Clinical role of *Ureaplasma parvum* and *Ureaplasma urealyticum* presence in female lower urogenital tract: Is there a place for routine screening and treatment?

Klinični pomen prisotnosti bakterij *Ureaplasma parvum* in *Ureaplasma urealyticum* v spodnjem urogenitalnem traktu žensk: Je potrebno rutinsko presejanje in zdravljenje?

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Abstract

Sexually transmitted infections represent major health problem in females all over the world if remained undiagnosed and untreated. They can have an adverse influence on the reproduction and health of the mother and the newborn. The development of molecular methods has permitted the detection of an array of microbes whose pathologic roles in urogenital infections need to be further studied. Ureaplasmas (*Ureaplasma* spp.), being originally found in 1954 from male urogenital tract, are prokaryotic cells without a cell wall, ranging from 0.1 to 1 μm in length. Fourteen known *Ureaplasma* serovars, named also serotypes, have been divided in two species based on their phenotypic and genotypic features, *Ureaplasma parvum* and *Ureaplasma urealyticum* detected and identified separately using polymerase chain reaction assays. Both are generally considered as genital tract commensals. *U. urealyticum* is most probably associated with male urethritis which has not been found for *U. parvum*. Recent studies with supposedly healthy women reported their detection rate between 18–87 % for *U. parvum* and 6–10 % for *U. urealyticum*. Even though they have been found to be associated with chorioamnionitis, preterm birth and perinatal complications more commonly then other commensals in this region, the rising question regarding their pathogenic role in females remains unresolved and the guidelines regarding the diagnostic screening and treatment are inconsistent.

The aim of our paper is to review the microbiological characteristics, diagnostic methods and epidemiology of newly differentiated *U. parvum* and *U. urealyticum*, and to assess evidence speaking for and against their clinical role in causing lower urogenital tract infection in women. Since both bacterial species are susceptible to antimicrobials, it is of utmost importance for clinicians to decide whether or not to search for one or both of them routinely and treat accordingly in order to prevent ascending upper genital tract infection as well as complications in pregnancy and newborns.

Izvleček

Spolno prenosljive okužbe žensk so velik javnozdravstveni problem povsod po svetu, če jih ne odkrijemo in ne zdravimo pravočasno. Nezdravljene lahko pomembno vplivajo na njihovo reproduktivno zdravje, zdravje nosečnic in novorojencev. Razvoj molekularnih diagnostičnih metod je omogočil odkritje številnih mikrobov, katerih klinični pomen pri ženskah z okužbo urogenitalnega trakta še ni pojasnjen. Ureaplazme (*Ureaplasma* spp.) je prvi odkril Shepard leta 1954 pri moških v vzorcih urogenitalnega trakta. Ureaplazme so prokariotske celice brez celične stene, velike od 0,1 do 1 μm. Na podlagi njihovih fenotipskih in genotipskih značilnosti ločimo štirinajst serovarjev, imenovanih tudi serotipi, ki so uvrščeni v dve ločeni vrsti, *Ureaplasma parvum* in *Ureaplasma urealyticum*. Posamezni vrsti dokazujemo z metodo verižne reakcije s polimerazo. Študije so pokazale povezavo med prisotnostjo *U. urealyticum* in uretritisom pri moških, medtem ko pri *U. parvum* te povezave ni bilo najti. Z nedavno opravljenimi raziskavami so v spodnjem urogenitalnem traktu domnevno zdravih

žensk dokazali *U. parvum* v 18–87 %, bakterijo *U. urealyticum* pa v 6–10 %. Kljub temu, da so raziskave pokazale povezavo med prisotnostjo ureaplazem in horioamnionitisom, prezgodnjim porodom in zapleti ob porodu pogosteje kot pri prisotnosti drugih komenzalov v tem področju, ostaja vprašanje o njihovi patogeni vlogi pri ženskah zaenkrat še nepojasnjeno. Prav tako pa ni ustreznih smernic glede presejalnega testiranja in morebitnega zdravljenja teh okužb.

Namen prispevka je predstaviti mikrobiološke značilnosti, diagnostične metode in epidemiološke posebnosti novo diferenciranih bakterij *U. parvum* in *U. urealyticum* ter na temelju dosedanjih dognanj oceniti njihov morebitni klinični pomen pri okužbi spodnjega urogenitalnega trakta žensk.

