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Retrospective Analysis of the *Ureaplasma* spp. Prevalence with Reference to Other Genital Tract Infections in Women of Reproductive Age

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Abstract

Ureaplasma spp. are frequently isolated from the genital tract of women of reproductive age. To date, it remains unclear whether they are commensal or pathogenic. In our study, we assessed the prevalence of *Ureaplasma* spp. in a group of 1,155 women of childbearing age. In addition, we assessed how often women with positive *Ureaplasma* spp. develop genital tract co-infections and how the vaginal pH changes. This study showed a relationship between colonization by *Ureaplasma* spp. and presenting symptoms. In fact, we showed that colonization of the genital tract by *Ureaplasma* spp.

can affect the occurrence of co-infections such as *Gardnerella vaginalis*. We also observed a relationship between increased pH values and the presence of *Ureaplasma* spp.

K e y w o r d s: female genital tract microbiota, *Ureaplasma* spp., bacterial vaginosis, vaginal infections

Introduction

The vaginal microbiota demonstrates its important role in the reproductive age women's health or disease. Many studies show that *Lactobacillus* spp. dominates the vaginal microbiota of healthy women (Ravel et al. 2011; Gajer et al. 2012; Kiecka et al. 2021). Due to *Lactobacillus* spp. colonization, a pH value up to 4 is maintained, and the binding of pathogenic microorganisms to vaginal epithelial cells is blocked. Bacteriocins, H_2O_2, and lactic acid produced by lactobacilli can inhibit the growth of different microorganisms (Aroutcheva et al. 2001; Vallor et al. 2001; Karaoğlu et al. 2003; Amabebe and Anumba 2018).

Ureaplasma spp. are frequently isolated from the genital tract of women at childbearing age (Leli et al. 2018). Based on nucleic acid amplification techniques (NAAT) *Ureaplasma urealyticum* species was split into *Ureaplasma parvum* and *U. urealyticum*. Despite the many studies conducted, it has not been resolved whether *Ureaplasma* spp. are pathogenic or commensal. Some authors describe *Ureaplasma* spp. as pathogenic in nongonococcal urethritis in men (NGU), pyelonephritis, endometritis (Kundsin et al. 1996; Dewan et al. 1997), and pregnancy complications (Horowitz et al. 1995; Abele-Horn et al. 1997; Harada et al. 2008) while others consider them commensal (Donders et al. 2017). These problems in determining their impact on women's health may be related to the design of a study and the diagnostic technique used: culture, serology, or polymerase chain reaction (PCR), among others.

The aim of this study was to assess the prevalence of *Ureaplasma* spp. colonization in a large group of women of childbearing age reporting to a microbiology laboratory for follow-up examinations or by presenting symptoms (itching, burning, discharge, pain, or discomfort). In addition, the objective was to determine the prevalence and co-occurrence of other microorganisms, both common components of the genital tract microbiota and pathogens.

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Experimental

Materials and Methods

Study population. This study was a retrospective analysis of the populations of women aged 18 to 49 who had microbiological tests of the genital tract in the Microbiology and Autovaccine Research Center in Cracow, Poland, from April to August 2019, as recommended by a gynecologist. All included patients had to undergo a set of tests such as pH determination, microscopic examination of vaginal smear, microbial culture, and molecular testing for *Chlamydia trachomatis*, *Mycoplasma hominis*, *U. urealyticum*, *U. parvum*, *Mycoplasma genitalium*. Moreover, a thorough history of reported symptoms and previous antibiotic treatment were recorded. Women with a history of antibiotic treatment within the past two months were excluded from the study. Information on antibiotic treatment was obtained from the worksheets and referrals for examinations from attending physicians. The following patient data were used for analysis: age, symptoms--reported complaints, pH of vaginal contents, *Lactobacillus* presence or absence, presence of other bacteria, yeast-like fungi, and *Trichomonas vaginalis*.

The clinical signs divide the patients into symptomatic and asymptomatic groups. The group of symptomatic women included those who reported at least one symptom in the genital tract: itching, burning, discharge, pain, or discomfort. The asymptomatic group reported no such complaints.

Microbiological methods. The material for microbiological examination consisted of two vaginal swabs (one for Gram-staining microscopy and one for culture) and one cervical swab for molecular testing (NAAT). The vaginal fluid pH was measurand during sample collecting. All tests were performed at the Microbiology and Autovaccination Research Center in Cracow, Poland.

Swabs were taken from the posterior vaginal vault and endocervical canal using a sterile speculum. The vaginal content remaining on the speculum was used to measure the pH value using pH indicator strips with a pH range of 2.0–9.0 (MERCK, Germany). One vaginal swab was used for Gram staining with PREVI® Color Gram stainer (bioMerieux, France). The vaginal Gram stain smears were assessed using the scale according to Kuczyńska modified by Kasprowicz (Kasprowicz and Białecka 2012). The presence of leukocytes, lactobacilli, other bacteria, fungi, and clue cells was assessed based on an abundance scale ranging from 0 to 3 in oil-immersion microscope fields (× 1000). The degrees III, III/IV, and IV with clue cells detected indicated BV, which is comparable to a score from 7 to 10 according to Nugent criteria (Nugent et al. 1991; Kuczyńska 2003; Kasprowicz and Białecka, 2008). The specimen from the first vaginal swab was also inoculated in Trichomedium (GRASO, Poland). After 48 hours of culture, a direct preparation was made and viewed under a microscope (200 × magnification) to identify *T. vaginalis*.

The second vaginal swab was used to perform cultures on TSA with 5% sheep blood (GRASO, Poland) for the detection of aerobic bacteria, Gardnerella Agar (bioMerieux, France) for anaerobic bacteria, CANDIselect (BioRad, USA) for yeasts, and Chocolate Agar + P.V.S. + VCAT (BioRad, USA) for *Neisseria gonorrhoeae*. The media were incubated for 24 h or 48 h at 35°C under appropriate conditions. The cultured microorganisms were isolated and identified using VITEK2 (bioMerieux, France) or MALDI-TOF MS (Brucker, USA).

