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The Prevalence and the Risk Factors of the Cervical Colonization by the Genital Mycoplasmas among Pregnant Women from Eastern Croatia

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A B S T R A C T

The aim of this study was to determine the prevalence of the genital mycoplasmas cervical colonization among the pregnant women from the Eastern Croatia and to evaluate its correlation with the demographic and the behavioural risk factors. Study was conducted from March 2010 to April 2011 at the Microbiology Department of the Institute of Public Health for the Osijek-Baranja County. The study included 456 pregnant women in the first and the second trimester of pregnancy. Demographic data and data on their possible risk behaviour during the lifetime were collected with the usage of an anonymous questionnaire consisting of the 16 questions. The Mycoplasma Duo kit test was used to diagnose the mycoplasma infections. Due to the fact that the results were generally similar for the women with U. urealyticum *or* M. hominis *and in order to dichotomize the analyses, 164 (36%) participants with* U. urealyticum, M. hominis *or both were considered positive, whereas 292 (64%) participants without* U. urealyticum *or* M. hominis *were considered negative. A higher genital mycoplasmas prevalence was statistically significantly associated with the younger group of the pregnant women (16–29 y), 5 or more sexual partners during lifetime and the age on the first intercourse ≤16. The study has showed that both the demographic and the behavioural risk factors were associated with the higher genital mycoplasmas colonization in the observed population.*

Key words*: genital mycoplasmas prevalence, pregnancy, risk factors, Croatia*

Introduction

Genital mycoplasmas *Mycoplasma hominis (M. hominis)* and *Ureaplasma urealyticum (U. urealyticum)* are often found in the genitourinary tract of the sexually active women and can be a part of the normal flora as common commensal inhabitants. *U. urealyticum* has been detected in the lower genital tract of the 40 to 80% sexually active women and is common in the vaginal flora of the pregnant women. Genital mycoplasmas although considered to be of a low pathogenicity, may also play a role in the many different infections and the multiple obstetrical complications. *U. urealyticum* seems to be more virulent and more associated with the vaginitis, cervicitis, salpingitis, infertility, funisitis, chorioamnionitis, preterm premature rupture of the membranes, preterm delivery and postpartum endometritis than *M. hominis*[1]. Spontaneous preterm labour and preterm birth is an important contributor to the perinatal mortality and morbidity. On this track the cervical colonization could be useful for predicting preterm birth because it is impossible to perform amniocentesis on every pregnant woman. Some earlier studies found no relationship between the Ureaplasma colonization and the premature birth, while others claims that *U. urealyticum* is the microbe most commonly found in the intra-amniotic infection and that it significantly increases the risk of the preterm birth and the preterm rupture of the membranes (PROM)[2–7]. Different study population and some risk factors could have masked the effect of *U. urealyticum* and may have been reasons for the mentioned different results. Recent study done by Abele-Horn showed that the high density of the vaginal colonization with *U. urealyticum* is an independent risk factor for the clinical chorioamnionitis and preterm delivery[8]. A joint effect of the vaginal *U. urealyticum* and the bacterial vaginosis on the adverse

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pregnancy outcome was also described[9]. *Ureaplasma urealyticum* include two different biovars. Biovar 1 includes 1, 3, 6 and the 14 serovars while the biovar 2 includes 2, 4, 5, 7–13 serovars. Recently, Kim at al. showed that the difference between the biovars in the cervix did not influence the occurrence of the preterm labor[10]. Intraamniotic infection with the genital mycoplasmas is more intense than the intraamnionitic infection with the other organisms with the preterm rupture of membranes[11]. The intrauterine presence of the mentioned organisms may results in the increase of the synthesis of prostaglandins, cytokines and metalloproteinases, and thus leads to the preterm labour and delivery[12]. *U. urealyticum* may be perinatally transmitted to the new-born with the transmission rates and the prevalence increasing inversely with the gestational age. Tracheal colonization with *U. urealyticum* increases the risk from respiratory complications such as the development of the respiratory distress syndrome, bronchopulmonary dysplasia and chronic lung disease. Invasive infection occurs commonly in the very low birth weight infants and may increase the risk for the severe intraventricular hemorrhage[6–8,12–16]. Previous studies have shown that the different prevalence of *U. urealyticum* and *M. hominis* in the pregnant women depends on the geographical areas. Despite the well-known possible consequences of the mycoplasmatic infections in pregnancy, there has not been any epidemiological report concerning the prevalence of these infections among the pregnant woman in Croatia. The aim of the present study was to determine the prevalence of the cervical colonization by *U. urealyticum* and *M. hominis* among the pregnant women from the Eastern Croatia and to evaluate its correlation with the demographic and the behavioural risk factors.