1. Introduction

In 2008 WHO estimated that 449 million new cases of curable sexually transmitted infections (STIs) (syphilis, gonorrhoea, chlamydial infections and trichomoniasis) occur each year all over the world in adults aged 15–49 years.[1] Females are severely and more commonly affected.[2] Untreated STIs can have an adverse influence on the reproduction and health of the mother and the newborn and therefore represent an important preventable cause of infertility.[1] Unresolved cervicitis may lead to ascending infection, resulting in endometritis and salpingitis or ascending infection during pregnancy, resulting in chorioamnionitis, premature rupture of membranes, premature delivery, amniotic fluid infection, and puerperal infection.[3] The development of molecular methods has permitted the detection of an array of microbes whose pathologic roles in urogenital infections need to be further studied, *Ureaplasma* spp. presenting the most interesting one.

Nowadays, evidence is accumulating that *Ureaplasma urealyticum* causes nongonococcal urethritis in males.[4-8] Unlike *U. urealyticum*, *Ureaplasma parvum* does not seem to be associated with male urethritis. However, clinical role of *U. parvum* and *U. urealyticum* in lower urogenital tract infections in females is blurred, lacking larger epidemiological and clinical studies in women with urogenital symptoms and those without them. Since both bacterial species are susceptible to certain antimicrobials, guidelines are needed to clarify whether one or both of them should be sought for routinely and treat accordingly to prevent further complications in females.

The aim of our paper is to review the microbiological characteristics, diagnostic methods and epidemiology of newly differentiated *U. parvum* and *U. urealyticum*, and to assess evidence speaking for and against their clinical role in causing lower urogenital tract infection in women.

2. Microbiological characteristics of ureaplasmas

2.1. Classification

Ureaplasmas (*Ureaplasma* spp.) were originally found by Shepard in 1954 from male urogenital tract[9] and the genus was established in 1974[10]. *Ureaplasma* spp. is included within the class *Mollicutes*, which contains four orders, five families, eight genera, and more than 200 species that have been detected in humans, vertebrate animals, arthropods, and plants.[11] Humans are the primary host for at least 17 species, primarily localized in the respiratory and urogenital tracts. Until 2002, *U. urealyticum* was considered to be the only species of this genus known to infect humans. By using polyclonal or monoclonal antibodies directed against whole cells or purified antigens, fourteen serovars (or serotypes) were recognized that were classified on the basis of 16S rRNA gene sequences into two biovars, biovar 1 and biovar 2. Biovars were later reclassified as two distinct species, *U. parvum* and *U. urealyticum*, based on genome size, 16S rRNA gene sequences, the 16S-23S rRNA intergenic region, enzyme polymorphisms, DNA--DNA hybridization, differential growth

responses to manganese, and differences in the multiple banded antigen (*mba*) genes.[12] *Ureaplasma parvum* now contains serotypes 1, 3, 6, and 14, while *U. urealyticum* includes the rest of 10 serotypes (2, 4, 5, 7, 8, 9, 10, 11, 12, and 13).

2.2. Bacterial characteristics

Ureaplasmas are particularly small prokaryotic cells without a cell wall, which makes them insensitive to the activity of beta-lactam antimicrobials, and precludes them from staining by Gram reaction.[13] They are spherical or coccobacillary-shaped cells and have a diameter ranging from 0.1 to 1 μm.[12] Colonies produced by ureaplasmas are typically 15 to 60 μm in diameter and require low-power microscopic magnification for visualization.[13] Ureaplasmas are the only prokaryotes that essentially need urea for their growth. Nearly all ATP synthesis results from urea hydrolysis.[14] *Ureaplasma parvum* was the third sequenced mycoplasma, and has the smallest sequenced genome among prokaryotes except *Mycoplasma genitalium*. It includes the serotypes with smaller genome (0.75–0.76 megabase pairs, Mbp), whereas *U. urealyticum* includes 10 serotypes with larger genome (0.88–1.2 Mbp).

