Journal of Microbiology, Immunology and Infection (2018) 51, 220–225

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Prevalence of cervical colonization by *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Mycoplasma genitalium* in childbearing age women by a commercially available multiplex real-time PCR: An Italian observational multicentre study

Christian Leli [a,*], Antonella Mencacci [a], Maria Agnese Latino [b], Pierangelo Clerici [c], Mario Rassu [d], Stefano Perito [a], Roberto Castronari [a], Eleonora Pistoni [a], Eugenio Luciano [a], Daniela De Maria [b], Cristina Morazzoni [c], Michela Pascarella [d], Silvia Bozza [a], Alessandra Sensini [a]

[a] *Microbiology Section, Department of Experimental Medicine, University of Perugia, Santa Maria della Misericordia Hospital, Sant'Andrea delle Fratte, 06129, Perugia, Italy*
[b] *AO City of Health and Science, Department of Laboratory Medicine, Sant'Anna Hospital, Turin, Italy*
[c] *Microbiology Unit, Hospital of Legnano, 20025 Legnano, Milan, Italy*
[d] *Department of Microbiology, S. Bortolo Hospital, Vicenza, Italy*

Received 9 July 2016; received in revised form 13 November 2016; accepted 7 May 2017
Available online 28 June 2017

KEYWORDS
Genital mycoplasmas;
Mycoplasma genitalium;
Mycoplasma hominis;
Ureaplasma parvum;

Abstract *Background:* Mycoplasmas are frequently isolated from the genital tract. New molecular PCR-based methods for the detection of mycoplasmas can better define the real epidemiology of these microorganisms. The aim of this study was to evaluate the prevalence of mycoplasmas in a population of childbearing age women by means of PCR.
Methods: This 21-month multicentre observational study was conducted at four Italian clinical microbiology laboratories. Women reporting symptoms of vaginitis/cervicitis, or with history of

* Corresponding author. Fax: +39 075 578 4298.
E-mail address: christian.leli@libero.it (C. Leli).

http://dx.doi.org/10.1016/j.jmii.2017.05.004

Ureaplasma urealyticum;
Real-time PCR

infertility, pregnancy, miscarriage or preterm birth were included. Detection of *Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma hominis, Mycoplasma genitalium* was performed from cervical swabs by means of a commercially available multiplex real-time PCR.
Results: a total of 1761 women fulfilled the inclusion criteria and were included in the study. The overall prevalence was: *U. parvum* 38.3%, *U. urealyticum* 9%, *M. hominis* 8.6% and *M. genitalium* 0.6%. The proportion of foreign patients positive for *U. parvum* was significantly higher compared to Italian patients (37% vs 30.1%, $p = 0.007$) and also for overall mycoplasma colonization (53.4% vs 45.8%, $p = 0.011$). The number of symptomatic patients positive for *M. hominis* was significantly higher than that of negative controls (2.9% vs 1%, $p = 0.036$). A significant positive trend in mycoplasma colonization was found in relation to the pregnancy week for *U. urealyticum* ($p = 0.015$), *M. hominis* ($p = 0.044$) and for overall mycoplasma colonization ($p = 0.002$).
Conclusion: multiplex RT-PCR can be a valuable tool to evaluate the real epidemiology of cervical mycoplasma colonization.

Introduction

Mycoplasmas are frequently isolated in the genital tract.[1] The introduction of nucleic acid amplification techniques (NAATs) has changed the epidemiology of these microorganisms and has allowed to distinguish the formerly known species *Ureaplasma urealyticum* into two new species, namely, *Ureaplasma parvum* (previously *U. urealyticum* biovar 1) and *U. urealyticum* (previously *U. urealyticum* biovar 2).[2] There is still not conclusive evidence whether these microorganisms should be considered pathogens, or mere co-factors associated with genital infections.[3,4] Indeed, if some authors described them as common commensal inhabitants,[5–13] others found them associated to pathologic conditions.[14–21] These conflicting results could be due to several factors, including study design, studied population, evaluated outcome, site of sampling, and diagnostic method used, such as: serology, culture or polymerase chain reaction (PCR).[22] The use of new PCR-based methods for their detection can be of help to better define the epidemiology of these microorganisms in the genital tract.

The aim of this study was to evaluate the prevalence of mycoplasma colonization in a large population of childbearing age women attending a clinical microbiology laboratory reporting symptoms of vaginitis/cervicitis or for infertility, pregnancy, history of miscarriage or preterm birth and in healthy controls.