Materials and Methods

Patients and specimens

This cross-sectional study was conducted from March 2010 to April 2011 and analysed at the Microbiology Department of the Institution for the Public Health Osijek-Baranja County. The study population included 456 pregnant women (from 4000 pregnant women in the Osijek-Baranja County) in the first and second trimester of pregnancy. Prior to the enrolment in the study, each women was informed on the objectives and the purpose of the study. With the approval of the Ethics Committee of Osijek University, Faculty of Medicine informed consent for the participation in the study and the anonymous questionnaire were obtained from each pregnant women. Anonymous questionnaire included demographic characteristics and the potential risk factors for the cervical colonization with the analysed microorganisms. Specimens were the cervical swabs for genital mycoplasmas. They were obtained during the routine prenatal examination at the primary health care gynaecologist offices. After the collection, the cervical swab for *U. urealyticum* and *M. hominis* was inserted into the Mycoplasma Duo suspension medium (BIO RAD). After the inoculation in the mentioned medium the specimen can be stored for 48 hours at room temperature or 72 hours at +2–8 °C.

TABLE 1
ANONYMOUS QUESTIONNAIRE FOR PREGNANT WOMEN-STUDY SUBJECTS IN THE STUDY ABOUT GENITAL MYCOPLASMAS COLONIZATION DURING PREGNANCY

Please circle or write your answer!
1. Place of residence: a) town b) village
2. The year of birth:
3. Marital status: a) married b) single c) divorced
4. Level of education: a) unfinished elementary school b) finished elementary school c) finished high school d) finished college e) finished faculty
5. This is my first pregnancy during lifetime a) YES b) NO
6. Have you ever had habitual abortion? a) YES b) NO
7. Have you ever had preterm delivery? a) YES b) NO
8. Have you ever had extra uterine pregnancy? a) YES b) NO
9. Age of first intercourse:
10. During the sexual intercourse with new partners during lifetime I have always used condoms: a) YES b) NO
11. Number of sexual partners during lifetime : a) 1–4 b) 5 or more
12. Have you ever use public swimming pool? a) YES b) NO
13. Have you ever use public gym? a) YES b) NO
14. Have you ever use public sauna? a) YES b) NO
15. Have you ever, during lifetime had genital mycoplasmas infection (*Ureaplasma Urealyticum, Mycoplasma hominis*)? a) YES b) NO
16. Have you ever, during lifetime had another sexually transmitted disease? a) YES b) NO
Thank you very much for your cooperation!

Anonymous questionnaire

Study subjects were asked to fill-in an anonymous questionnaire in order to identify the different demographic and the behavioural risk factors that may have implied their exposure to genital mycoplasmas. There were 16 questions, 14 of them of the closed type (with the optional answers to be marked) and two of the open type (the year of the birth and the age of the first intercourse). Demographic data included the place of residence, age, level of education and marital status. Data of their previous pregnancies included the questions about the parity, previous habitual abortion, previous preterm delivery and previous extra uterine pregnancy. Data on the sexual preferences included the questions on the sexual activity during the lifetime, age of the first intercourse, number of sexual partners during the lifetime, regular condom use during the sexual contacts with new partners and lifetime history of the sexually transmitted diseases (STDs). Data on their possible risk behaviour included the public swimming pool, public gym and public sauna use. The questionnaire used in the study is presented in the Table 1.