2.3. Virulence factors

Attachment to host cell surfaces is essential for ureaplasmas to colonize and afterwards produce pathological changes. Factors involved with their attachment to mucosal surfaces have so far not been extensively studied, but they are known to adhere to erythrocytes[15], spermatozoa[16], urethral epithelial cells[17], and neutrophils.[18]

Five proteins such as urease, immunoglobulin-α (IgA) protease, phospholipases A and C, and multiple banded antigen (MBA), along with the ureaplasma enzymes for producing hydrogen peroxide have been suggested as virulence factors.[14]

Genes involved in pathogenicity have not been conclusively identified. Xiao and colleagues have recently shown that individual serovars are not likely to be associated with differential pathogenicity.[19]

3. Diagnostic methods for ureaplasmas

Methods for laboratory detection of ureaplasmas have been greatly improved over the past years because of effective molecular-based techniques. Relatively rapid bacterial growth makes the identification of most positive cultures possible within two to four days, but culture cannot differentiate between the two species.[13]

Molecular-based methods, such as PCR, are able to detect and identify *U. parvum* and *U. urealyticum* separately. For target sequences, 16S rRNA gene, 16S rRNA to 23S rRNA intergenic spacer regions, the urease gene, and *mba* gene are mainly used.[20] In addition, a number of diverse genotyping methods have been developed for identification of *Ureaplasma* serotypes: restriction fragment length polymorphism (RFLP), pulsed-field gel electrophoresis (PFGE), a high-resolution melt (HRM) PCR assay, real-time PCR, and multilocus sequence typing (MLST) assay.[21,22] In 2010 Xiao and colleagues showed originally that all 14 serotype strains of ureaplasmas can be distinctly differentiated from one another by using real-time PCR technology.[23] Polymerase chain reaction seems to be more sensitive for diagnostic purposes compared to culture; among 132 clinical specimens, PCR detected 20 (15.2 %) positive samples more compared to culture.[23] Advantages of molecular-based methods compared to culture and serological analysis for the detection of ureaplasmas and mycoplasmas are discussed in detail by Waites and colleagues.[20]

While using PCR as the gold standard, first-voided urine specimens from women were reported having the lowest overall sensitivity (84.6 %) when compared to endocervical swab (98.1 %) and the sensitivity of self-collected vaginal specimens (99.3 %).[24]

Culture remains the most economical and practical means of detection for laboratories with a low to moderate sample volume. Culture also has an advantage of providing antimicrobial susceptibility testing.[20] Whether culture- or non-culture-based detection methods should be used for diagnostic purposes depends on the resources and

facilities available in individual laboratories and the species being sought.[20]

Indirect, serological test methods for ureaplasmas include microimmunofluorescence, metabolism inhibition, and enzyme immunoassay[13], but the interpretation of antibody titers is difficult because of their ubiquity in healthy people so it is of limited clinical use.[20]

4. Epidemiology of ureaplasmas

Ureaplasmas can be detected in the cervix or vagina of 40 to 80 % of sexually mature asymptomatic women.[25] Colonization is more common in females of younger age, lower socioeconomic status, with multiple sex partners, black ethnicity, and those using oral contraceptives. Incidence of ureaplasmas in female genital tract is most probably dependent on hormonal status; incidences in the prepuberty, puerperium, postmenopause, in pregnant women, in sexually inactive women, and in sexually active nonpregnant women were 5 %, 24 %, 25 %, 82 %, 40 %, and 67 %, respectively.[26]

Studies with supposedly healthy women reported *Ureaplasma* spp. detection rate at approximately 18–87 % for *U. parvum* and 6–10 % for *U. urealyticum* (Table 1).

5. Clinical role of ureaplasmas in females

5.1. Infection of the lower urogenital tract

Both *U. parvum* and *U. urealyticum* are generally considered as female urogenital tract commensals. Even though they are more commonly than other normal flora in the urogenital region associated with some clinical syndromes such as chorioamnionitis and preterm birth as well as perinatal morbidity and mortality, the rising question regarding their pathogenic role in females remains unresolved.[30] Recent study in 303 presumably healthy women from Japan attending their first prenatal visit has shown that there was a significant association between urogenital presence of *Chlamydia trachomatis* and either *U. parvum* ($p = 0.023$) or *Ureaplasma* spp. ($p = 0.013$), but not *U. urealyticum* ($p = 0.275$).[30] This finding suggests that ureaplasmas might change the urogenital microenvironment and enable the survival of *C. trachomatis*. However, data on the mutual effect of urogenital flora is limited.