Material collected from the cervical swabs was preserved on UTM medium (Copan Italia, Italy) and tested for *C. trachomatis*, *M. hominis*, *U. urealyticum*, *U. parvum,* and *M. genitalium*. The DNA was isolated with croBEE 201A Nucleic Acid Extraction Kit (GeneProof, Czech Republic) with croBEE NA16 Nucleic Acid Extraction System (GeneProof, Czech Republic) according to the manufacturer's instructions. The NAAT was performed with a commercially available PCR – AmpliSens test, according to the manufacturer's instructions in a Cobas Z480 real-time thermocycler (ROCHE, Switzerland).

Statistical analysis. A language and environment for statistical computing R were used for statistical analysis (R Core Team 2022). The significance level was assumed at $p = 0.05$. The Chi-square test of independence, Fisher exact test (for qualitative features) and Mann-Whitney U test (for quantitative features) were used to compare distributions of variables across two independent groups. The odds ratios [ORs] with 95% confidence intervals [CIs] were calculated with Firth's bias reduction method of logistic regression with the Wald test (Wang 2014, Heinze et al. 2022). The multivariable logistic regression model was built and evaluated to associate various independent factors, such as the presence of different microorganisms or the age of women, with clinical symptoms or elevated pH. The Akaike information criterion was extracted and based on this statistic best-suited (with minimal AIC), the most parsimonious models were selected from a collection of fitted models. Bearing in mind the possible coexistence of some microorganisms, we tested second-order interactions between them. Due to non-significant results, none of the interaction terms was incorporated into the final model.

Results

Study patients' population. A total of 1,155 women aged from 18 to 49 years were included in the study. Seven hundred fifty-six women in the study group did not report symptoms, while 399 women presented symptoms.

Among 1,155 women, *Lactobacillus* spp. was found in vaginal swabs of 1,107 (95.8%) (single/few/numerous lactobacilli). The differences in the abundance of *Lactobacillus* spp. in the patients' group (n = 1,107) were analyzed; 964 women presented the vaginal smear with few or numerous lactobacilli, and 143 presented only single lactobacilli.

Ureaplasma spp. was detected in 274 women (23.7%). Aerobic bacteria were identified in 153 vaginal swabs (13.2%), and yeast-like fungi in 192 (16.6%). *Gardnerella vaginalis* was found in 56 specimens (4.8%) from patients with vaginosis. *M. hominis* (1.8%) and sexually transmitted pathogens *C. trachomatis* (0.7%) and *M. genitalium* (0.2%) were found rarely in the study group. *T. vaginalis* and *N. gonorrhoeae*, two other etiological agents of STI, were not identified in any specimen in this study. Complete data on genital tract microorganisms' prevalence are summarized in Table I.

The occurrence of urogenital mycoplasmas (*U. parvum, U. urealyticum,* and *M. hominis*) was analyzed regarding multiple colonization with these species and co-infection with *C. trachomatis* and *M. genitalium*. The overall incidence of urogenital mycoplasmas in this study group was 24.22%. Most patients (23.02%) presented single mycoplasma species in the genital tract. Both double (1.03%) and triple mycoplasma colonization (0.17%) and co-infections of urogenital mycoplasmas with *C. trachomatis* and *M. genitalium* (0.35%) were very rarely observed (Fig. 1).

Urogenital microorganisms and symptoms. Our data show that the presence of different microorganisms in the genital tract is associated with symptoms including itching, burning, discharge, pain, and discomfort. The results of univariable and multivariable logistic regression models are depicted in Table I and II, respectively.

Univariable analysis showed that women in whom *G. vaginalis* was isolated were more likely to report symptoms (OR = 8.32, 95% CI: 4.30–16.08). Women with identified *Ureaplasma* spp. were about four times more likely to be symptomatic (OR = 4.26, 95% CI: 3.21–5.66), and a similar trend was found for *C. trachomatis*, but the group of *C. trachomatis* positives

Table I
The prevalence of specific microorganisms across groups of women manifesting or not clinical symptoms.

Microorganisms	Microbiological method	Total	Without symptoms (n = 756)	With symptoms (n = 399)	p^*	OR*** (95% CI)	p^{**}
		n (%)	n (%)	n (%)			
Lactobacillus spp.	microscopic	1,107 (95.8)	737 (97.5)	370 (92.7)	< 0.001	0.33 (0.18–0.60)	< 0.001
Gardnerella vaginalis	microscopic, culture	56 (4.8)	11 (1.5)	45 (11.3)	< 0.001	8.32 (4.30–16.08)	< 0.001
Ureaplasma spp.	PCR	276 (23.9)	108 (14.3)	166 (41.6)	< 0.001	4.26 (3.21–5.66)	< 0.001
Ureaplasma parvum	PCR	187 (16.2)	81 (10.7)	106 (26.6)	< 0.001	3.01 (2.19–4.14)	< 0.001
Ureaplasma urealyticum	PCR	89 (7.7)	27 (3.6)	62 (15.5)	< 0.001	4.91 (3.08–7.83)	< 0.001
Mycoplasma hominis	PCR	21 (1.8)	0 (0.0)	21 (5.3)	< 0.001#	85.94 (5.19–1,422.62)	0.002
Mycoplasma genitalium	PCR	2 (0.2)	0 (0.0)	2 (0.5)	0.119#	9.52 (0.46–198.69)	0.146
Chlamydia trachomatis	PCR	8 (0.7)	1 (0.1)	7 (1.8)	0.003#	9.62 (1.66–55.79)	0.012
Aerobic bacteria (with GBS)	culture	153 (13.2)	95 (12.6)	58 (14.5)	0.396	1.19 (0.84–1.68)	0.339
Streptococcus group B (GBS)	culture	88 (7.6)	57 (7.5)	31 (7.8)	0.981	1.04 (0.66–1.63)	0.865
Yeast	microscopic, culture	192 (16.6)	100 (13.2)	92 (23.1)	< 0.001	1.97 (1.44–2.69)	< 0.001
Neisseria gonorrhoeae	culture		–	–	–	–	–
Trichomonas vaginalis	culture		–	–	–	–	–

* – all *p*-values denoted by # are from Fisher's exact test; the remaining comparisons are made with the chi-square test of independence
** – *p*-values calculated by Wald method using Firth's logistic regression
*** – OR of prevalence symptoms in women with specific microorganisms detected when compared with those without symptoms

Table II
Association between the prevalence of microorganisms and clinical symptoms – multivariable analyses. The fully adjusted model and the most parsimonious model.

