatic women and controls. (C) Concentration of IL-1β (pg/ml) in cervical samples of women qPCR positive and negative *U. urealyticum*. (D) Concentration of IL-6 (pg/ml) in cervical samples of women qPCR positive and negative *U. urealyticum*. (E) Concentration of IL-1β (pg/ml) in cervical samples of women qPCR positive and negative *U. parvum*. (F) Concentration of IL-6 (pg/ml) in cervical samples of women qPCR positive and negative *U. parvum*. Statistical analysis by Mann–Whitney. $P < 0{\cdot}05$.

by Fethers *et al.* [38] observed an increase in prevalence of this parasite in women according to their number of sexual partners.

The difference in prevalence between the ureaplasmas and the searched microorganisms and risk factors compared with the literature could be related to the population studied. Some authors confirm that the prevalence of genital microorganisms is related to regional differences [39–44]. However, explanations for these differences are not clear, are variable and require more study. The women studied live in the Northeast region of Brazil, which has a semi-arid climate, with high rates of poverty. This population depends strictly on basic governmental health services. At the time of collection of clinical material, the healthcare service for women was not in service for several months. We believe this factor associated with lack of knowledge regarding prevention and treatment of STIs may have contributed to this prevalence obtained.

Bacterial infections in the vagina typically induce a local immune response in the host. The pro-inflammatory cytokine, IL-1β, is induced in the lower genital tract in women with vaginal infection [45, 46]. The vaginal production of additional cytokines, both pro- and anti-inflammatory, in relation to the local immune response to bacterial vaginosis has not been thoroughly investigated [47]. In this study, a statistically significant association was not found between both IL-6 and IL-1β and ureaplasma infection compared with women without infection.

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The proinflammatory cytokine IL-6 is a component of mechanisms that regulate innate immunity. The IL-6 is produced in the early phase of infections, and several studies have shown that preterm labor is associated with increased levels of IL-6 in maternal cervicovaginal and amniotic fluid [48]. A difference in the IL-6 levels was also detected among women with *U. parvum* infection. Some studies have shown a positive association between levels of IL-6 in cervicovaginal fluid with preterm labor [49] and presence of cervical inflammation, altered vaginal microbiota and pregnancy [50].

In the present study, a difference in the IL-6 and IL-1β levels was observed among women with *U. urealyticum* and *U. parvum* infection. The IL-1β cytokines are present intracellularly in the epithelial cells at the cell damage. These cytokines play a central role in regulating the inflammatory response in damaged tissue and assist in the development of the preterm labor when associated with an infection, with prostaglandin production by amniotic epithelium and stimulate myometrial contractily [51]. According to Patterson *et al.* [52] isolation of *Ureaplasma* from the respiratory tract of preterm infants is associated with increased IL-1β concentrations. However, another study found no relationship between *U. urealyticum* detection and the concentration of any cytokine IL-1β, IL-4 and IL-6 in asymptomatic pregnant women [53].

The high prevalence of microorganisms involved with STIs underscores the urgent need for comprehensive prevention and control programs including not only behavioral interventions but also screening and improvements in medical care. Sexually active women, with more than one sexual partner in the last 3 months, living in a rural area were associated with an increase of odds for ureaplasma infection. This study detected 50 positive samples for *U. urealyticum* and 183 for *U. parvum* and significant differences were observed between IL-6 and IL-1β concentrations in cervical samples among symptomatic and non-symptomatic women. Moreover, IL-1β was observed in higher concentration in women with *U. urealyticum* and *U. parvum* infection. Higher IL-6 only was observed in women with *U. parvum*.

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DECLARATION OF INTEREST

None.

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