Methods

Study design

This was a multicentre observational study conducted from February 2013 to October 2014. This study was conducted at four Italian clinical microbiology laboratories: the Microbiology Section of the Department of Experimental Medicine of Santa Maria della Misericordia Hospital in Perugia, the Department of Laboratory Medicine of Sant'Anna Hospital in Turin, the Microbiology Unit of the Hospital of Legnano and the Department of Microbiology of S. Bortolo Hospital in Vicenza. All women attending any of the four centres involved, and fulfilling inclusion criteria, were included. Inclusion criteria were childbearing age (from 15 to 50 years of age), and only one of the following criteria for each patient: symptoms of vaginitis/cervicitis, infertility, pregnancy, history of miscarriage or preterm birth. Vaginal pain and/or discharge, pain during intercourse, itching, burning were considered as symptoms of vaginitis/cervicitis.[23] Exclusion criteria were: the presence of more than one of the above mentioned inclusion criteria in the same patient, antimicrobial therapy within 30 days prior to evaluation, language barrier, psychiatric conditions. Healthy asymptomatic patients referring to the laboratory because of a symptomatic partner, for a check-up, or for personal motivation (therefore without any of the above described inclusion criteria) were used as negative controls.

Ethic statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Research ethics committee approval for the study was obtained from the institutional review board (Comitato Etico Aziende Sanitarie No. 2396/14). Informed consent was obtained from all individual participants included in the study.

Anamnestic data

Anamnestic data were collected on a detailed standard questionnaire, administered by a physician, concerning biographical data and the reasons for the medical evaluation and sampling.

Vaginal sample collection, microscopic examination and culture

Three vaginal samples were obtained from each patient, collected with the aid of a disposable vaginal speculum and by sterile swabs: one sample for wet mount examination, one for Gram-staining, and one for culture. Vaginal pH was measured using indicator strips. All swabs were plated within two hours from sampling and cultured on different media. For yeast culture, Saboraud Dextrose Agar plates with 40 mg/ml of chloramphenicol (Becton Dickinson, Sparks, MD) were incubated at 37 °C and analyzed after 24 and 48 h. Wet mounts were examined for the presence of leukocytes, clue cells, *Trichomonas vaginalis* and yeasts. Bacterial vaginosis (BV) was diagnosed according to Amsel criteria.[24] The clinical diagnosis of BV required that three of four conditions be present: vaginal pH $>$ 4.7, abnormal vaginal discharge, positive whiff-amine test or clue cells on microscopy. Diagnosis of yeast colonization was done by microscopic examination and yeast identification was performed by the ID 32 C (bioMérieux, Marcy-l'Etoile, France) from colonies grown on solid media.

Molecular test for genital mycoplasmas detection

Cervical swab specimens were placed in 3-mL UTM Transport medium (Copan Italia S.p.A., Brescia, Italy). The collection tubes were equilibrated to room temperature and mixed by vortexing, and 1-mL mixed specimens were transferred to the sample cartridge. The DNA was extracted from the specimens using the instrument Nuclisense easyMag (bioMérieux, Marcy-l'Etoile, France), in accordance with the manufacturer's instructions, and stored frozen at −20 °C until testing. Detection of mycoplasmas was performed by means of the Anyplex™ II STI-7 Kit (STI-7 Seegene, Seoul, Korea), a commercially available multiplex real-time PCR relying on a newly developed TOCE™ (Tagging Oligonucleotide Cleavage and Extension) technology which allows to detect simultaneously seven microorganisms (*U. urealyticum, U. parvum, Mycoplasma hominis, Mycoplasma genitalium, Chlamydia trachomatis, Neisseria gonorrhoeae, T. vaginalis)*. Tests were performed according to the manufacturer's protocol. Briefly, the amplification was performed in a CFX96 real-time thermocycler (Bio-Rad, Hercules, CA, USA). Each PCR was performed in 5-μL of extracted DNA and 15-μL of Anyplex PCR Mix in a 20-μL final volume per reaction. The thermal cycle conditions consisted of an initial incubation at 50 °C for 4 min to activate the UDG system and prevent contamination, predenaturation at 95 °C for 15 min, followed by 50 cycles of alternating incubations: 95 °C for 30 s, 60 °C for 1 min, and 72 °C for 30 s. The melting temperature was analysed by increasing the reaction temperature from 55 °C to 85 °C (5 s/0.5 °C). The whole process was monitored adding to each sample 1 μL of internal control (provided by the manufacturer) before the DNA extraction, to confirm the DNA extraction and to exclude PCR inhibition. DNA quantification was performed using the default algorithm that calculates the amount of DNA denaturation by means of analysis of melting temperatures after 8, 14, and 20 cycles, and is expressed as number of DNA copies/reaction.

