European Journal of Obstetrics & Gynecology and Reproductive Biology 220 (2018) 44–49

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

A twelve–year retrospective analysis of prevalence and antimicrobial susceptibility patterns of *Ureaplasma* spp. and *Mycoplasma hominis* in the province of Lower Silesia in Poland

Urszula Kasprzykowska[a], Beata Sobieszczańska[a,*], Anna Duda-Madej[a], Anna Secewicz[b], Joanna Nowicka[a], Grażyna Gościniak[a]

[a] Department of Microbiology, Medical University, Wrocław, Poland
[b] Department of Pharmaceutical Microbiology and Parasitology, Medical University, Wrocław, Poland

ARTICLE INFO

Article history:
Received 17 October 2016
Received in revised form 21 August 2017
Accepted 10 November 2017

Keywords:
Genital mycoplasmas
Ureaplasma spp.
Mycoplasma hominis
Antimicrobial susceptibility

ABSTRACT

Objective: Genital mycoplasmas are opportunistic pathogens that have been associated with urogenital infections in humans. Only a few groups of antimicrobials are available for treatment of urogenital tract infections caused by genital mycoplasmas. However, emerging resistance of mycoplasmas to antimicrobial agents has been reported worldwide. The aim of the study was a retrospective analysis of the prevalence and antimicrobial susceptibility patterns of *M. hominis* and *Ureaplasma* spp. in patients with urogenital tract infections during a twelve-year period between 2003 and 2015.
Study design: Mycoplasma IST2 test was used for the detection, enumeration, identification and antimicrobial susceptibility testing of genital mycoplasmas in 1182 samples from 778 women and 404 men with genitourinary tract infection. Indicative enumeration in the test determines whether the mycoplasma count in the sample is equal or higher than the threshold set at 10^4 colony forming units.
Results: A total of 152 (12.8%) samples were found to be positive for genital mycoplasmas. *M. hominis* was detected only in three samples and *Ureaplasma* spp. in 141 samples. Both, *M. hominis* and *Ureaplasma* spp. were detected in the remaining eight samples. In the analyzed period between 2003 and 2015, a gradually increasing resistance of ureaplasmas to ciprofloxacin and clarithromycin and decreasing resistance to ofloxacin, erythromycin and tetracycline were observed. Pristinamycin, josamycin and doxycycline were most active against *Ureaplasma* spp. In contrast, fluoroquinolones had the lowest efficacy against *Ureaplasma* spp. and as many as 116 (82.3%) and 77 (54.6%) of *Ureaplasma* spp. isolates were resistant to ciprofloxacin and ofloxacin, respectively. *M. hominis* isolates were uniformly resistant to azithromycin, clarithromycin and erythromycin but susceptible to josamycin, ofloxacin, doxycycline and pristinamycin. One-third of these isolates were resistant to ciprofloxacin and tetracycline.
Conclusion: In the study *Ureaplasma* spp. and *M. hominis* were detected with relatively low frequency in comparison with other studies however, most of these isolates were resistant to ciprofloxacin indicating the need for better management of ciprofloxacin prescription. Important limitations of Mycoplasma IST2 assay concerning antimicrobial susceptibility testing and divergences between breakpoints in the test and EUCAST guidelines point the need to introduce new methodologies to improve evaluation of resistant strains at our region.

Introduction

Genital mycoplasmas are the group of organisms that includes saprophytic species such as *Mycoplasma primatum*, *M. penetrans*, *M. sparmatophilum* as well as opportunistic one e.g. *M. hominis*, *M. genitalium*, *Ureaplasma parvum* and *U. urealyticum* [1,2]. Although many of these potentially pathogenic species are often isolated from the lower urogenital tract of healthy individuals, they are also

* Corresponding author at: Department of Microbiology, University of Medicine, Chałubińskiego 4 Street, Wrocław 50-368, Poland.
E-mail addresses: bmsobie@gmail.com, beata.sobieszczanska@um.wroc.pl (B. Sobieszczańska).

https://doi.org/10.1016/j.ejogrb.2017.11.010
0301-2115/

implicated in urogenital infections such as non-gonococcal urethritis (NGU), pelvic inflammatory disease (PID), infertility, adverse outcomes of pregnancy and neonatal diseases [3–6].

Prevalence of genital mycoplasmas varies from one study to another, depending upon type of investigated populations, their socioeconomic status, sexual activity and numbers of sexual partners [7]. According to Cassell et al. [5] ureaplasmas can be found in cervicovaginal samples of 40% to 80% of asymptomatic sexually mature women. The prevalence of *M. hominis* is lower and may occur in 21% to 53% of asymptomatic women [1,5]. The prevalence of these both species is lower in the urethra of males as well as among sexually inactive individuals [1,5].

Infections caused by genital mycoplasmas can be treated with tetracyclines, fluoroquinolones and macrolides. Moreover, pristinamycin, an oral streptogramin is recommended for the treatment of urogenital infections although, in Europe it is registered only in France [8]. However, because of unpredictable therapeutic efficacy of these antimicrobials due to persistence of mycoplasmas after therapy and their increasing resistance, other treatment alternatives are considered e.g. oligosaccharide antibiotic evernimicin [9], ketolides (telithromycin) [10], glicylcyclines (tigecycline and new glicylcycline GAR-936) [9,11], linezolid [9], streptogramins (quinupristin-dalfopristin) and newer quinolones such as gatifloxacin, difloxacin, pefloxacin, grepafloxacin, sperfloxacin, moxifloxacin and trovafloxacin [9,12].