Culture procedure

The Mycoplasma Duo kit (BIO RAD) was used to diagnose mycoplasma infections[17]. It enabled cultivation, identification and indicative enumeration of *U. urealyticum* and *M. hominis*. The combination of three antibiotics and an antifungal agent provided the selectivity, ensuring that any contaminant flora in the specimen does not affect the test. Identification of the urogenital mycoplasmas was based on the specific metabolic properties of each organism: the hydrolysis of urea by *U. urealyticum* and the hydrolysis of arginine by *M. hominis* with the release of ammonia and the alkalisation of the medium. The reaction is visualized by a change in the colour of the pH indicator from yellow to red (phenol red). Yellow micro well suggested no mycoplasmas in the sample and red micro well suggested presence of the mycoplasmas in the sample. Results were first read after the incubation for 24 hours on 37 °C and provided the definitive result in the high-titre specimens ($\geq 10^4$ CCU/mL). A second reading performed after 48 hours incubation on 37 °C was needed to detect the strains present in the high titres ($\geq 10^4$ CCU/mL) witch were characterized by a slow metabolic rate. A Mycoplasma Duo test allows differential titration of the two mycoplasma species which may have both been present in the same specimen. The Mycoplasma S.I.R. kit (BIO RAD) was used for the antibiotic susceptibility testing.

Statistics

Descriptive statistics was used for the data processing and analysed using the SPSS Statistical Package for the Windows, version 13.0 (SPSS Inc., Chicago, IL, USA). The χ^2- test and the Fisher exact test were used in order to determine the differences in the distribution of the qualitative variables. In all statistical analyses, two-sided p-values of 0.05 and the lesser ones were considered significant.

Results

A total of 456 pregnant women were analysed in the present study. A positive cervical culture for *U. urealyticum* or *M. hominis* or both was present in 164 (36 %) of pregnant women. Of the women with the positive cervical culture for *U. urealyticum* and/or *M. hominis,*154 *(*93.9%) had *U. urealyticum* only, 2 (1.2%) had *M. hominis* only and 8 (4.9%) had both *U. urealyticum* and *M. hominis* (Table 2). Because the results were generally similar for women with *U. urealyticum* or *M. hominis*, the analyses was dichotomised that 164 (36%) participants with *U. urealyticum*, *M. hominis* or both were considered positive, whereas 292 (64%) participants without *U. urealyticum* or *M. hominis* were considered negative (Table 3). Their data, obtained from each pregnant women by an anonymous questionnaire are shown in the Table 4. There were no significant differences between the women with the positive and the women with the negative cervical culture for *U. urealyticum* and/or *M. hominis* regard to the basic characteristics like the age, place of residence, marital status and level of education. Since an age is an important determinant of the sexual behaviour[18], the study subjects were divided into two age groups: the younger group, aged 16–29 (N=266) and the

TABLE 2
POSITIVE CERVICAL CULTURE OF U.UREALYTICUM AND/OR M.HOMINIS COLONIZATION AMONG PREGNANT WOMEN FROM THE EASTERN CROATIA

Positive cervical colonization	N (%)
Solely *U. urealyticum*	154 (93.9)
Solely *M. hominis*	2 (1.2)
Both *U. urealyticum and M. hominis*	8 (4.9)
Total	164 (100)

TABLE 3
PREVALENCE OF U.UREALYTICUM AND/OR M.HOMINIS COLONIZATION AMONG PREGNANT WOMEN FROM THE EASTERN CROATIA

Cervical colonization	Positive (%)	Negative (%)	Total (%)
Solely *U. urealyticum*	154 (33.7)	302 (66.3)	456 (100)
Solely *M. hominis*	2 (0.4)	454 (99.6)	456 (100)
Both *U. urealyticum* and *M. hominis*	8 (1.7)	448 (98.3)	456 (100)
Total	164 (36)	292 (64)	456 (100)