Microorganisms	Model 1*		Model 2**	
	OR*** (95% CI)	p**	OR*** (95% CI)	p**
Ureaplasma parvum	3.06 (2.17–4.31)	< 0.001	3.07 (2.18–4.31)	< 0.001
Ureaplasma urealyticum	5.25 (3.20–8.62)	< 0.001	5.20 (3.17–8.51)	< 0.001
Mycoplasma hominis	49.30 (3.09–787.71)	0.006	48.97 (2.94–815.88)	0.007
Chlamydia trachomatis	10.03 (1.66–60.42)	0.012	10.04 (1.66–60.66)	0.012
Lactobacillus spp.	0.47 (0.23–0.98)	0.045	0.48 (0.24–0.94)	0.033
Gardnerella vaginalis	3.92 (1.89–8.14)	< 0.001	3.87 (1.90–7.91)	< 0.001
Yeast	1.73 (1.23–2.43)	0.002	1.73 (1.23–2.43)	0.002
DF	12		7	
AICc	207.5		197.8	
ΔAICc	9.7		0.0	

* – Model 1 – a fully adjusted model to variables presented in Table II and additionally to *Mycoplasma genitalium*, *Streptococcus* group B, aerobic bacteria (with GBS), age, pH > 4.5
** – Model 1 – the most parsimonious model adjusted to variables presented in Table II
*** – OR of prevalence symptoms in women with detected specific microorganisms compared with those without symptoms

was small, so estimates were imprecise (OR = 9.62, 95% CI: 1.66–55.79). All 21 women diagnosed with *M. hominis* were symptomatic in the study group. The data on the diagnosis of *M. genitalium* was not significant, as it was identified in only two patients. The results also showed a positive correlation between candidiasis and symptoms in the analyzed group of women. In the present study, neither the prevalence of aerobic bacteria, nor Gram-negative and Gram-positive, like *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp., nor the prevalence of GBS was statistically different in women manifesting symptoms compared to the non-symptomatic women. Complete data on the presence of specific microorganisms in the genital tract and their relationship to symptoms are presented in Table I.

Multivariable analysis found the vaginal colonization by *G. vaginalis*, urogenital *Mycoplasma* spp., *Chlamydia*, and yeast-like fungi as independent predictors of vaginitis symptoms, while *Lactobacillus* spp. was shown as protective against it (Table II).

***Ureaplasma parvum* and co-infections.** Among 1,155 examined women, 274 women had a positive PCR result for *Ureaplasma* spp., 187 for *U. parvum* (68%), and 89 cases for *U. urealyticum* (32%). Both *U. urealyticum* and *U. parvum* were diagnosed in two women. Genital tract colonization by *U. parvum* more than for times increased the chance of bacterial vaginosis with *G. vaginalis* (OR = 4.30, 95% CI: 2.44–7.49), almost tripled the prevalence of *M. hominis* infection (1.4% vs. 3.7%), and contributed to the more frequently recorded infection by yeast-like fungi (OR = 1.58, 95% CI: 1.07–2.32). However, it also significantly reduced the chance of *U. urealyticum* detection (OR = 0.11, 95% CI: 0.03–0.45) (Table III).

Among the 187 women with positive *U. parvum*, 81 showed no symptoms, and 106 reported symptoms. The presence of *Lactobacillus* spp. was analyzed in both groups and was at a similar level (Table I). The prevalence of *U. parvum* increased the chance of clinical symptoms about three times (OR = 3.07, 95% CI: 2.18–4.31) (Table II).

***Ureaplasma urealyticum* and co-infections.** The colonization of the genital tract by *U. urealyticum* increased four times the chance of bacterial vaginosis (OR = 4.07, 95% CI: 2.10–7.89). The possibility of *M. hominis* detection was also significantly increased (OR = 8.00, 95% CI: 3.22–19.86*)* (Table IV).

Among 89 women with positive *U. urealyticum*, 27 were asymptomatic, while 62 women were symptomatic. In all asymptomatic women with *U. urealyticum*, the presence of *Lactobacillus* spp. in the vagina was

Table III
Relationship between the presence of *U. parvum* and other microorganisms.

Microorganisms	Without *Ureaplasma parvum* (n = 968)	With *Ureaplasma parvum* (n = 187)	p^*	OR*** (95% CI)	p^{**}
	n (%)	n (%)			
Lactobacillus spp.	930 (96.1)	177 (94.7)	0.489	0.72 (0.35–1.48)	0.372
Garderella vaginalis	32 (3.3)	24 (12.8)	< 0.001	4.30 (2.44–7.49)	< 0.001
Chlamydia trachomatis	5 (0.5)	3 (1.6)	0.125#	2.60 (0.86–12.81)	0.101
Mycoplasma hominis	14 (1.4)	7 (3.7)	0.064#	2.65 (1.05–6.66)	0.0313
Mycoplasma genitalium	2 (0.2)	0 (0.0)	1.000#	0.00 (0.05–21.56)	0.534
Ureaplasma urealyticum	87 (9.0)	2 (1.1)	< 0.001	0.11 (0.03–0.45)	< 0.001
Aerobic bacteria	134 (13.8)	19 (10.2)	0.214	0.70 (0.42–1.17)	0.174
Yeast	150 (15.5)	42 (22.5)	0.025	1.58 (1.07–2.32)	0.019
Streptococcus group B	82 (8.5)	6 (3.2)	0.020	0.36 (0.14–0.77)	0.017

* – all *p*-values denoted by # are from Fisher's exact test; the remaining comparisons are made with the chi-square test of independence
** – *p*-values calculated by Wald method using Firth's logistic regression
*** – OR of prevalence of specific microorganisms in women with *Ureaplasma parvum* compared with those without *Ureaplasma parvum*

Table IV
Relationship between the presence of *U. urealyticum* and other microorganisms.