Statistics

Categorical variables were expressed as count and percentage. Tests for association were performed by chi-square or Fisher's exact test, as appropriate. Continuous variables were expressed as median and interquartile range (IQR), and median values were compared by means of the Mann−Whitney U-test. Statistical significance was assumed if a null hypothesis could be rejected at a p value of $\leq$0.05. Multivariate logistic regression analysis was performed to determine factors that were independently associated with symptoms of vaginitis/cervicitis. Covariates associated with symptoms of vaginitis/cervicitis at a level of significance $p \leq 0.05$ were selected for the multivariate analysis. The analyses were performed by SPSS 13.0 version.

Results

A total of 1761 women fulfilled the inclusion criteria and were included in the study. Demographic, anamnestic, and clinical characteristics of the population are summarized in Table 1. Among the 363 foreign patients, 197 (54.3%) were from eastern Europe, 69 (19%) from South America, 38 (10.5%) from North Africa, 25 (6.9%) from Sub-Saharan Africa, 14 (3.9%) from western Europe, 13 (3.6%) from Asia, 2 (0.5%) from Oceania, 2 (0.5%) from Middle East, 2 (0.5%) from North America, and 1 (0.3%) from Australia. Median age value of patients positive for mycoplasmas was significantly lower than that of patients negative [33 years, (IQR 25-38) vs 34 years (IQR 29-39), $p < 0.0001$]. Likewise, foreign patients were significantly younger than Italian patients [32 years (IQR 27-38) vs 34 years (IQR 27-39); $p = 0.014$]. Among the whole population, 994/1761 (56.4%) women were colonized by genital mycoplasmas, either by a single or by multiple species (Table 2). The mycoplasmas cervical prevalence according to nationality, pregnancy, infertility, history of miscarriage, preterm birth or presence of symptoms of vaginitis/cervicitis is described in Table 3. The proportion of foreign patients positive for *U. parvum* colonization was significantly higher compared to Italian patients (37% vs 30.1%, $p = 0.007$) and also for overall cervical colonization (53.4% vs 45.8%, $p = 0.011$). The rate

Table 1 Demographic, anamnestic and clinical characteristics of the whole population (n = 1761).

Variable	Values (IQR; %)
Age (years)	34 (27−39)
Foreign	363 (20.6)
Pregnant	210 (11.9)
Infertility	232 (13.2)
Miscarriage	104 (5.9)
Preterm birth	32 (1.8)
Bacterial vaginosis	193 (10.9)
Vaginal yeast colonization	276 (15.7)
Symptomatic	791 (44.9)
Negative controls	392 (22.3)

Values are expressed as median or count; IQR: interquartile range.

Table 2 Prevalence of mycoplasma colonization in the whole population (n = 1761).

Variable	Values, (%)
Overall prevalence (single + multiple colonization)	
Ureaplasma parvum	675 (38.3)
Ureaplasma urealyticum	159 (9.0)
Mycoplasma hominis	151 (8.6)
Mycoplasma genitalium	9 (0.5)
Single colonization	
Ureaplasma parvum	555 (31.5)
Ureaplasma urealyticum	94 (5.3)
Mycoplasma hominis	44 (2.5)
Mycoplasma genitalium	2 (0.1)
Double colonization	
Ureaplasma parvum + *Mycoplasma hominis*	68 (3.8)
Ureaplasma parvum + *Ureaplasma urealyticum*	29 (1.6)
Ureaplasma urealyticum + *Mycoplasma hominis*	20 (1.1)
Ureaplasma parvum + *Mycoplasma genitalium*	4 (0.2)
Triple colonization	
Ureaplasma parvum + *Ureaplasma urealyticum* + *Mycoplasma hominis*	16 (0.9)
Ureaplasma parvum + *Mycoplasma hominis* + *Mycoplasma genitalium*	3 (0.2)

Values are expressed as count (%).

of symptomatic patients positive for *M. hominis* was significantly higher than that of negative controls (2.9% vs 1%, $p = 0.036$). With respect to the other mycoplasmas, no association was found with symptoms of vaginitis/cervicitis (Table 3). Likewise, in regard to infertility, miscarriage and preterm birth, no significant association was found with the colonization rates by all the four mycoplasmas evaluated (Table 3).