TABLE 4
DEMOGRAPHIC AND BEHAVIOURAL RISK FACTORS FOR GENITAL MYCOPLASMAS COLONIZATION AMONG PREGNANT WOMEN FROM THE EASTERN CROATIA

Characteristics	Positive (%) N=164	Negative (%) N=292
Age (years / $\overline{X}$±SD)	27.9±5.3	28.8±4.8
Age group*		
16–29y	106 (64.6)	160 (54.8)
30–43y	58 (35.4)	132 (45.2)
Place of residence		
Town	78 (47.6)	132 (45.2)
Village	86 (52.4)	160 (54.8)
Marital status		
Married	122 (74.4)	255 (87.3)
Single	41 (25.0)	36 (12.3)
Divorced	1 (0.6)	1 (0.4)
Years of education		
<12	131 (79.8)	206 (70.5)
>12	33 (20.2)	86 (29.5)
Parity		
0	83 (50.6)	112 (38.3)
≥1	81 (49.4)	180 (61.7)
Previous habitual abortion*		
Yes	18 (10.9)	60 (20.5)
No	146 (89.1)	232 (79.5)
Previous preterm delivery		
Yes	20 (12.1)	56 (19.1)
No	144 (87.9)	236 (80.9)
Previous extra uterine pregnancy		
Yes	2 (1.2)	2 (0.7)
No	162 (98.8)	290 (99.3)
Age on first intercourse*		
≤16	45 (27.4)	54 (18.5)
≥17	119 (72.6)	238 (81.5)
Condom use		
Yes	84 (51.2)	133 (45.5)
No	80 (48.8)	159 (54.5)
Number of sexual partners during lifetime*		
1–4	138 (84.1)	267 (91.4)
5 or more	26 (15.9)	25 (8.6)
Public swimming pool use		
Yes	62 (37.8)	90 (30.8)
No	102 (62.2)	202 (69.2)
Public gym use		
Yes	52 (31.7)	95 (32.5)
No	112 (68.3)	197 (67.5)
Public sauna use		
Yes	30 (18.3)	36 (12.3)
No	134 (81.7)	256 (87.7)
Previous genital mycoplasmas infections		
Yes	21 (12.8)	45 (15.4)
No	143 (87.2)	247 (84.6)
Previous other STDs*		
Yes	14 (6.1)	45 (15.4)
No	150 (93.9)	247 (84.6)

* statistically significant difference

older group, aged 30–43 (n=190) years. Positive cervical culture for *U. urealyticum* and/or *M. hominis* was recorded in 106/266 (39.8%) subjects from the younger group and 58/190 (30.5%) subjects from the older group. Significantly more frequent positive cervical culture for *U. urealyticum* and/or *M. hominis* were in the younger group (p= 0.04, χ^2=4.183). Higher number (five or more) of sexual partners during lifetime and the age of the first intercourse ≤16 were sexual risk behaviour connected with the genital mycoplasmas positivity (p=0.01; χ^2= 5.622 and p=0.03; χ^2=4.945). History of the habitual abortion (p=0.009; χ^2=6.786), preterm delivery and other STDs (p=0.03; χ^2=4.406) were more frequent among the women with the negative cervical culture than the women with the positive cervical culture for *U. urealyticum* and/or *M. hominis*. Other possible risk factors include the parity, extra uterine pregnancy, use of condom on sexual contact with new partner, public swimming pool, public gym and public sauna use have not provoked any differences between the women. Considering the prevalence of the possible risk factors for the genital mycoplasmas infections in *U. urealyticum* and/or *M. hominis* positive and negative subjects, the younger group of pregnant women (16–29 y), five or more sexual partners during lifetime, the age on the first intercourse ≤16, no history of habitual abortion and no history of other STDs were more common in the pregnant women with the positive cervical culture for *U. urealyticum* and/or *M. hominis*.