Microorganisms	Without *Ureaplasma urealyticum* (n = 1,066)	With *Ureaplasma urealyticum* (n = 89)	p^*	OR*** (95% CI)	p^{**}
	n (%)	n (%)			
Lactobacillus spp.	1,024 (96.1)	83 (93.3)	0.319	0.57 (0.23–1.37)	0.203
Garderella vaginalis	43 (4.0)	13 (14.6)	< 0.001	4.07 (2.10–7.89)	< 0.001
Chlamydia trachomatis	8 (0.8)	0 (0.0)	1.000#	0.00 (0.04–12.15)	0.412
Mycoplasma hominis	13 (1.2)	8 (9.0)	< 0.001#	8 (3.22–19.86)	< 0.001
Mycoplasma genitalium	1 (0.1)	1 (1.1)	0.148#	5.98 (1.24–116.95)	0.025
Ureaplasma parvum	185 (17.4)	2 (2.2)	< 0.001	0.11 (0.03–0.45)	< 0.001
Aerobic bacteria	137 (12.9)	16 (18.0)	0.227	1.48 (0.84–2.63)	0.171
Yeast	173 (16.2)	19 (21.3)	0.272	1.40 (0.82–2.39)	0.213
Streptococcus group B	77 (7.2)	11 (12.4)	0.122	1.81 (0.88–3.42)	0.083

* – all *p*-values denoted by # are from Fisher's exact test; the remaining comparisons are made with the chi-square test of independence
** – *p*-values calculated by Wald method using Firth's logistic regression
*** – OR of prevalence specific microorganisms in women with *Ureaplasma urealyticum* compared with those without *Ureaplasma urealyticum*

Table V
Relationship between the detection of *Ureaplasma* spp. and pH > 4.5 regarding *Gardnerella vaginalis* coinfections.

	pH ≤ 4.5 n (%)	pH > 4.5 n (%)	OR*** (95% CI)	*p***
Ureaplasma spp.	180 (15.6%)	94 (8.1%)	2.1 (1.56–2.84)	< 0.005
Ureaplasma spp. without *Garnerella vaginalis* coinfection	173 (15.7%)	65 (5.9%)	1.60 (1.15–2.24)	0.004

** – *p*-values calculated by Wald method using Firth's logistic regression
*** – OR of prevalence pH > 4.5 in women with *Ureaplasma* spp. (without *Garderella vaginalis* coinfection) compared with those without *Ureaplasma* spp. infection

Table VI
Distribution of vaginal pH values in women with and without the presence of specific microorganisms.

Microorganisms	Not prevalent		Prevalent		*p**	pH > 4.5 OR (95% CI)**	*p***
	n	Q2 (Q1-Q3)	n	Q2 (Q1-Q3)			
Lactobacillus spp.	48	5.5 (5.0–5.5)	1,107	4.5 (4.0–4.5)	< 0.001	0.01 (0.00–0.05)	< 0.001
Gardnerella vaginalis	1,099	4.5 (4.0–4.5)	56	5.0 (4.9–5.5)	< 0.001	11.18 (6.05–20.65)	< 0.001
Ureaplasma spp.	881	4.5 (4.0–4.5)	274	4.5 (4.5–5.0)	< 0.001	2.09 (1.55–2.82)	< 0.001
Ureaplasma parvum	968	4.5 (4.0–4.5)	187	4.5 (4.5–5.0)	0.001	1.71 (1.21–2.41)	0.002
Ureaplasma urealyticum	1,066	4.5 (4.0–4.5)	89	4.5 (4.5–5.0)	0.004	2.42 (1.55–3.78)	< 0.001
Mycoplasma hominis	1,134	4.5 (4.0–4.5)	21	4.5 (4.5–5.0)	0.008	3.07 (1.31–7.16)	0.010
Aerobic bacteria (with GBS)	1,002	4.5 (4.0–4.5)	153	5.0 (4.5–5.0)	< 0.001	7.44 (5.17–10.69)	< 0.001
Streptococcus group B	1,067	4.5 (4.0–4.5)	88	5.0 (4.5–5.1)	< 0.001	5.89 (3.76–9.24)	< 0.001
Yeast	963	4.5 (4.0–4.5)	192	4.5 (4.5–5.0)	0.003	1.49 (1.06–2.10)	0.023

* – based on Mann-Whitney *U* test
** – based on logistic regression, crude models, Firth method, OR of prevalence pH > 4.5 in women with specific microorganisms compared with those without their presence Q2 (Q1-Q3) – median (interquartile range)

Table VII
Association between the prevalence of microorganisms and pH > 4.5 – multivariable analyses. The fully adjusted model and the most parsimonious model.

Microorganisms	Model 1*		Model 2**	
	OR (95% CI)	*p****	OR (95% CI)	*p****
Ureaplasma parvum	1.81 (1.21–2.73)	0.004	1.86 (1.24–2.79)	0.003
Ureaplasma urealyticum	2.23 (1.31–3.78)	0.003	2.32 (1.37–3.92)	0.002
Lactobacillus spp.	0.03 (0.01–0.11)	< 0.001	0.03 (0.01–0.11)	< 0.001
Gardnerella vaginalis	5.91 (2.91–12.00)	< 0.001	6.54 (3.25–13.18)	< 0.001
Aerobic bacteria (with GBS)	7.31 (4.14–12.91)	< 0.001	6.85 (4.61–10.18)	< 0.001
DF	11		5	
AICc	287.7		275.0	
ΔAICc	12.7		0.0	

* – Model 1 – fully adjusted model variables presented in the Table and additionally to yeast, Streptococcus group B, *Mycoplasma hominis*, *Mycoplasma genitalium, Chlamydia trachomatis*, and age
** – Model 2 – the most parsimonious model, adjusted to variables presented in the Table; OR of prevalence pH > 4.5
*** – *p*-values calculated by the Wald method using Firth's logistic regression

demonstrated (Table I). The prevalence of *U. urealyticum* increased about five times the chance of clinical symptoms (OR = 5.20, 95% CI: 3.17–8.51) (Table II).