5.1.1. Symptomatic lower urogenital tract infection in ureaplasma-positive females

Using culture, Schlicht and colleagues (2004) found out *Ureaplasma* spp. in 21/39 (54 %) of symptomatic and only in 4/25 (16 %) of asymptomatic women, the difference being significant.[33] However, the number of asymptomatic females was low. In 2009, De Francesco reported that *U. parvum* serovar 3 and *U. urealyticum* were significantly associated with symptomatic women compared to asymptomatic ones ($p < 0.05$).[34]

Table 1: Prevalence of *Ureaplasma parvum* and *Ureaplasma urealyticum* in supposedly healthy female population.

Author	Country	N	*U. parvum*	*U. urealyticum*
McIver, 2009[27]	Australia	233	57 %	6.1 %
Kong, 2000[28]	Australia	263	87 %	19 %
Kataoka, 2006[29]	Japan	877	52 %	8.7 %
Yamazaki, 2012[30]	Japan	303	41.7 %	8.9 %
Cao, 2007[31]	China	128	53.1 %	7.8 %
Ekiel, 2009[32]	Poland	39	17.9 %	2.6 %

In this study only the samples with isolated ureaplasmas and no other bacteria were evaluated. *U. parvum* was found in 64/80 (80 %) of symptomatic compared to 56/59 (95 %) of asymptomatic women whereas *U. urealyticum* was found in 16/80 (20 %) of symptomatic compared to 3/59 (5 %) of asymptomatic women. *U. parvum* serovar 3 was present most commonly in the 21 to 25-year-old age group, while *U. urealyticum* was distributed with quite similar frequency in women of 26 to 30 and > 40 years of age. *U. parvum* serovar 3 and *U. urealyticum* were found to be significantly associated with loss of lactobacilli, while *U. parvum* serovar 6 was significantly correlated to normal vaginal flora. In a study by McKechnie and colleagues in 2011, *U. parvum* was found in 51/111 (45.9 %) of symptomatic compared to 52/105 (49.5 %) of asymptomatic women and *U. urealyticum* was found in 30/111 (27 %) of symptomatic compared to 23/105 (21.9 %) of asymptomatic women.[24] Researchers did not find any significant differences in detection rates when comparing symptomatic and asymptomatic women neither for *U. parvum* nor for *U. urealyticum*. Women were classified as cases when reporting one or more of the following symptoms: vaginal discharge, irritation, dysuria, urinary frequency or pelvic pain, and as controls when presenting with none of these specific symptoms. In 2013, Hunjak and colleagues isolated ureaplasmas from cervicovaginal or urethral swab in 34.4 % of 1370 women visiting gynecological practice (28.5 % being pregnant).[35] Out of 244 samples 18 (7.4 %) were identified as *U. urealyticum* and 226 (92.6 %) as *U. parvum*, 15/18 *U. urealyticum* cases (83.3 %) being isolated in symptomatic and 3/18 (16.7 %) in asymptomatic women, while 179/226 (79.2 %) and 47/226 (20.8 %) of *U. parvum* cases were isolated in symptomatic and asymptomatic women, respectively. There were no statistically significant differences in the incidence of either *U. urealyticum* or *U. parvum* regarding the presence of symptoms or pregnancy.

Studies on the association between ureaplasmas and cervicitis in particular are sparse. In 1985 research by Paavonen and colleagues, *Ureaplasma* spp. was the only organism significantly associated with mucopurulent cervicitis after adjustment for the results of cervical culture for *C. trachomatis*.[36] No other studies on the matter have been found so far.

In several studies ureaplasmas have been shown to be associated with bacterial vaginosis (BV).[37] However, studies regarding the association between newly differentiated ureaplasmas and BV are sparse. In women who delivered preterm, *U. urealyticum* was detected significantly more often in specimens from those with clinical diagnosis of BV (3/7), compared to those without it (2/42) (OR 15.95 % CI 1.2–209) as was shown by Povlsen and colleagues in 2001.[38] The group of women with BV who delivered preterm was limited. There was no difference in the proportion of biovar strains when comparing women who delivered preterm and women who delivered at term. *U. urealyticum* was present more frequently in women with BV (57/70) compared to women without it (223/414) (OR 3–7, 95 % CI 2.0–7.0). In 2008 Haggerty and colleagues defined BV by Amsel's and Nugent's criteria and detected them both more frequently among women who were positive for *U. urealyticum*, compared to women who were *U. urealyticum* negative (57 % vs. 50 % and 64 % vs. 53 %, respectively), however, the differences were small and did not reach statistical significance.[39]

