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**MYCOPLASMA HOMINIS
IN FEMALE GENITAL TRACT
AND ITS SUSCEPTIBILITY TO ANTIBIOTICS**

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Mycoplasma hominis is a common inhabitant of the vagina of healthy women. It has been linked to pelvic inflammatory diseases such as tubo-ovarian abscess and salpingitis. *Mycoplasma hominis* is also associated with bacterial vaginosis but it is still unclear whether the organism really contributes to a pathological process in which so many different bacteria are involved. The aim of this study was to determine the incidence of *Mycoplasma hominis* in the genital tract of women with different clinical symptoms. A total of 351 cervical swabs were investigated. *Mycoplasma hominis* was isolated in 83 cases (23.6%). The positive samples were compared with regard to genital symptoms and age of the women. *Mycoplasma hominis* was isolated in 33.3 % from pregnant women and 27.5 % from group of 31-40 years old women. Susceptibility of clinical isolates of *Mycoplasma hominis* was studied to doxycycline, ciprofloxacin and erythromycin.

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Introduction

Mycoplasma hominis (*M. hominis*) was the first mycoplasma isolated from humans, as reported in 1937 by Dienes and Edsall [1]. This microorganism belongs to the class Mollicutes [2]. Members of this taxonomic class are known to be the smallest free-living microorganisms without rigid cell wall which makes them insensitive to beta-lactam antibiotics. They possess the smallest genome among all prokaryotes, which is about one-sixth the size of *Escherichia coli* genome. However, they are still able to replicate on cell-free media [3].

M. hominis is a common inhabitant of the vagina of healthy sexually active women [4,5]. The prevalence of this organism is significantly associated with socio-economic status [6], increasing number of sexual partners [7], age [8] and bacterial [9] and protozoan infection [10]. Reports from various studies show that colonisation rates range from 6 % to 40 % [7,11]. *M. hominis* is frequently isolated in association with bacterial vaginosis [12,13], pelvic inflammatory diseases and salpingitis [3]. *M. hominis* infection is a potential risk factor for the premature delivery [14], endometritis and postpartum or postabortal fever [6]. Pregnant women colonized with *M. hominis* are likely to have low-birth-weight infants [15].

Materials and Methods

M. hominis belongs to the sensitive microorganisms and its determination is, therefore, very difficult [4]. *M. hominis* possesses the smallest genome of all prokaryotes and, to ensure them the best growth conditions, their cultivation medium should contain additional supplements. The basic medium is a beef-heart infusion broth, available commercially as the PPLO broth (pleuropneumonia-like-organism) with yeast extract and horse serum. The main diagnostic property of *M. hominis* is its ability to metabolize arginine to ammonia and form a characteristic fried egg appearance on agar [16]. The characteristic colonial morphology is shown in Fig. 1.

The specimens were collected from 351 randomly selected women who visited private gynecology practice G-med in Pardubice, Czech Republic. Specimens were taken by cotton-tipped swabs from the posterior vaginal fornix and placed in mycoplasma transport medium (PPLO broth - Difco, horse serum, yeast extract, thallium acetate and ampicillin). The pH of the medium was adjusted at 6.5. Samples in transport medium were stored at 4 °C until transported to the laboratory.

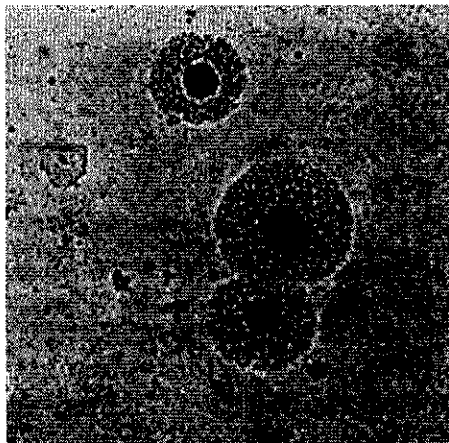


Fig. 1 Colonies of *Mycoplasma hominis* exhibiting a characteristic “fried-egg” appearance ($\times 200$)

Laboratory Processing

All swabs were processed within 4 hours. A volume of 200 μl of transport medium was inoculated into 1.8 ml of arginine broth (the same composition as transport medium with addition of arginine and phenol red) for cultivation of *M. hominis*. The swabs were spread on PPLO agar plates at the same time. This agar contains PPLO agar, horse serum, yeast extract, thallium acetate and ampicillin. Both cultivation media were incubated at 37 °C in the atmosphere of 5 % CO_2 . PPLO agar plates were cultivated for 2-4 days. The broths were cultivated until the change of pH and subsequent alteration of colour of phenol red from orange to pink. The samples of broths with colour change were identified as positive and were spread on PPLO agar for further confirmation. The plates were examined microscopically (100 \times magnification) after 2-4 days incubation period.