***Ureaplasma* spp. and the pH value.** Among 1,155 vaginal smears, 76.6% of samples showed the correct vaginal pH range from 3.5 to 4.5. In 23.3% of samples,

pH was increased > 4.5. The statistical analysis indicated a strong association between *Ureaplasma* spp. presence in the genital tract with higher pH (Fig. 2, Table VI). According to our data, a higher pH value (pH > 4.5) increases the chance of *Ureaplasma* spp. detection regardless *G. vaginalis* infection (Table V).

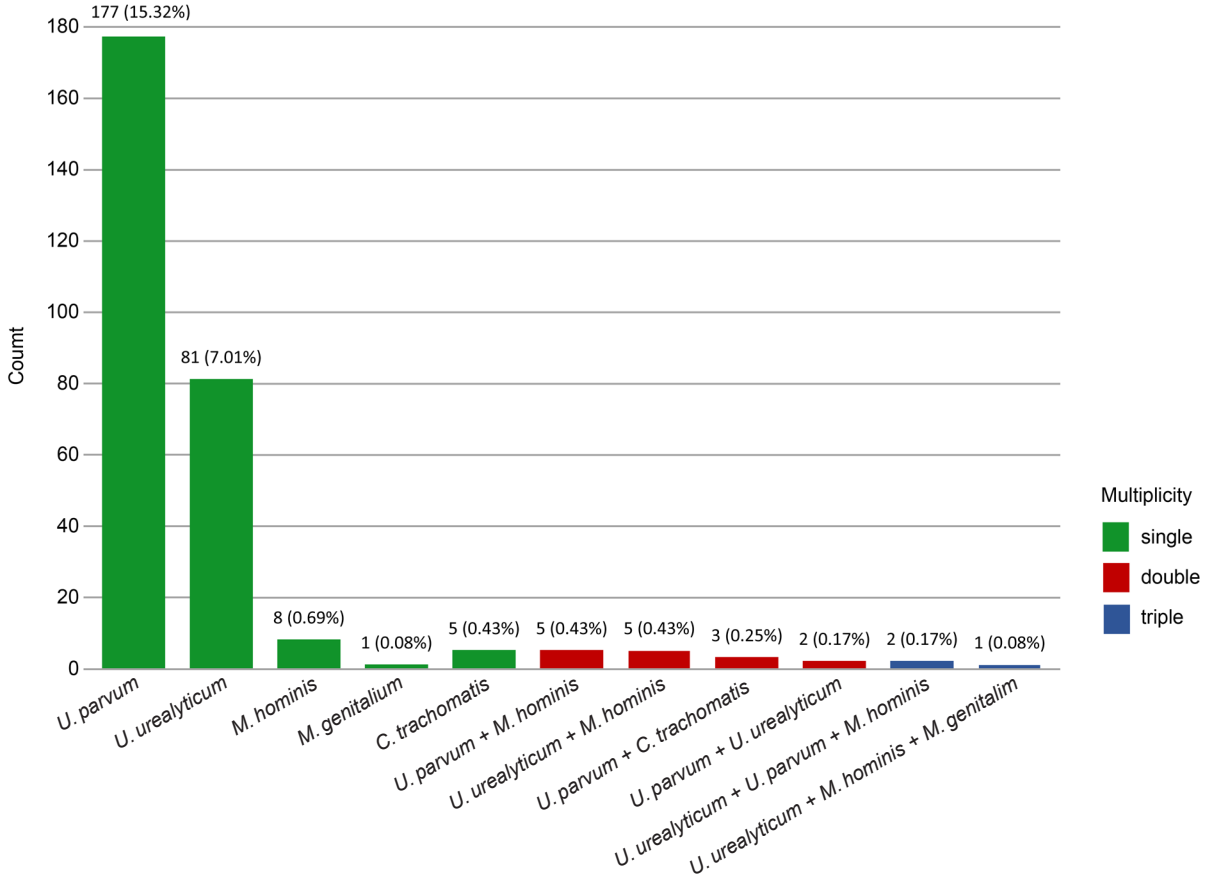

Fig. 1. The prevalence of mycoplasmas and *C. trachomatis* according to the multiplicity of colonization in the population studied (n = 1,155).

