Detection of Mycoplasma genitalium in female cervical samples by Multitarget Real-Time PCR

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Abstract

Mycoplasma genitalum (MG) is associated with variety of urogenital infections such as non-gonococcal urethritis (NGU), endometritis and cervicitis. The objective of this study was to demonstrate and evaluate a research polymerase chain reaction (PCR) assay, for the detection of MG in cervical samples of a tested population of women attending gynecology clinics in Bosnia and Herzegovina. The Multitarget Real-Time (MTRT) PCR, utilizing the ABI 7900HT, the sequence detection system, was performed for the detection of MG. Cervical samples (N=97) from females were divided into three types of patient groups: Group 1: patients who had known abnormal clinical cytology reports (N=34); Group 2: patients who reported a history of genitourinary infections (N=22); and Group 3: patients not in either groups 1 or 2 (N=41). Overall, 14,43% (14/97) of those tested were positive for MG. A positive sample was defined as having a cycle threshold cross point (Ct) < 40,0 with a fluorescent detection comparable to the low positive control utilized during the run. This study validated the use of MTRT PCR as a reliable method for the detection of MG in clinical specimens and should facilitate large-scale screening for this organism.

KEY WORDS: *Mycoplasma genitalium*, Multitarget real-time PCR

Introduction

Mycoplasma genitalum (MG) is associated with variety of urogenital infections such as nongonococcal urethritis –NGU (5-10% of cases), endometritis and cervicitis (1). Mycoplasmas are the smallest free-living prokaryotic microorganisms. They posess unique characteristics among prokaryotes such as the lack of cell walls and complete insensitivity to the penicillins and cephalosporines. These organisms have sizes of about 0,2-0,3 μm, but they are highly plastic and pleomorphic and may appear as coccoid bodies, filaments, and large multinucleoid forms. They belong to the family of *Mycoplasmatacae* which are divided into two geneses: *Mycoplasma* and *Ureaplasma*. Most mycoplasmas are comensals for humans with exception of the following pathogens: *M. pneumoniae, M. hominis, M. genitalium* and *U. urealiticum* (Table 1.). MG was first isolated from urethral specimens from 2 of 13 men with urethritis in 1981 (2, 3). Laboratory detection includes culture and molecular techniques. Different polymerase chain reaction (PCR) assays for detection of MG have been developed, but there are some limitations including the potential for false positive results, with PCR techniques, as well as false negative results when using culture. The multi-target real-time (MTRT) PCR for MG utilizes two different gene targets; the MgPa gene and 16S ribosomal gene and was developed to limit the number of false positives generated (4).

Patients and Methods

DNA extractions of each concentration of the positive control standards (starting with 1X105 colony forming units (CCU/PCR reaction and ending with 100 CCU/PCR) were performed utilizing the Roche MagNA Pure LC® robotic instrument (Roche Molecular Diagnostics, Indianapolis, IN). DNA extraction was carried out according to the manufacturer's instructions supplied for the MagNA Pure LC® program «DNA I Blood Cells High performance Serum» protocol. Negative controls were compared in a similar manner using molecular grade water as the negative template. Cervical swab samples were collected from 97 women from three disease defined groups:

Group 1: patients who had an abnormal Papanicolaou (PAP) cytology report (N= 34);

Group 2: patients who had a history of genitourinary infections (N=22),

Group 3: patients not in either group 1 or 2 (N=41) (Table 2).

Age groups were defined as Group A (20-24yr), Group B (25-29yr), Group C (30-34 yr), Group D (35-39yr), Group E (40-44 yr), Group F (45-49 yr), Group G (>50 yr). Swab samples were collected from December 2004 to January 2005 at two sites in Sarajevo: the Department of Gyneacology of the University of Sarajevo Clinics Center and the Institute for Health Protection of Women and Motherhood. Specimens were shipped frozen to Johns Hopkins University, Baltimore, MD, for final testing (samples kept at -80°C until testing). Samples were extracted in a manner identical to the positive and negative controls and standards utilizing the Roche MagNA Pure LC ® robotic instrument.