M. hominis was identified by the means of arginine hydrolysis, colonial morphology and polymerase chain reaction (PCR).

Polymerase Chain Reaction

DNA was extracted from 100 μl of positive broth cultures of *M. hominis* using standard phenol-chloroform extraction [17].

The nucleotide sequences of the primers used in this study are shown in Table I. The oligonucleotides were synthesized by the firm Generi-Biotech, Hradec Králové. The following primers were used for detection of *M. hominis*: RNAH1 and RNAH2, specific for the 16S rRNA gene [15].

Table I Nucleotide sequence of the primers [15]

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Primers for target DNA-16S rRNA gene of <i>M. hominis</i>	Nucleotide sequence (5'-3')	Amplicon length
RNAH1	CAATGGCTAATGCCGG ATACGC	334 bp
RNAH2	GGTACCGTCAGTCTG	

The PCR solution (25 μ l) contained 2.5 μ l of 10 \times PCR buffer, 2 μ l $MgCl_2$, 2 μ l of deoxynucleotide triphosphate, 0.25 μ l of each primer, 1.25 units of Taq DNA polymerase and 2 μ l of the template DNA. PCR reactions were performed in a gradient Robocycler (Stratagene). After the initial denaturation (95 $^{\circ}C$, 5 min), the tubes with PCR solution were subjected to 30 cycles with following program: 95 $^{\circ}C$ for 1 min, 62 $^{\circ}C$ for 1 min and 72 $^{\circ}C$ for 1 min. The last cycle was followed by an extension step at 72 $^{\circ}C$ for 10 min. The tubes were stored at 4 $^{\circ}C$ until further analysis. Amplified products were visualized in ultraviolet light after electrophoresis for 50 min at 80 V through a 1.5 % (w/v) agarose gel (Biotech) containing 0.5 μ g ml^{-1} of ethidium bromide.

Antibiotic Susceptibility Testing of *Mycoplasma Hominis* Strains

The susceptibility of isolated strains was determined by tube broth dilution method. Erythromycin, ciprofloxacin and doxycycline (Sigma-Aldrich Ltd.) powders were used to prepare fresh stock solution in water except erythromycin which was first diluted in 95 % ethanol [18]. Clinical isolates were suspended in fresh PPLO medium with supplements (horse serum, yeast extract, arginine and phenol red) and incubated at 37 $^{\circ}C$ in 5 % CO_2 , until medium changed the colour. A volume of 200 μ l aliquot of these cultures was used to inoculate the tubes containing twofold decreasing concentrations of particular antimicrobial agent in PPLO broth (Difco) with supplements (horse serum, yeast extract, arginine and phenol red).

Every strain was inoculated into identical medium without antibiotic at the same time. This tube was a positive control of growth of testing strains. All tubes were incubated 24-48 hours until control tube changed the colour. The MIC was defined as the lowest concentration of antibiotic causing a colour change which remains stable after 48 hours incubation.

Results

M. hominis was isolated from 83 (23.6 %) of 351 randomly selected women. The women were divided according to diagnosis into five groups — women without problems, pregnant women without problems, women with bacterial vaginosis, precancerosis and other clinical symptoms. The results are shown in Table II. *M. hominis* was more often isolated from pregnant women (33.3 %) without clinical symptoms. There was no significant difference between the studied categories.

Table II Prevalence of *Mycoplasma hominis* according to diagnosis

Diagnosis	Number of samples	Positive	
		Number	%
Women without problems	127	27	21.3
Pregnant women without problems	39	13	33.3
Bacterial vaginosis	15	3	20.0
Precancerosis	102	24	23.5
Other clinical symptoms	68	16	23.5

We also studied the occurrence of *M. hominis* in samples taken from the cervix of women whose pregnancy ended in abortion. Another group involved infertile women. The results are shown in Table III.

Table III Occurrence of *Mycoplasma hominis* according to anamnesis

Anamnesis	Number of samples	Positive	
		Number	%
Abortion	43	15	34.9
Infertility	9	4	44.4
Without anamnesis	291	62	21.3

The prevalence of *M. hominis* according to age of women is presented in Fig. 2. *M. hominis* was found in the women of all ages.

The susceptibility of isolated strains was tested to several antimicrobial agents — ciprofloxacin, doxycycline and erythromycin. The results are summarized in Table IV. Our strains were susceptible to ciprofloxacin,

doxycycline and resistant to erythromycin.

