Polish Journal of Microbiology
2016, Vol. 65, No 1, 93–95

SHORT COMMUNICATION

Prevalence of Urogenital Mycoplasmas Among Men with NGU in Upper Silesia, Poland. Preliminary Study

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Submitted 17 September 2013, revised 28 September 2013, accepted 3 September 2015

Abstract

The prevalence of urogenital mycoplasmas in men with NGU in Upper Silesia (Poland) was studied. Mycoplasmas were detected in 36.7% men (*Ureaplasma parvum* and *Mycoplasma genitalium* were found in 30% and 16.7% respectively). *Urealyticum urealyticum* was not detected. We suggest including *M. genitalium* in the diagnostic scheme for nongonococcal urethritis (NGU).

Key words: *Mycoplasma genitalium*, nongonococcal urethritis, urogenital mycoplasmas

The importance of ureaplasmas as a cause of nongonococcal urethritis (NGU) is discussed. The topic of this discussion has taken on a new meaning since 1999, when Kong *et al.* (1999) divided the genera *Ureaplasma* into two new species *Ureaplasma parvum* and *Ureaplasma urealyticum*. *Mycoplasma genitalium* was being isolated from urethra of men with NGU until 1980, but only recently was confirmed as an important etiology of NGU in men (Workowski and Berman, 2010). In Poland detection of urogenital mycoplasmas is not a part of routine diagnostic procedure. Few published papers cover mainly pregnant women and newborns.

The aim of this study was to evaluate the prevalence of *M. genitalium* and *Ureaplasmas* in men with NGU, taking into account the diversity of the species (*U. parvum* and *U. urealyticum*). According to our knowledge, this is the first such study in Poland.

Thirty male patients aged 30–55 years (mean age 38 ± 5.68 years) diagnosed with NGU (clinical symptoms, microscopic preparation, after excluding *Chlamydia trachomatis)* were directed for detection of urogenital mycoplasmas. All men gave informed consent for this study. Patients were advised of the need to maintain sexual abstinence for 3–4 days and were obliged to come to testing at minimum 4 h after the last voiding. This study was approved by the Bioethical Committee of Medical University of Silesia.

Isolation of mycoplasmal DNA was performed from a pellet obtained from culture in Mycoplasma IST 2 (bioMérieux, Marcy I'Etoile, France). Identification of *U. parvum*, *U. urealyticum* and intraspecific diversity of *U. parvum* was done using species-specific primers according to Kong *et al.* (2000). Detection of *M. genitalium* was conducted using primers for adhesin genes: MgPa-1 – MgPa-3 and for 16S rRNA gene MG16-45F – MG16-447R; MG16-1204F – MG16-1301R primers were designed according to Jensen *et al.* (1991; 2003).

In 9/30 (30%) samples urogenital mycoplasmas were detected with Mycoplasma IST 2 test. PCR amplification confirmed the presence of mycoplasmas in all 9 cases and did not increase the number of positive results for ureaplasmas. However, detection of *M. genitalium* DNA by PCR increased the number of positive results for urogenital mycoplasmas from 9 cases to 11 (36.7%). Co-occurrence of two different species of urogenital mycoplasmas was shown in the majority of samples (Table I). Interestingly, species identification

Table I
Occurrence of urogenital mycoplasmas in the study group (n = 30).

	No (%)
M. genitalium	**2** (6.7)
U. parvum + *M. genitalium*	**3** (10)
U. parvum + *M. hominis*	**2** (6.7)
U. parvum	**4** (13.3)
Total	**11** (36.7)

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Table II
Results of *M. genitalium* DNA detection by PCR with selected primers in men with NGU (n = 30).