Oligonucleotides primers and probes were as follows:

MgPa gene target (Ref 1, MgPa gene)

MgPa-355F 5' gagaaataccttgatggtcagcca 3'

MgPa-432R 5'gttaatatcatataaagctctaccgttgttgttatc 3'

MgPa-380 5'TET-actttgcaatcagaaggt-MGBNFQ 3'

16S rRNA gene target (Ref 2,16S rRNA)

My-INS 5'gtaatacataggtcgcaagcgttatc 3'

MGSO 2 5' caccacctgtcactcggttaacctc 3'

Mgen P1 probe 5' 6FAM-ctgtcggagcgatcccttcggt-MGBNFQ 3'

MTRT PCR reactions were performed with 80 μl of master mix and 20 μl of template DNA. The master mix contained: 10 μl of 10X Qiagen HotStarTaq polymerase buffer, 20 μl of 25mMCl_2, 8 μl of 25 mM dNTPs, 0.5 μl of each 50 uM primer and probe, 36 μl of PCR-grade H2O, 1 μl of Qiagen Q solution, 2 μl of Qiagen HotStar-

Organism	Site	Prevalence	Disease
M.salivarium	Peridontal suci	Very common	None
M. orale	Upper respiratory tract	Very common	None
M. pneumoniae	Upper and lower respiratory tract	Common	Primary atypical pneumonia
M. fermentas	Genitourinary tract	Rare	None
M. hominis	Genitourinary tract	Common	Postpartum fever; pelvic inflammatory disease
U. urealiticum	Genitourinary tract	Very common	NGU
M. genitalium	Genitourinary tract	Undetermined	NGU, endometritis, cervicitis

TABLE 1. Common Mycoplasma and Ureaplasma Species of Humans

Age	Group 1	Group 2	Group 3	Total	Percent (%)
20-24	2	2	0	4	4,12
25-29	9	6	6	21	21,65
30-34	7	4	9	20	20,61
35-39	4	1	10	15	15,47
40-44	6	1	8	15	15,47
45-49	2	4	5	11	11,35
> 50	4	2	2	8	8,24
No data	0	2	1	3	3,09
TOTAL	34	22	41	97	100

TABLE 2. *Mycoplasma genitalium* positive samples by age And disease defined groups (1-3).

MTRT PCR (MG)	No. of patients	Percent (%)
Negative test	83	85,57
Positive test	14	14,43
Total	97	100

TABLE 3. MTRT PCR for *Mycoplasma genitalium* MG) in all women using the ABI7900 HT

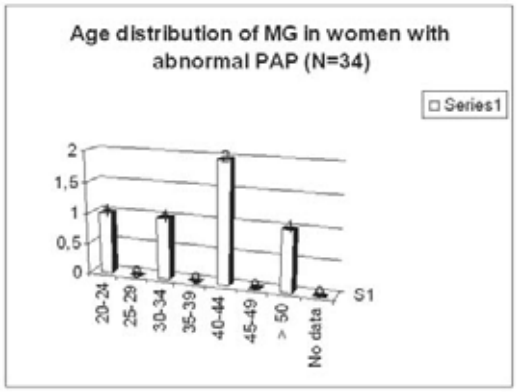

FIGURE 1. Age distribution of MG in women with abnormal PAP (N=34)

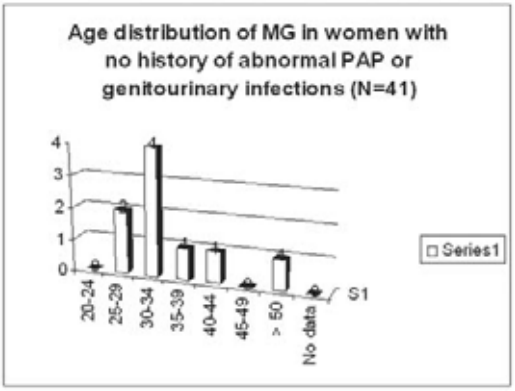

FIGURE 2. Age distribution of MG in women with no history of abnormal PAP or genitourinary infection