Concerning the other pathogens identified by the multiplex real-time PCR, their prevalence was: *C. trachomatis* 46/1761 (2.6%), *N. gonorrhoeae* 1/1761 (0.06%), *T. vaginalis* 13/1761 (0.7%). The only significant association found was between the infection by *C. trachomatis* and symptoms of vaginitis/cervicitis (3.5% vs 1.9%, $p = 0.04$), no other significant association was found between these pathogens and symptoms of vaginitis/cervicitis, infertility, miscarriage or preterm birth.

Overall, 193 patients out of 1761 (10.9%) were positive for BV (Table 1). A significantly greater proportion of patients positive for BV reported symptoms of vaginitis/cervicitis (15.4%) compared to patients positive for BV without symptoms of vaginitis/cervicitis (7.3%, $p < 0.0001$). No association was found between BV and infertility (7.3% vs 11.5%, $p = 0.074$), miscarriage (9.6% vs 11%, $p = 0.771$) or preterm birth (9.4% vs 10.9%, $p = 1.0$).

With respect to vaginal yeast colonization, a total of 276 patients out of 1761 (15.7%) were positive (Table 1), and a

Table 3 Prevalence of mycoplasma colonization in the whole population (n = 1761) according to demographic, anamnestic and clinical data.

	UP only	UU only	MH only	MG only	UP + UU	UP + MH	UP + MG	UU + MH	UP + UU + MH	UP + MH + MG	Total positives	Negatives
Italian (n = 1398)	421 (30.1)	73 (5.2)	35 (2.5)	2 (0.1)	25 (1.8)	56 (4)	3 (0.2)	14 (1)	10 (0.7)	2 (0.1)	641 (45.8)	757 (54.2)
Foreigns (n = 363)	134 (37)*	21 (5.8)	9 (2.5)	0 (0)	4 (1.1)	12 (3.3)	1 (0.3)	6 (1.6)	6 (1.6)	1 (0.3)	194 (53.4)*	169 (46.6)
Pregnancy (n = 210)	59 (28)	11 (5.2)	5 (2.4)	0 (0)	3 (1.4)	7 (3.3)	0 (0)	1 (0.5)	0 (0)	0 (0)	86 (41)	124 (59)
Infertility (n = 232)	77 (33.2)	11 (4.7)	8 (3.4)	0 (0)	2 (0.9)	5 (2.1)	0 ()	1 (0.4)	1 (0.4)	0 (0)	105 (45.3)	127 (54.7)
Miscarriage (n = 104)	29 (27.8)	4 (3.8)	3 (2.8)	0 (0)	2 (1.9)	3 (2.8)	0 (0)	1 (0.9)	1 (0.9)	0 (0)	43 (41.3)	61 (58.7)
Preterm birth (n = 32)	11 (34.8)	1 (3.1)	1 (3.1)	0 (0)	0 (0)	2 (6.2)	0 (0)	0 (0)	0 (0)	0 (0)	15 (46.9)	17 (53.1)
Symptomatic (n = 791)	252 (31.8)	51 (6.4)	23 (2.9)*	0 (0)	12 (1.5)	39 (4.9)	2 (0.2)	15 (1.9)	9 (1.1)	3 (0.4)	406 (51.3)	385 (48.7)
Negative controls (n = 392)	127 (32.4)	16 (4.1)	4 (1)	2 (0.5)	10 (2.5)	12 (3.1)	2 (0.5)	2 (0.5)	5 (1.3)	0 (0)	180 (46)	212 (54)
Pregnancy trimester												
1st (n = 35)	10 (28.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (28.6)	25 (71.4)
2nd (n = 100)	24 (24)	4 (4)	1 (1)	0 (0)	2 (2)	3 (3)	0 (0)	0 (0)	0 (0)	0 (0)	34 (34)	66 (66)
3rd (n = 75)	25 (33.3)	7 (9.3)*	4 (5.3)*	0 (0)	1 (1.4)	4 (5.3)	0 (0)	1 (1.4)	0 (0)	0 (0)	42 (56)*	33 (44)