Number of studied cases	Primers			Final interpretation
	Mg16-45F + Mg16-447R	Mg16-1204F + MG16-1301R	MgPa-1 + MgPa-3	
1	Positive	Positive	Positive	Positive
4	Negative	Positive	Posititve	Positive
4	Negative	Negative	Positive	Negative
21	Negative	Negative	Negative	Negative

revealed the presence of *U. parvum* in all cases, in contrast to *U. urealyticum* strains, which were not detected. Domination of *U. parvum* in our study group was concordant with others (Tang *et al*., 2011; Vancutsem *et al*., 2011). Several studies have shown that *U. urealyticum* was significantly more common in men with NGU (Maeda *et al*., 2004; Manhart *et al*., 2013). Among isolated mycoplasmas *U. parvum* was detected significantly more often than *M. hominis* (p = 0.0453). However domination of *U. parvum* in comparison to *M. genitalium* was not significant (p = 0.3598). Although the presence of *M. genitalium* DNA by PCR using primers for adhesin genes MgPa was shown in specimens from 9 (30%) men with NGU, primers for 16S rRNA gene confirmed the presence of *M. genitalium* in only 5 samples (16.7%, Table II). The rule that double-positive amplicon for adhesin gene with primers MgPa-1/MgPa-3 and double-positive for one of the primers for 16S rRNA gene could be considered as positive was used in the interpretation of the obtained results (Tabele II). The decrease in the number of positive cases from 9/30 to 5/30 in the study group of men was not significant. According to other authors, confirmation of positive results through repetition or the use of different primers are required (Manhart *et al*., 2003). Gaydos *et al.* (2009) recognized a patient's infection only when positive results with primers for both MgPa and 16S rRNA genes were achieved. Among the analysed specimens from 719 women (Manhart *et al*., 2003) 51 were double-positive using primer pairs for MgPa genes. Furthermore, only 45 (88.2%) of 51 double-positive results were confirmed using PCR for 16S rRNA gene. At the same time, for none of 49 selected MgPa PCR-negative specimens, positive results with primers for 16S rRNA gene were demonstrated. Edberg *et al.* (2008) achieved higher sensitivity by real-time PCR for MgPa gene, compared to conventional PCR for 16S rRNA gene. Moreover, the authors demonstrated much higher sensitivity in contrary to real-time PCR for 16S rRNA gene.

The urogenital mycoplasmas are recognized in a large percentage of men with NGU. However, in order to properly interpret the presence and role of urogenital mycoplasmas in the etiology of infection, the result of the test should be complete. The outcome has to contain both: detection of *M. genitalium* DNA and identification of ureaplasmas to *U. parvum* and *U. urealyticum*. Japanese authors identified *M. genitalium*, *U. urealyticum* and *U. parvum* in men with NGU with a frequency of 17%, 16.3% and 7.8% respectively; among the patients with non-chlamydial NGU – 23.8%, 18.8% and 8.8% respectively (Maeda *et al*., 2004). In a study from Denmark, the prevalence of *M. genitalium* infection was 2.3% and 1.1% respectively in 731 men and 921 women aged 21–23 years, not seeking medical assistance (Andersen *et al*., 2007). Detection rate of *M. genitalium* DNA in urine from asymptomatic healthy young Japanese men was only 1% (Takahashi *et al*., 2006).

The absence of *U. urealyticum* in our specimens was probably due first of all to small size of the study group and secondly, the low frequency of isolation of *U. urealyticum* in the Polish population, demonstrated previously in a group of women (Ekiel *et al*., 2009). Similar results of low percentage of *U. urealyticum* were indicated in other countries (Tang *et al*., 2011; Vancutsem *et al*., 2011). A limitation of our study was the absence of a control group due to the fact that taking urethral swabs is invasive and poorly accepted by men.

The usefulness of the first – void urine (FVU) in molecular biology studies was shown by other authors. Thanks to this, availability of research on urogenital mycoplasmas certainly will increase (Takahashi *et al*., 2006; Wroblewski *et al*., 2006).

M. genitalium is now an important and established cause of approximately 9–25% cases of NGU (Workowski and Berman, 2010; Manhart *et al*., 2013). In spite of that fact that our study group was limited, 16.7% positive *M. genitalium* cases confirmed the role of this microorganism as an important causative agent of NGU. Furthermore, our study points to the requirement of including *M. genitalium* in the diagnostic scheme for patients with non-chlamydial NGUs.

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