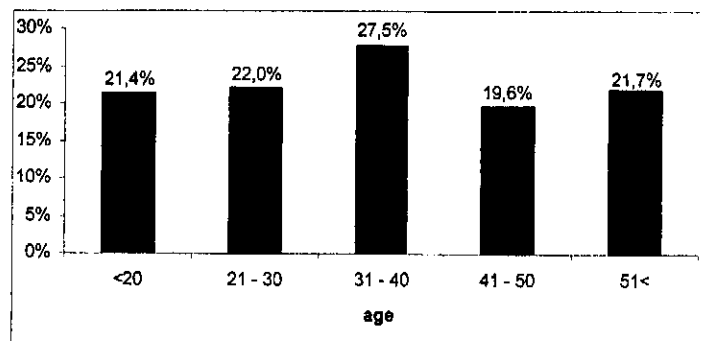


Fig. 2 Distribution of *Mycoplasma hominis* according to age of the women

Table IV Antibiotic susceptibility of *Mycoplasma hominis* determined by broth dilution method

Drug	Number of strains <i>M. hominis</i>	Range mg l ⁻¹	MIC 5 mg l ⁻¹	MIC 90 mg l ⁻¹
Ciprofloxacin	65	0.5-2	1	2
Erythromycin	44	62.5-1000	250	500
Doxycycline	77	0.016-2.5	0.125	0.5

Discussion

M. hominis is often isolated from the genital tract of women with different kinds of disease such as pelvic inflammatory disease [19,20], bacterial vaginosis [12,13] and salpingitis [3]. The presence of this microorganism in human genital tract is also linked to sexual activity, number of partners, younger age, lower socioeconomic status and oral contraceptive use [8]. The role of this microorganism in genital diseases is still unclear because of so many bacteria participating in these processes.

All the women were divided according to their diagnosis. The highest occurrence was found in the group of pregnant women (33.3 %). Almost the same results were reported by other authors. Lamont R.F. *et al.* [21] isolated *M. hominis* from 17 (24 %) pregnant women. Cedillo-Ramirez L. *et al.* [22] from 12 % and Chua K.B. *et al.* [23] from 17.7 % of pregnant women.

Out of total 15 women with bacterial vaginosis (BV) 20 % was positive for *M. hominis*. It is not possible to estimate the contribution of *M. hominis* to BV due to small number of women in our study. However, many authors found association

between *M. hominis* and BV [12,13]. On the other hand, Demba *et al.* [24] examined samples from 227 women. According to their study, *M. hominis* is not associated with BV.

Very interesting is the number of isolated strains from the group of infertile women (44.4 %). Although this group of sterile women is too small, there exist studies of other authors which confirm the presence of *M. hominis* as an independent predictor of tubal factor infertility [25].

M. hominis was more frequently isolated from women 31-40 years old (27.5 %). A higher incidence of genital mycoplasma was found in women between 26 and 30 years (34 %) as reported by Nunez-Troconis J.T. [26]. Occurrence of *M. hominis* in genital tract of women around 30 years could relate to higher number of sexual partners or sexual promiscuity [16].

The absence of cell wall makes *M. hominis* insensitive to beta-lactam antibiotics. The most useful in treatment of infections with these organisms are the tetracyclines, the macrolides and related antibiotics and some fluoroquinolones [27].

The susceptibility of isolated strains of *M. hominis* to ciprofloxacin, doxycycline and erythromycin were tested. Every strain was susceptible to ciprofloxacin (MIC_{90} , 2 mg l⁻¹) and doxycycline (MIC_{90} , 0.5 mg l⁻¹). These results compare with those of Bebear *et al.* [27] — ciprofloxacin (MIC_{90} , 1 mg l⁻¹), doxycycline (MIC_{90} , 0.12 mg l⁻¹), erythromycin (MIC_{90} , > 64 mg l⁻¹) and those of Kenny *et al.* [28] — ciprofloxacin (MIC_{90} , 2 mg l⁻¹), erythromycin (MIC_{90} , > 32 mg l⁻¹). The resistance to erythromycin (MIC_{90} , 500 mg l⁻¹) was confirmed in our study.

In conclusion, the results of this study indicate that *M. hominis* was frequently isolated from genital tract of pregnant women, infertile women and women around 30 years. Every isolate was confirmed by PCR method. The isolated strains of *M. hominis* were susceptible to ciprofloxacin and doxycycline and resistant to erythromycin.

Acknowledgments

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