Discussion and Conclusion

There is no doubt that *U. urealyticum* and *M. hominis* are associated with the adverse pregnancy outcomes. But in reality, the mentioned organisms are rarely cultivated routinely in the clinical practice and because of the low awareness are often undiagnosed[19]. The role of the genital mycoplasmas in the ascending genital tract infection is logical and the colonized pregnant women possess bacteria in their vaginal flora that are at least potentially capable of ascending through the cervix. It remains obscure why they causes colonization in some pregnant women and leads to the infection in the others. There might be other associated factors which increase the capacity of the microorganism to invade the uterine cavity including stress on the mother or fetus, the blood borne infection of the placenta, the short cervix allowing the vaginal flora to be close to the foetal membranes, the over distension of the uterus and the altered vaginal flora with the elevated concentrations of the proinflammatory microorganisms. Colonization density, virulence variability and infection sensitivity of the host may play a role in development of the infection[20]. In Croatia, the data on the prevalence of *U. urealyticum* and *M. hominis* colonization in the pregnant women are not reported. There is no consensus on the monitoring of the pregnant women with the colonization. Also, there are no systematic monitoring of the prevalence of the neonatal disease including the prevalence of the neonatal infection caused by *U. urealyticum* and *M. hominis*[21]. The present study

that included that pregnant women from the Eastern Croatia was the first study specifically investigated the genital mycoplasmas prevalence in the population of the Croatian pregnant women. A positive cervical culture of *U. urealyticum* or *M. hominis* or both was present in 164/456 (36 %) of the pregnant women. Prevalence of *U. urealyticum* and *M. hominis* colonization showed regional variations from 11 to 63% and from 5 to 48% (Czech Republic, Hungary, Belgium, Chile, Argentina, Japan, Israel, South Africa). The mentioned values are comparable to the Croatian samples despite the fact that genital mycoplasmas colonization in the Croatian pregnant women was a little bit lower[22–28]. The problem of establishing a role of the genital mycoplasmas depends partly on the laboratory diagnostics. Genital mycoplasmas presence was first based on culture but now molecular methods such as polymerase chain reaction (PCR) and DNA hybridization are commonly used[29,30.] They are rapid and reliable tests for the detection of these organisms with the great specificity and have the ability to be used in the quantitative mode. Due to their costs, there are the differences in the technical expertise between the laboratories. The limitation of the present study is the fact that The Mycoplasma Duo kit (BIO RAD), which is not molecular method, was used for the cultivation and the detection of the genital mycoplasmas. Because of that we probably have lower number of the positive subjects in our study. Only the Czech Republic and Israel studies had used the same diagnostic method, while others studies used molecular method (PCR) for a detection of the genital mycoplasmas. In the present study, the higher genital mycoplasmas colonization (106/266; 39.8%, p= 0.04, χ^2=4.183) was associated with the younger group of pregnant women (16–29 y) which is in agreement with the other report[28]. From the sexual risk behaviour, five or more sexual partners during lifetime (26/164; 15.9%, p=0.01, χ^2=5.622) and the age at the first intercourse ≤16 (45/164; 27.4%, p=0.03, χ^2=4.945) were more common in the pregnant women with the positive cervical culture for *U. urealyticum* and/or *M. hominis*. The mentioned findings confirm the fact that the number of sexual partners during a lifetime and the duration of sexual activity represent greater risk for STDs[31,32]. Overall number of the extra uterine pregnancy (4/456; 0.8%), genital mycoplasmas infections (66/456; 14.4%), and other STDs (59/456; 13%) are very low for all study subjects and because of that we have probably failed to succeed in our attempt to confirm the association between genital mycoplasmas positivity and these potential risk factors. History of the preterm deliveries (56/292; 19.1%) and the habitual abortion (60/292; 20.5% and p=0.009, χ^2=6.786) were more common in the pregnant women with the negative cervical culture for *U. urealyticum* and/or *M. hominis* than the women with the positive cervical culture. In Croatia it is not mandatory to obtain the cervical swabs in all pregnant women but there are recommendations to obtain the mentioned swabs and to analyse them in the mentioned pregnant women who are considered to have high-risk pregnancies[33]. In our study, we showed that pregnant women who have no history of spontaneous abortions and preterm delivery and according to those criteria in Croatia they are not classified in the group of women with the high-risk pregnancies ultimately have a positive finding for genital mycoplasmas in their cervical swabs. With our research we thus confirmed the findings of some authors who had discovered that in the most pregnancies that are considered to be the high-risk complications in fact do not develop, as opposed to the low-risk pregnancies in which the complications often develop resulting in a various adverse outcomes[34–36]. Data obtained by the anonymous questionnaire showed that all the study subjects do not use the public swimming pool (152/456; 33.3%), public gym (147/456; 32.2%) and public sauna (66/456; 14.4%) often and because of that the present study did not show the connection between the genital mycoplasmas colonization and some of the above mentioned behaviours. In conclusion, the importance of the genital mycoplasmas in the pregnancy requires further investigations. The group of the pregnant women with genital mycoplasmas colonization (36%) is far too large to be suitable for an intervention strategy, because the administration of the antibiotics could also cause more harm than benefit. Screening for the genital mycoplasmas and the other risk factors for the spontaneous preterm birth should allow a selection of women who may benefit from the prenatal treatment. In the era characterized by the need for the most cost-effective health-care on the one hand and at the same time highly efficient and efficacious health care on the other hand, the respective and comprehensive studies should be continued and extended in Croatia. We suggest a study predicting the costs of the routine screenings of the pregnant women and comparing them with the current treatment the costs of the genital mycoplasmas infections and their secondary consequences in Croatia. The issues of the infectious diseases leading to the preterm birth and the other complications of pregnancy do not end with delivery. The new-born may be born with the infection and in this respect we should not forget the potential severe sequels for the child. It is necessary for the obstetricians, neonatal intesivists and public health professionals to be involved in solving the issue because the definitive conclusion on the development of the national strategy can only be made upon the large-scale and comprehensive epidemiological studies, done by the interdisciplinary teams of experts.