5.2. Infection in pregnancy and neonates

Possible association between ureaplasmas and adverse pregnancy outcome is a topic of great interest and has not been resolved satisfactorily. Studies that were limited to sampling the lower genital tract of women have yielded inconclusive results, mainly because not all women who are colonized in the lower tract will develop infection in the upper tract.[11] Occurrence of ureaplasmas in pregnant women provides a reservoir for transmission to the fetus and neonate.[40] It can occur as an ascending intrauterine infection, through a hematogenous route or acquisition by the neonate through passage of an infected maternal birth canal.[11]

The most conclusive data associating adverse pregnancy outcomes with ureaplasmas were obtained from prospective studies in which ureaplasmas were detected in the amniotic fluid.[41-43] *Ureaplasma* spp. are most frequently isolated from the amniotic fluid or placenta in women who deliver prematurely, either with preterm premature rupture of membranes or in preterm labor with intact membranes, and isolation of *Ureaplasma* spp. has been consistently associated with histological chorioamnionitis.[11]

There is significant association and/or strong suggestive evidence without proven causal role between *Ureaplasma* spp. and congenital pneumonia as well as neonatal bacteremia, neonatal meningitis and neonatal abscesses.[40]

6. Treatment of ureaplasma infection

Mollicutes are innately resistant to all beta-lactams, sulfonamides, trimethoprim, and rifampin. *Ureaplasma* spp. is susceptible to erythromycin and other 14- and 15-membered macrolides but resistant to clindamycin.[13] Tetracycline resistance has been well documented in *Ureaplasma* spp. since the mid-eighties, mediated by the *tet*(M) determinant which codes for a protein that binds to the ribosomes, protecting them from the actions of these drugs.[13] The extent to which tetracycline resistance occurs in *Ureaplasma* spp. varies geographically and according to prior exposure in different populations but may approach 40 do 50 %.[11] High-level macrolide-resistant *U. parvum* was recently reported from the United Kingdom[44], but such resistance is believed to be rare.[13] Fluoroquinolones such as the latest levofloxacin and moxifloxacin are usually active against all human mycoplasmal and ureaplasmal species.[13] Infrequent fluoroquinolone-resistant strains of *Ureaplasma* spp. have first been reported from China, France and the USA.[45,46] However, in a 2013 study from Croatia, all of the 424 ureaplasma strains were susceptible to doxycycline, tetracycline, erythromycin and clarithromycin, whereas the susceptibilities to ofloxacin and ciprofloxacin were 42.9 % and 24.5 %, respectively.[35]

7. Future implications for clinicians

Because several investigators have proposed an association between certain ureaplasma serotypes and certain diseases, and others were not able to affirm that association, an association between ureaplasmas and clinically important infection should probably be understood in terms of biovars (now newly recognized species) rather than serotypes.[47] Studies have shown that some strains are more firmly associated with the disease. However, more studies are needed to find out whether this is due to either bacterial virulence factors, host response, local environment or their combination. Information gained from animal models[48,49] and humans[50,51] suggests that intact host response is essential in overcoming the disease.

Presuming that non-specific cervicitis represents an analog of male non-gonococcal urethritis, can we assume that in females *U. urealyticum* is more pathogenic than *U. parvum*? Or: are certain *U. parvum* serovars in females clinically more important than others? Is there a difference between males and females regarding ureaplasmas? Since infection of the female lower urogenital tract can lead to ascending upper genital tract infection and causes complications in the infected women, pregnancy as well as in the newborn, it is of essential importance to recognize all clinically important pathogens and treat the condition accordingly. Considering the low cost of sequencing nowadays, the genomes of ureaplasma isolates from women with different clinical conditions, including those who deliver prematurely, should be sequenced routinely.[52] The comparison of sequences should further aid the identification of genes involved in differential pathogenicity.

Since both bacteria are susceptible to macrolides, tetracyclines and the latest fluoroquinolones, consistent guidelines should be available to clinicians enabling them to decide whether or not to search for one or

both bacteria including their serotypes routinely and treat the condition accordingly, regardless the presence or absence of symptoms to prevent complications. Clinicians should be aware of the possibility in expanding diagnostic means in view of better clinical management of females.

8. Conclusion

Limited number of studies investigating the clinical role of presence of *U. parvum* and *U. urealyticum* as well as some of their serovars in the female lower urogenital tract gave no conclusive results. Future studies are warranted. So far, clinicians should be aware of the possibility in expanding diagnostic means in view of better clinical management of their female patients.

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