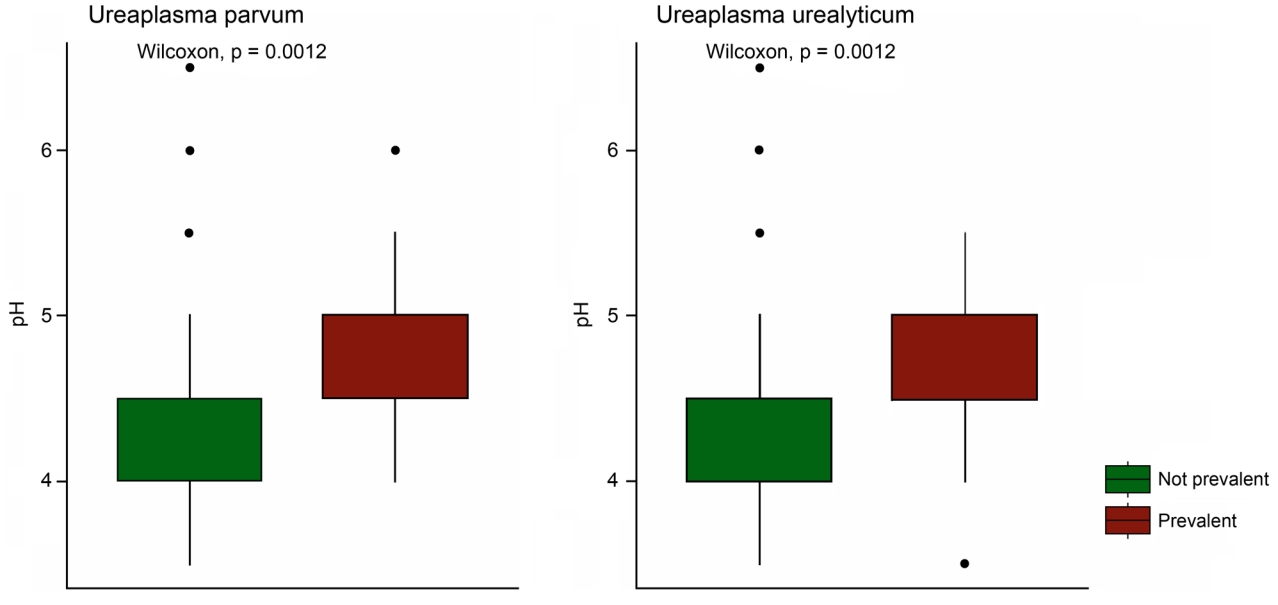

Fig. 2. Distribution of vaginal pH values in women with and without the presence of specific microorganisms. Dots denote extreme values; vertical segments are placed at median values, and boxes are located at quartile values. All differences in pH levels between groups were statistically significant.

Discussion

This presented retrospective study showed a set of results from women in reproductive age diagnosed at the Microbiology and Autovaccine Research Center in Cracow, Poland. Gynecologists referred patients participating in the study to the laboratory for preventive or diagnostic examinations in case of suspected infection in the genital tract. The relationship between the presence of specific microorganisms and the manifestation of various symptoms was investigated. The significant positive associations between vaginal infection of *G. vaginalis* or *Candida* spp. with symptoms such as itching, burning, discomfort, discharge, and pain were detected. The results connecting BV or candidiasis with typical vaginitis or cervicitis symptoms have also been described by Leli et al. (2018). This study also found that in women with symptoms, *C. trachomatis* genital tract infections were over nine times more often identified than in the group without symptoms. It may be related to the previously studied occurrence of the pelvic inflammatory disease reported after *C. trachomatis* infection (O'Connell and Ferone 2016). Statistically significant differences in our study were observed in colonization by *U. urealyticum* of symptomatic or asymptomatic women as others reported (Hunjak et al. 2014; Marovt et al. 2015). Similarly, our results conformed the observations by De Francesco et al. (2013) on a relationship between symptom onset and infection by *Ureaplasma* spp.

Among 1,155 women in our study, 24,2% were colonized by single or multiple species of genital mycoplasmas. Most patients (23.02%) presented single mycoplasma species versus infrequent double and triple colonization, which was detected only in 1.2% of women. The other observational study conducted on a group of 1,761 women showed a much higher general prevalence of mycoplasmas (56.4%). In 39.4% of patients, single species colonization was detected (Leli el al. 2018). Contrary to our results, no association between *Ureaplasma* spp. and symptoms of vaginitis or cervicitis was reported in the study mentioned above.

The study shows that women with *Lactobacillus* spp. in the vagina reported symptoms less frequently, confirming the long-known protective role of *Lactobacillus* spp. (Boskey et al. 2001; Ravel et al. 2011; Witkin and Linhares 2017). No relationship was shown between *Lactobacillus* spp. and infection by *Ureaplasma* spp. *Lactobacillus* spp. was presented in women without *Ureaplasma* spp. and in women with *Ureaplasma* spp. symptomatic and asymptomatic. There was also no relationship between *Ureaplasma* spp. and *C. trachomatis* infection, as identified by Yamazaki et al. (2012) with only a statistically insignificant trend. A similar trend was shown by Leli et al. (2013). Moreover, *U. urealyticum* colonization in symptomatic women increases the prevalence of *M. hominis* (Leli et al. 2013).

Several observational studies have shown that BV and undetectable vaginal lactobacilli are important risk factors for STIs, such as HIV (Martin et al. 1999) and HPV infections (Watts et al. 2005; Biernat-Sudolska et al. 2011). BV microbiota assessed by Gram staining is associated with a significantly increased risk for acquisition of *T. vaginalis, N. gonorrhoeae,* and *C. trachomatis* infection (Schwebke and Desmond 2007; Brotman et al. 2010). Our results show that the colonization of the genital tract with *U. urealyticum* and *U. parvum* increased four times the chance of bacterial vaginosis. These results are consistent with reports on the epidemiology of *U. urealyticum* and *M. hominis* infections associated with the presence of BV and BV markers in pregnant women (Donders et al. 2017).

Excessive growth of *G. vaginalis* with a reduced number of *Lactobacillus* spp. leads to an increase of vaginal pH (Mączyńska 2008; Kasprowicz and Białecka 2012). The correlation between increased vaginal pH and BV is well established, making pH testing one of the predictors of BV according to Amsel's criteria (Amsel et al. 1983). In our study, the interesting association between colonization by *Ureaplasma* spp. and higher pH was detected regardless of co-infection with *G. vaginalis* indicating bacterial vaginosis. During *Ureaplasma* spp. colonization, the increase of vaginal pH may be partially mediated by urea hydrolysis into ammonia. Also, *M. hominis* produces ammonia from arginine, thus alkalizing the environment. Alkalization of the vaginal environment by mycoplasmas is opposite to the acidifying effect of *Lactobacillus*. Since our retrospective study lacks data on urogenital mycoplasmas and *Lactobacillus* load, quantitatively comparing their presence with a pH value is impossible. In addition to the microbiota, the vaginal pH can also be affected by hormonal status, sexual, and hygiene behavior. Our results show an interesting relationship between genital mycoplasmas, bacterial vaginosis, and symptoms of genital tract infections that needs further confirmation.