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UČESTALOST I RIZIČNI ČIMBENICI CERVIKALNE KOLONIZACIJE GENITALNIM MIKOPLAZMAMA TRUDNICA U ISTOČNOJ HRVATSKOJ

SAŽETAK

Cilj ovoga istraživanja bio je utvrditi učestalost i rizične čimbenike kolonizacije cerviksa genitalnim mikoplazmama kod trudnica s područja istočne Hrvatske, te procijeniti njezinu povezanost s demografskim osobinama i rizičnim ponašanjima ispitanica. Studija je provedena u razdoblju od ožujka 2010. godine do travnja 2011. godine u Zavodu za javno zdravstvo Osječko-baranjske županije, Služba za mikrobiologiju. U istraživanje je uključeno 456 trudnica u prvom i drugom trimestru trudnoće. Demografski podatci o ispitanicama, te podatci o njihovom eventualnom rizičnom ponašanju tijekom života dobiveni su pomoću anonimnog anketnog upitnika koji se sastojao od 16 pitanja. Za detekciju genitalnih mikoplazmi korišten je Mycoplasma duo Kit test. Budući da su rezultati općenito slični za žene s *U. urealyticum* ili *M. hominis* kolonizacijom odlučili smo dihotomizirati naše rezultate, tako da smo ispitanice s *U. urealyticum, M. hominis* kolonizacijom ili oboje (164; 36%) smatrali pozitivnima, a ispitanice bez *U. urealyticum* ili *M. hominis* kolonizacije (292; 64%) smo smatrali negativnima. Viša prevalencija je statistički značajno bila povezana s: mlađom dobnom skupinom trudnica (16–29), s većim brojem (pet ili više) spolnih partnera tijekom života te s ranijim stupanjem u spolne odnose (≤16 godina prvi spolni odnos). Studija je pokazala da su demografske karakteristike i rizično ponašanje ispitanica povezani s višom prevalencijom genitalnih mikoplazmi u promatranoj populaciji.