

DETECTION OF GENITAL MYCOPLASMAS IN MEN ATTENDING MEDICAL CLINICS IN SOFIA, BULGARIA

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ABSTRACT

PURPOSE: The role of *Ureaplasma urealyticum* (*U. urealyticum*), *Mycoplasma hominis* (*M. hominis*) and *Mycoplasma genitalium* (*M. genitalium*) in male genital disorders deserves a more intensive research. The aim of this study was to determine the prevalence rate of these pathogens in symptomatic and asymptomatic sexually active males in Bulgaria.

MATERIAL AND METHODS: A total of 260 symptomatic and asymptomatic men were included in the study. Their urethral swabs were examined for the presence of the mentioned microorganisms by using polymerase chain reaction (PCR).

RESULTS: The prevalence rates of these pathogens were the following: *U. urealyticum* - 4,6%; *M. hominis* - 0,8%, and *M. genitalium* - 3,1%. No coinfections were registered at all.

CONCLUSION: The present investigation of these mycoplasmas is the first attempt to provide certain epidemiological information about these infections in Bulgaria. Their continuous monitoring is of practical importance for public health.

Key words: *U. urealyticum*, *M. hominis*, *M. genitalium*, prevalence, males, Bulgaria

INTRODUCTION

Genital mycoplasmas belong to the sexually transmitted infections that more and more frequently cause acute diseases, long-term disability and infertility. However, these pathogens are not included in the routine microbiological diagnosis. Nowadays polymerase chain reaction (PCR) is the method of choice for their identification.

The aim of this study was to establish the prevalence and clinical significance of *U. urealyticum*, *M. hominis* and *M. genitalium* in symptomatic and as-

ymptomatic sexually active men in Bulgaria by PCR examinations of their urethral swabs.

MATERIAL AND METHODS

The study covered a total of 260 males. A written informed consent to participate in the study was obtained from each person. Dacron swabs were used to collect urethral specimens for the detection of the bacteria. They were put into a special transport medium. The samples were transported within ~1 hour to the laboratory and examined either within the same day, or no longer than one week later on.

Aliquots of the transport medium with the sample were used for PCR testing for the detection of fragments of *U. urealyticum*, *M. hominis* and *M. genitalium*. After vortexing the diluted sample was subjected to a DNA extraction using DNA extraction kit according to manufacturer's requirements (Sacace Biotechnologies, Como, Italy). Detection of the pathogens was performed by applying PCR spe-

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cific primers according to Lee *et al.* (11). PCR was carried out with 4 µL of the template DNA, 0,25 µL of each primer (Alpha DNA, Canada), 0,2 mM (each) of the deoxyribonucleoside triphosphates, 1x Reaction Buffer, MgCl₂ (2 mM) and 1,5 U of Taq DNA polymerase (Prime Taq™ DNA Polymerase; GEN-ET BIO) in a total volume of 25 µL. The DNA was amplified in a Techgen PCR thermocycler (Techne, England) using the following protocol: an initial denaturation 94 °C, 5 min; 35 cycles (94 °C, 40 sec; 54 °C, 45 sec; 72 °C, 1 min 5 sec) and final extension 72 °C, 7 min. PCR products (12,5 µL of each amplification) were separated in a 1,5% agarose gel for 45 min at 150 V, stained with ethidium bromide (0,5 µg/mL) and detected using UV transillumination (λ=312 nm). Each PCR run was accompanied by a quality control. For positive control, *U. urealyticum* ATCC-33699, *M. hominis* ATCC-14027 and *M. genitalium* ATCC-33530 were used. The negative controls included distilled water and negative clinical samples selected at random.

Each DNA was subjected to a human β-globin PCR to ensure that amplifiable DNA was successfully extracted from the sample and to monitor for PCR inhibitors (4). This β-globin PCR protocol was the same one listed for bacterial PCR, with the exception that the following primers were employed: GH20, 5'-GAAGAGCCAAGGACAGG TAC-3', and PCO4, 5'-CAACTTCATCCACGTTCCACC-3'.

RESULTS

The swabs received from persons with or without any genital complaints were used to evaluate the prevalence rate of *U. urealyticum*, *M. hominis* and *M. genitalium* infection.

U. urealyticum was found out in 12 swabs (in 4,6%), *M. hominis* - in two samples (in 0,8%), and *M. genitalium* - in 8 symptomatic males (in 3,1%) (Table 1). Coinfections were not detected at all. The high-

er relative share of positive findings was established in the symptomatic group of *U. urealyticum* and *M. genitalium*.

DISCUSSION

In men, *Ureaplasma* spp. are the main cause of nonchlamydial, nongonococcal urethritis and acute prostatitis (3) *M. genitalium* is associated with urethritis (7,8,10), too, whereas *M. hominis* is related to pyelonephritis (3). Although genital mycoplasmas are established as relevant sexually transmitted pathogens little is known about these infections in Bulgarian males.

It is considered that *M. hominis* does not provoke urogenital disorders except in 5% of the cases with pyelonephritis (17). There are contradictory opinions about the infertility caused by this microorganism. Some investigators register a prevalence rate of 1,3% of the infection among patients attending infertility clinics (9), while others detect a higher one - of 10,8% (6). In our study, *M. hominis* is proved in one male attending the Clinic for a prophylactic check-up.

U. urealyticum is another genital microorganism which prevalence rate among healthy men varies in a wide range - between 0% and 56% (12). The pathogen is detected in 5,6-42% among the patients with urethritis (5) and in 15-20,1% among those with infertility. In our study, its prevalence rate is 4,6%.

In our study, *M. genitalium* prevalence rate is 3,1%. Other authors establish a low prevalence rate of the infection in sexually active persons, too - 0,98% (13); 0,8% (2); 1,3% (15); 2,3% (1), and 3,7% (14). Significantly higher literature data are reported concerning the symptomatic patients with acute nongonococcal urethritis between 13,2% and 33,3% (16) and the persons with complains but without urethritis - between 0% and 9,1% (18).

Table 1. Detection of *U. urealyticum*, *M. hominis* and *M. genitalium* in males from different groups

Clinical groups	n	<i>U. urealyticum</i>	<i>M. hominis</i>	<i>M. genitalium</i>
symptomatic*	200	8	0	6
infertility	48	4	0	2
prophylactic check-up	12	0	2	0

*predominant diagnosis of urethritis

There are scanty data about the prevalence rates, diagnostic methods and clinical significance of mycoplasmas in Bulgaria. In this respect, males are first examined in our country. The relatively lower prevalence rates of these three infections in Bulgaria than those reported elsewhere could be due to the less expressed circulation of these pathogens as well as to the widespread usage of macrolides in our clinical practice.

CONCLUSION

U. urealyticum and *M. genitalium* are established in symptomatic patients only while *M. hominis* is detected in two patients attending the clinic for prophylactic checkup. The present investigation of these mycoplasmas is the first attempt to provide certain epidemiological information about these infections in Bulgaria. Their continuous monitoring is of practical importance for public health.

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