Our many years of experience in microbial diagnostics were starting points for retrospective analysis of microorganisms' prevalence, including urogenital mycoplasmas, in the south Poland population of women. The paper is retrospective, based on patients' charts who underwent routine microbiological diagnosis of the genital tract and answered questions regarding symptoms and antibiotic treatment and medical referrals. The paper attempts to use a large pool of data on the microbiota of the genital tract in a population of women in the south of Poland. Information on the prevalence of specific etiological factors of reproductive tract infections could be valuable for clinics. As a part of routine work, our diagnostic laboratory of the CBMiA

carries out diagnostics of the reproductive tract, including microscopic technique, microbial culture technique, and molecular methods (NAAT).

Infections of *C. trachomatis, N. gonorrhoeae*, and *M. genitalium* developing in the cervix cause many complications. Colonization of lower genital tract with mycoplasmas can lead to asymptomatic infections of the woman's upper reproductive system (Kasprzykowska et al. 2014). Often asymptomatic or sparsely symptomatic cervical infections cause complications such as pelvic inflammatory disease (PID), fallopian tube inflammation, fallopian tube obstruction, and ectopic pregnancies. Also, the incidences of sexually transmitted diseases differ regionally; therefore, the knowledge of the occurrence of STI pathogens is valuable. In Poland, women of childbearing age who are sexually active are often referred by gynecologists for prophylactic testing to diagnose infections with *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, but also *Ureaplasma* spp., and *Mycoplasma* spp. However, women reporting complaints in the genitourinary tract such as increased vaginal discharge, soreness on urination, soreness during intercourse, and lower abdominal pain are often treated empirically based on the reported symptoms.

The strength of our study is the numerous patient group and the broad picture of microorganisms that have been investigated. On the other hand, the impact of microbiota on women's health and often co-infections certainly cannot be solved by methods used in routine microbiological diagnostics. The diversity of the vaginal microbiota requires further studies with high-throughput sequencing. However, classic diagnostic microbiology can still broaden the knowledge of the microorganism prevalence in the genital tract and support women's reproductive health.

In addition, the group of women with symptoms was smaller than the asymptomatic group. It is mainly because of the retrospective character of the analysis and the chosen exclusions parameters. Some women who reported symptoms were not tested with all three microbiological methods; microscopy, culture, and NAAT, and, therefore, were excluded from the analysis. In some cases of STI risk, only NAAT was proceed according to the physician's prescription. Also, when candidiasis or BV were suspected, only microscopic evaluation of vaginal smear and culture were conducted along with the gynecologist's recommendation. In our opinion, the picture of coinfections needs more attention from physicians, diagnosticians, and scientists. Future studies should include more numerous groups of women with particular attention to symptoms. The symptoms assessment is an important factor in the analysis. It may result in bias since not every woman experiences the symptoms to the same degree, and many factors can influence this assessment.

In conclusion, a relationship between colonization of the female genital tract by *Ureaplasma* spp. and coinfection by *G. vaginalis* were observed in our study group. It was shown that the presence of *Lactobacillus* spp. significantly reduces the risk of symptoms in women. It was also assessed that BV with *G. vaginalis* isolation was more often detected in the group of symptomatic women. However, with a view to studies from other groups, further analyses of coinfections and potential associations with symptoms occurring in genital tract infections are needed.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Abele-Horn M, Peters J, Genzel-Boroviczény O, Wolff C, Zimmermann A, Gottschling W. Vaginal *Ureaplasma urealyticum* colonization: Influence on pregnancy outcome and neonatal morbidity. Infection. 1997 Sep–Oct;25(5):286–291. https://doi.org/10.1007/BF01720398

Amabebe E, Anumba DOC. The vaginal microenvironment: The physiologic role of *Lactobacilli*. Front Med (Lausanne). 2018 Jun 13;5:181. https://doi.org/10.3389/fmed.2018.00181

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 Jan;74(1):14–22. https://doi.org/10.1016/0002-9343(83)91112-9

Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, Gurguis A, Faro S. Defense factors of vaginal lactobacilli. Am J Obstet Gynecol. 2001 Aug;185(2):375–379. https://doi.org/10.1067/mob.2001.115867

Biernat-Sudolska M, Szostek S, Rojek-Zakrzewska D, Klimek M, Kosz-Vnenchak M. Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology. Adv Med Sci. 2011;56(2):299–303. https://doi.org/10.2478/v10039-011-0028-9

Boskey ER, Cone RA, Whaley KJ, Moench TR. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Hum Reprod. 2001 Sep;16(9):1809–1813. https://doi.org/10.1093/humrep/16.9.1809

Brotman RM, Klebanoff MA, Nansel TR, Yu KF, Andrews WW, Zhang J, Schwebke JR. Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. J Infect Dis. 2010 Dec 15;202(12):1907–1915. https://doi.org/10.1086/657320

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 Aug;19(4):621–627. https://doi.org/10.1007/s10156-012-0527-z

Dewan B, Sharma M, Nayak N, Sharma SK. Upper urinary tract stones and *Ureaplasma urealyticum*. Indian J Med Res. 1997 Jan; 105:15–21.