UP: *Ureaplasma parvum*; UU: *Ureaplasma urealyticum*; MH: *Mycoplasma hominis*; MG: *Mycoplasma genitalium*.
Data are expressed as count (%); * Statistically significant difference.

significantly greater proportion of patients positive for yeast colonization reported symptoms of vaginitis/cervicitis (19.8%) compared to patients without symptoms of vaginitis/cervicitis (12.3%, $p < 0.0001$). A greater proportion of patients positive for vaginal yeast colonization did not report problems of infertility (16.7%) compared to patients suffering from infertility (9%, $p = 0.004$). On the other hand, no association was found between yeast colonization and miscarriage (14.4% vs 15.7%, $p = 0.824$) or preterm birth (6.3% vs 15.8%, $p = 0.215$).

A significant positive trend in cervical mycoplasma colonization was found in relation to the pregnancy week (Table 3) for *U. urealyticum* ($p = 0.015$), *M. hominis* ($p = 0.044$) and for overall cervical colonization ($p = 0.002$).

The multivariate logistic regression analysis (Table 4) showed BV and vaginal yeast colonization as independent predictors of symptoms of vaginitis/cervicitis. Both *M. hominis* colonization and *C. trachomatis* infection were excluded from the regression model.

Discussion

The main aim of this study was to assess the prevalence of cervical colonization by *U. parvum*, *U. urealyticum*, *M. hominis* and *M. genitalium* in a population of childbearing outpatients, by means of a molecular test. The prevalence of genital mycoplasmas found in this study matches that found by Kim et al.[25] from 799 endocervical swab samples collected from healthy women. By means of the same multiplex real-time PCR assay, a prevalence of 32.5% for *U. parvum*, of 3.5% for *U. urealyticum* and of 1% for *M. hominis*, as single colonization, were found.[25] Kasprzykowska et al.[26] in a study carried out on consecutive 40 women admitted to a Gynecology Clinic for diagnostics laparoscopy, found an overall prevalence of 52.5%, similar to that of this study. On the other hand, McIver et al.[27] on a total of 233 cervical swabs from 175 women attending a sexual-health clinic, found a different prevalence: *U. parvum* 48%, *U. urealyticum* 2.8%, *M. hominis* 6.4%, *M. genitalium* 1.3%. Nevertheless, in that study, another RT-PCR assay was used, and the population evaluated was different. Similarly, Choe et al.[28] on 201 endocervical swabs found a higher prevalence of colonization, but in that study the specimens were tested in parallel using four NAATs and one mycoplasma detection kit, and the criteria to define a positive result were also different. It has been clearly demonstrated that PCR method has a higher sensitivity than culture,[29] consequently some studies based on mycoplasma culture reported lower prevalence values. Indeed, De Francesco et al.[30] in a previous Italian study, found a prevalence of 18.6% for genital mycoplasmas, and Wang et al.[31] in a study on 6051 female outpatients, found a prevalence of 33.9%. In our study *M. hominis* cervical colonization was associated with symptoms of vaginitis/cervicitis. This result could be due to BV. It has been already shown indeed that *M. hominis* is more often identified in women with BV,[32] and the multivariate logistic regression analysis showed that BV is a variable independently associated with symptoms of vaginitis/cervicitis, whereas *M. hominis* colonization was excluded from the model. On the other hand, the lack of association between symptoms of vaginitis/cervicitis and *U. parvum* and/or *U. urealyticum* colonization rates has been already reported by Kasprzykowska et al.[26] Likewise, a recent review[22] highlighted how in several reports no association between *M. hominis* and/or *U. urealyticum* colonization rates and adverse pregnancy outcomes was found.

Table 4 Multivariate logistic regression of predictive variables of symptoms of vaginitis/cervicitis.

Variable	Odds ratio	95% confidence interval	*P*
Bacterial vaginosis	2.132	1.404—3.237	<0.0001
Vaginal yeast colonization	1.890	1.327—2.692	<0.0001
Mycoplasma hominis colonization	2.860	0.974—8.393	0.057
Chlamydia trachomatis infection	1.343	0.639—2.827	0.437

The significant positive association between BV or vaginal yeast colonization and symptoms of vaginitis/cervicitis was expected, since both BV and vaginal yeast colonization are the most common infectious causes of that clinical picture.[33] Indeed, these two variables were the only two independently associated with symptoms of vaginitis/cervicitis.