Several diagnostic commercial assays (e.g. Mycoplasma IST2, Mycoplasma Duo, Mycofast Revolution, Mycoplasma IES) based on liquid broth cultures are currently available for the rapid detection and identification of genital mycoplasmas in urethral and cervicovaginal swabs. These assays rely on biochemical properties of mycoplasmas i.e. *Ureaplasma* spp. and *M. hominis* metabolize urea and ariginine, respectively, resulting in a color change of the growth medium containing phenol red as pH indicator. These tests present a range of results for specificity and include a reference or two although, their sensitivity is lower than that of culture on selective media [13]. Moreover, none of these tests allow to detect *M. genitalium*, involved in urogenital infections in humans [14].

Reports on the prevalence and antimicrobial options for treating infections caused by genital mycoplasmas in the province of Lower Silesia in Poland are limited therefore the aim of this twelve-year retrospective analysis was to evaluate the prevalence and antimicrobial susceptibility patterns of *Ureaplasma* spp. and *M. hominis* in patients with clinically relevant symptoms of urogenital infection.

Materials and methods

The study included 1182 outpatients (778 non-pregnant women and 404 men, aged from 19 to 81 years) with symptoms of urogenital tract infection (e.g. abnormal discharge, dysuria or other voiding symptoms, dyspareunia, bleeding between periods or after sex, fever and low abdominal pain) who visited Internal Medicine and Gynecological and Obstetrics Clinics of Medical University of Wrocław, Poland between 2003 and 2015 (without 2006 year). A commercial Mycoplasma IST2 assay (M-IST2; bioMérieux, France) was used for the detection, enumeration, identification and antimicrobial susceptibility testing of genital mycoplasmas i.e. *M. hominis*, *U. urealyticum,* and *U. parvum* (collectively referred to as *Ureaplasma* spp.) in patients samples according to the manufacturer's instructions. *Ureaplasma parvum* strain ATCC 27813/7 was used as an internal laboratory quality control. The urethral samples from men were collected using Dacron swabs introduced ca. 3–4 cm into the urethra and rotated for 5 s to obtain urethral epithelial cells, whereas cervicovaginal specimens from women were taken with Dacron swabs inserted 2 cm into the cervical canal and rotated for 5 s to scrape the endocervical epithelial cells. For testing genital mycoplasmas only one swab was taken from each patient. All samples were collected by physicians or well–trained nurses into liquid transport medium R1 that contains selective agents inhibiting the growth of contaminating flora present in the sample. Samples in R1 medium were transported to the laboratory within 4 h of collection and subcultured on arrival. The swabs in the R1 transport medium were vortexed for 10 s and 3 ml of the R1 medium was used to rehydrate the lyophilized selective growth medium R2 containing urea/arginine. The R2 medium was then dispensed into wells 1–5 on the strip providing information on the presence or absence of genital mycoplasmas and 22 test wells, and overlaid with mineral oil to prevent drying. The strips were incubated at 37 °C for 48 h and observed for color changes at 24 h and 48 h. Each test was read independently by two workers and the quality control for subjectivity of interpreting color changes was performed independently by two trained laboratory workers. Change of color in the culture medium from yellow to orange-red is related to an increase of pH and indicates the growth of mycoplasmas with an estimate of the density of each organism $\geq 10^4$ CFU. The development of orange to red color in the medium on the antimicrobial susceptibility testing panel of the test strip provides an index of resistance or susceptibility of growing mycoplasmas to each antimicrobial agent. The antimicrobial resistance breakpoints (mg/L) for the nine antimicrobials were interpreted according to the manufacturer's guide as follows: doxycycline (DOX) $S \leq 4$, $R \geq 8$; josamycin (JOS) $S \leq 2$, $R \geq 8$; ofloxacin (OFX) $S \leq 1$, $R \geq 4$; erythromycin (ERY) $S \leq 1$, $R \geq 4$; tetracycline (TET) $S \leq 4$, $R \geq 8$; ciprofloxacin (CIP) $S \leq 1$, $R \geq 2$; azithromycin (AZM) $S \leq 0.12$, $R \geq 4$; clarythromycin (CLR) $S \leq 1$, $R \geq 4$; pristinamycin (PRI) $S < 2$, $R \geq 2$ where R means resistant and S susceptible. Exclusion criteria were: duplicate samples from the same patients, e.g. samples of the same patient taken at the same

Table 1
Distribution and prevalence of *Ureaplasma* spp. and *M. hominis* according to age group and sex.

Specimen	Frequency of isolation (%)			Prevalence rate (%) n = 1182
	Ureaplasma spp.	Mh	*Ureaplasma* spp.+ Mh	
Men (yrs)				
<25	0	0	1 (0.2)	1 (0.2)
25–50	16 (3.9)	1 (0.2)	2 (0.5)	19 (4.7)
>50	3 (0.7)	0	0	3 (0.7)
n = 404	**19 (4.7)**	**1 (0.2)**	**3 (0.7)**	**23 (5.7)**
Women (yrs)				
<25	6 (0.8)	0	0	6 (0.8)
25–50	112 (14.4)	2 (0.2)	5 (1.2)	119 (15.3)
>50	4 (0.5)	0	0	4 (0.5)
n = 778	**122 (15.7)**	**2 (0.2)**	**5 (1.2)**	**129 (16.6)**
Prevalence rate (%) n = 1182	**141 (11.9)**	**3 (0.2)**	**8 (0.7)**	**152 (12.9)**

Mh, *Mycoplasma hominis*; yrs, years.