Donders GGG, Ruban K, Bellen G, Petricevic L. Mycoplasma/Ureaplasma infection in pregnancy: To screen or not to screen. J Perinat Med. 2017 Jul 26;45(5):505–515. https://doi.org/10.1515/jpm-2016-0111

Gajer P, Brotman RM, Bai G, Sakamoto J, Schütte UM, Zhong X, Koenig SS, Fu L, Ma ZS, Zhou X, et al. Temporal dynamics of the human vaginal microbiota. Sci Transl Med. 2012 May 2; 4(132): 132ra52. https://doi.org/10.1126/scitranslmed.3003605

Harada K, Tanaka H, Komori S, Tsuji Y, Nagata K, Tsutsui H, Koyama K. Vaginal infection with *Ureaplasma urealyticum* accounts for preterm delivery via induction of inflammatory responses. Microbiol Immunol. 2008 Jun;52(6):297–304. https://doi.org/10.1111/j.1348-0421.2008.00039.x

Heinze G, Ploner M, Dunkler D, Southworth H, Jiricka L. Firth's bias-reduced logistic regression [Internet]. Vienna (Austria): Center for Medical Statistics, Informatics and Intelligent Systems, Medical University of Vienna; 2022 [cited 2022 Mar 1]. Available from https://cran.r-project.org/web/packages/logistf/logistf.pdf

Horowitz S, Horowitz J, Mazor M, Porath A, Glezerman M. *Ureaplasma urealyticum* cervical colonization as a marker for pregnancy complications. Int J Gynaecol Obstet. 1995 Jan;48(1):15–19. https://doi.org/10.1016/0020-7292(94)02236-4

Hunjak B, Sabol I, Vojnović G, Fistonić I, Erceg AB, Peršić Z, Grce M. *Ureaplasma urealyticum* and *Ureaplasma parvum* in women of reproductive age. Arch Gynecol Obstet. 2014 Feb;289(2):407–412. https://doi.org/10.1007/s00404-013-2980-z

Karaoğlu ŞA, Aydin F, Kiliç SS, Kiliç AO. Antimicrobial activity and characteristics of bacteriocins produced by vaginal lactobacilli. Turk J Med Sci. 2003;33(1):7–13.

Kasprowicz A, Białecka A. [Assessment of vaginal biocenosis, degrees of vaginal cleanliness] (in Polish). Diagnosta laboratoryjny. 2008;6(2):21–24.

Kasprowicz A, Białecka A. [Atlas of vaginal purity degrees. Research procedures, interpretation of results] (in Polish). Wrocław (Poland): MesPharm; 2012. p. 5–45.

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 May;289(5):1129–34. https://doi.org/10.1007/s00404-013-3102-7

Kiecka A, Macura B, Szczepanik M. Can *Lactobacillus* spp. be a factor reducing the risk of miscarriage? Pol J Microbiol. 2021 Dec; 70(4):431–446. https://doi.org/10.33073/pjm-2021-043

Kuczyńska K. [Female vaginal biocenosis] (in Polish). Studia Medyczne Akademii Świętokrzyskiej. 2003;11:17–21.

Kundsin RB, Leviton A, Allred EN, Poulin SA. *Ureaplasma urealyticum* infection of the placenta in pregnancies that ended prematurely. Obstet Gynecol. 1996 Jan;87(1):122–127. https://doi.org/10.1016/0029-7844(95)00376-2

Leli C, Mencacci A, Latino MA, Clerici P, Rassu M, Perito S, Castronari R, Pistoni E, Luciano E, De Maria D, et al. 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. J Microbiol Immunol Infect. 2018 Apr;51(2):220–225. https://doi.org/10.1016/j.jmii.2017.05.004

Leli C, Meucci M, Vento S, D'Alò F, Farinelli S, Perito S, Bistoni F, Mencacci A. Microbial and vaginal determinants influencing *Mycoplasma hominis* and *Ureaplasma urealyticum* genital colonization in a population of female patients. Infez Med. 2013 Sep;21(3):201–206.

Mączyńska B. [Diagnostics of bacterial vaginosis and atypical sexually transmitted infections] (in Polish). Diagnosta laboratoryjny. 2008;6(2):13–17.

Marovt M, Keše D, Kotar T, Kmet N, Miljković J, Šoba B, Matičič M. *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 Jun;34(6):1237–45. https://doi.org/10.1007/s10096-015-2351-8

Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J, Kreiss J. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis. 1999 Dec;180(6):1863–1868. https://doi.org/10.1086/315127

Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol. 1991 Feb;29(2):297–301. https://doi.org/10.1128/jcm.29.2.297-301.1991

O'Connell CM, Ferone ME. *Chlamydia trachomatis* Genital Infections. Microb Cell. 2016 Sep 5;3(9):390–403. https://doi.org/10.15698/mic2016.09.525

R Core Team. A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2022 [cited 2022 Mar 1]. Available from https://www.R-project.org

Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA. 2011 Mar 15;108(Suppl 1):4680–4687. https://doi.org/10.1073/pnas.1002611107

Schwebke JR, Desmond R. A randomized trial of metronidazole in asymptomatic bacterial vaginosis to prevent the acquisition of sexually transmitted diseases. Am J Obstet Gynecol. 2007 Jun;196(6):517.e1–517.e6. https://doi.org/10.1016/j.ajog.2007.02.048

Vallor AC, Antonio MA, Hawes SE, Hillier SL. Factors associated with acquisition of, or persistent colonization by, vaginal lactobacilli: Role of hydrogen peroxide production. J Infect Dis. 2001 Dec 1; 184(11):1431–1436. https://doi.org/10.1086/324445

Wang X. Firth logistic regression for rare variant association tests. Front Genet. 2014 Jun 19;5:187. https://doi.org/10.3389/fgene.2014.00187

Watts DH, Fazzari M, Minkoff H, Hillier SL, Sha B, Glesby M, Levine AM, Burk R, Palefsky JM, Moxley M, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. J Infect Dis. 2005 Apr 1;191(7):1129–1139. https://doi.org/10.1086/427777

Witkin SS, Linhares IM. Why do lactobacilli dominate the human vaginal microbiota? BJOG. 2017 Mar;124(4):606–611. https://doi.org/10.1111/1471-0528.14390

Yamazaki T, Matsumoto M, Matsuo J, Abe K, Minami K, Yamaguchi H. Frequency of *Chlamydia trachomatis* in *Ureaplasma*-positive healthy women attending their first prenatal visit in a community hospital in Sapporo, Japan. BMC Infect Dis. 2012 Apr 2;12:82. https://doi.org/10.1186/1471-2334-12-82