The significant increase in the prevalence of cervical colonization by mycoplasmas throughout pregnancy has already been described by Luton et al.[34] in 218 pregnant women followed from before 20 weeks gestational age through delivery.

The strengths of this study are represented by: the large sample size, that gives enough statistical power; the use of a standardized questionnaire, unique for all centres and administered by the same physician in each centre; the use of the same diagnostic procedure in all the centres. In addition, periodic revisions of the quality of the data (processing and editing), and the final analysis were centralized and performed in only one of the centres by the same principal investigator, to avoid the possible variability of interpretation.

The limitations of the study were that we could not compare the RT-PCR results with the reference culture method,[1] and we could not follow the patients for the possible occurrence of pregnancy adverse events, due to the cross-sectional nature of the study.

In conclusion, the use of RT-PCR for identification of mycoplasmas on cervical samples showed a higher rate of recovery in comparison to culture methods. A future better clarification of the clinical significance of these microorganisms will guide the possible treatment of asymptomatic patients colonized by mycoplasmas and reduce the risk of obstetric complications.

Conflict of interest

The authors have no conflicts of interest to declare.

References

1. Waites KB, Taylor-Robinson D. Mycoplasma and ureaplasma. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. *Manual of clinical microbiology*. 11th ed. Washington DC: ASM Press; 2015. p. 1088—105.
2. Kong F, James G, Ma Z, Gordon S, Bin W, Gilbert GL. Phylogenetic analysis of *Ureaplasma urealyticum*—support for the establishment of a new species, Ureaplasma *parvum*. *Int J Syst Bacteriol* 1999;**49**:1879—89.
3. Patel MA, Nyirjesy P. Role of Mycoplasma and Ureaplasma species in female lower genital tract infections. *Curr Infect Dis Rep* 2010;**12**:417—22.
4. Taylor-Robinson D, Lamont RF. Mycoplasmas in pregnancy. *BJOG* 2011;**118**:164—74.
5. Romero R, Mazor M, Oyarzun E, Sirtori M, Wu YK, Hobbins JC. Is genital colonization with *Mycoplasma hominis* or *Ureaplasma urealyticum* associated with prematurity/low birth weight? *Obstet Gynecol* 1989;**73**:532—6.
6. Carey JC, Blackwelder WC, Nugent RP, Matteson MA, Rao AV, Eschenbach DA, et al. Antepartum cultures for *Ureaplasma urealyticum* are not useful in predicting pregnancy outcome. The Vaginal Infections and Prematurity Study Group. *Am J Obstet Gynecol* 1991;**164**:728—33.
7. Arya OP, Tong CY, Hart CA, Pratt BC, Hughes S, Roberts P, et al. Is *Mycoplasma hominis* a vaginal pathogen? *Sex Transm Infect* 2001;**77**:58—62.
8. Guven MA, Dilek U, Pata O, Dilek S, Ciragil P. Prevalence of *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* infections in the unexplained infertile women. *Arch Gynecol Obstet* 2007;**276**:219—23.
9. Rezeberga D, Lazdane G, Kroica J, Sokolova L, Donders GG. Placental histological inflammation and reproductive tract infections in a low risk pregnant population in Latvia. *Acta Obstet Gynecol Scand* 2008;**87**:360—5.
10. Govender S, Theron GB, Odendaal HJ, Chalkley LJ. Prevalence of genital mycoplasmas, ureaplasmas and chlamydia in pregnancy. *J Obstet Gynaecol* 2009;**29**:698—701.
11. Günyeli I, Abike F, Dünder I, Aslan C, Tapısız OL, Temizkan O, et al. Chlamydia, Mycoplasma and Ureaplasma infections in infertile couples and effects of these infections on fertility. *Arch Gynecol Obstet* 2011;**283**:379—85.
12. Choi SJ, Park SD, Jang IH, Uh Y, Lee A. The prevalence of vaginal microorganisms in pregnant women with preterm labor and preterm birth. *Ann Lab Med* 2012;**32**:194—200.
13. Marovt M, Keše D, Kotar T, Kmet N, Miljković J, Šoba B, et al. *Ureaplasma parvum* and *Ureaplasma urealyticum* detected with the same frequency among women with and without symptoms of urogenital tract infection. *Eur J Clin Microbiol Infect Dis* 2015;**34**:1237—45.
14. Mårdh PA, Weström L. Tubal and cervical cultures in acute salpingitis with special reference to *Mycoplasma hominis* and T-strain mycoplasmas. *Br J Vener Dis* 1970;**46**:179—86.
15. Friberg J. Genital mycoplasma infections. *Am J Obstet Gynecol* 1978;**132**:573—8.
16. Stray-Pedersen B, Bruu AL, Molne K. Infertility and uterine colonization with *Ureaplasma urealyticum*. *Acta Obstet Gynecol Scand* 1982;**61**:21—4.
17. Donders GG, Bosmans E, Dekeersmaecker A, Vereecken A, Van Bulck B, Spitz B. Pathogenesis of abnormal vaginal bacterial flora. *Am J Obstet Gynecol* 2000;**182**:872—8.
18. Gerber S, Vial Y, Hohlfeld P, Witkin SS. Detection of *Ureaplasma urealyticum* in second-trimester amniotic fluid by polymerase chain reaction correlates with subsequent preterm labor and delivery. *J Infect Dis* 2003;**187**:518—21.
19. Nguyen DP, Gerber S, Hohlfeld P, Sandrine G, Witkin SS. *Mycoplasma hominis* in mid-trimester amniotic fluid: relation to pregnancy outcome. *J Perinat Med* 2004;**32**:323—6.
20. Gupta A, Gupta A, Gupta S, Mittal A, Chandra P, Gill AK. Correlation of mycoplasma with unexplained infertility. *Arch Gynecol Obstet* 2009;**280**:981—5.
21. Vouga M, Greub G, Prod'hom G, Durussel C, Roth-Kleiner M, Vasilevsky S, et al. Treatment of genital mycoplasma in colonized pregnant women in late pregnancy is associated with a lower rate of premature labour and neonatal complications. *Clin Microbiol Infect* 2014;**20**:1074—9.
22. Capoccia R, Greub G, Baud D. *Ureaplasma urealyticum*, *Mycoplasma hominis* and adverse pregnancy outcomes. *Curr Opin Infect Dis* 2013;**26**:231—40.
23. Landers DV. Vaginitis/cervicitis: diagnosis and treatment options in a limited resource environment. *Womens Health Issues* 1996;**6**:342—8.
24. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;**1**:1422.
25. Kim Y, Kim J, Lee KA. Prevalence of sexually transmitted infections among healthy Korean women: implications of multiplex PCR pathogen detection on antibiotic therapy. *J Infect Chemother* 2014;**20**:74—6.
26. Kasprzykowska U, Elias J, Elias M, Mączyńska B, Sobieszczańska BM. Colonization of the lower urogenital tract with *Ureaplasma parvum* can cause asymptomatic infection of the upper reproductive system in women: a preliminary study. *Arch Gynecol Obstet* 2014;**289**:1129—34.
27. McIver CJ, Rismanto N, Smith C, Naing ZW, Rayner B, Lusk MJ, et al. Multiplex PCR testing detection of higher-than-expected rates of cervical mycoplasma, ureaplasma, and trichomonas and viral agent infections in sexually active australian women. *J Clin Microbiol* 2009;**47**:1358—63.
28. Choe HS, Lee DS, Lee SJ, Hong SH, Park DC, Lee MK, 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**: e1134—1140.
29. Stellrecht KA, Woron AM, Mishrik NG, Venezia RA. Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. *J Clin Microbiol* 2004;**42**:1528—33.
30. De Francesco MA, Caracciolo S, Bonfanti C, Manca N. Incidence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* isolated in Brescia, Italy, over 7 years. *J Infect Chemother* 2013;**19**:621—7.
31. Wang QY, Li RH, Zheng LQ, Shang XH. Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in female outpatients, 2009-2013. *J Microbiol Immunol Infect* 2016;**49**:359—62.
32. Keane FE, Thomas BJ, Gilroy CB, Renton A, Taylor-Robinson D. The association of *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Mycoplasma genitalium* with bacterial vaginosis: observations on heterosexual women and their male partners. *Int J STD AIDS* 2000;**11**:356—60.
33. Hainer BL, Gibson MV. Vaginitis. *Am Fam Physician* 2011;**83**: 807—15.
34. Luton D, Ville Y, Luton-Sigy A, Cousin C, Narraido B, Fassasi-Jarretou A, et al. Prevalence and influence of *Mycoplasma hominis* and *Ureaplasma urealyticum* in 218 African pregnant women and their infants. *Eur J Obstet Gynecol Reprod Biol* 1994;**56**:95—101.