Taq polymerase and 20 µl of template DNA. The 10X buffer, MgCl2, Q solution and HotStarTaq polymerase kit were obtain from the (Qiagen, Valencia, CA). The dNTPs were obtained from the 100 mM dNTP set PCR Grade (Invitrogen). PCR-grade water was obtained from Quality Biologics Incorporated (Quality Biologics Incorporated, Gaithersburg, MD). PCR was performed in a 96 well plate format on the ABI 7900 HT Sequence detection system (Applied BioSystems, Foster City, CA) with ROX reference removed under the following conditions: 50 °C for 2 min., 95 °C for 10 minutes followed by 50 cycles of 95 °C for 15 sec., 60 °C for 1 min. and 72 °C for 30 sec. A cooling hold of 4 °C for 2 minutes was added at the end of the cycling protocol for ease of plate handling (4). A positive sample was defined as having a Ct ° 40,0 and having a overall fluorescent increases comparable to the low positive control utilized during the run. Negative samples demonstrated no increase in fluorescence and a Ct > 40,0. The association between the patients age, diagnosis and MG findings were examined by Chi square (x^2) test with SPSS 11.0.

Results

Numbers of women in each age group by disease category are shown in Table 2. For the whole population 14,43% (14/97) of the population were MG positive by MTRT PCR (Table 3). For the three types of disease groups, 5/14 of the positive samples were from to disease Group 1, while 9/14 positive samples were from disease defined Group 3. The most positives were from age category 40-44 yr. in disease group 1 (2 positives) and age category 30-34 yr. (4 positives) in disease defined group 3 (Figure 1, 2). Of the 14 women who were positive for MG, one was diagnosed with ASCUS (Atypical Squamous Cells of Uncertain Significance), four had CIN 1(Cervical intraepithelial neoplasia grade 1), and two had CIN 2(Cervical intrepithelial neoplasia rade 2).

Discussion

The 97 cervical samples of examined female population divided into three disease groups were analyzed. There was a significant prevalence (14,43%) of detected MG in the study population. All MG infections were found in the disease group 1 of 34 women with an abnormal PAP and in the group of 41 women in disease group 3 with no abnormal PAP or genitourinary infections. There were no MG positives in the group with a history of genitourinary infections (group 2). In one Swedish study, 85 women were examined dur-

ing a period of 11 months and the prevalence of MG was 3,5% respectively (5). Another Swedish study reported an MG prevalence of 5% (6). In a retrospective study of women attending an STD clinic in Seattle, Washington, stored samples from 1984 to 1986 were tested in 2001 and the MG prevalence was 7% compared with 11% for *Chlamydia trachomatis* (7). It may be likely that MG is less prevalent than *C. trachomatis*, and that MG is not widely spread in a low risk asymptomatic population. This notion is supported by the absence of MG infection among the women in the screening group. Only one study conducted in 1994-1996 in France has shown a completely different pattern with an MG prevalence among symptomatic female STD clinic attendees of 38% (65/170), and a *C. trachomatis* prevalence of 8% (14/171) (8). Two previous studies comparing MG prevalence among women with and women without cervicitis produced conflicting results (8). Morever, no adjustment for other cervical pathogens or potential confounding variables was performed, nor were risk factors for infection identified. Casin et al. (8) found no association between MG and cervicitis among women presenting with symptoms to an STD clinic. Unlike Manharts study (7), Casins study (8) did not include asymptomatic women, mucopurulent cervicitis, the (MPC) prevalence was extremely high (30% vs. 85%), and samples for MG detection were taken from the cervix, urethra, and/or vagina (vs. the cervix in Manharts study), which may account for the difference in findings.

Conclusion

Several PCR assays have been described to detect *Mycoplasma genitalium* in vaginal, cervical and urine specimens from infected patients (1-3). Traditional PCR methods require postamplification detection of products, which can be error prone, laborious, a time consuming (1). The recent advent of real-time PCR has improved accuracy and eliminated the need for any postamplification processing. Real-time PCRs couple amplification with detection and are reported by the threshold cycle number (Ct), or the point at which the PCR product accumulates significantly over baseline levels, as detected by interaction with fluoregenic probes. The MTRT PCR did limit the number of potential false positive results during the study with high-throughput detection of *Mycoplasma genitalium* because two gene targets were evaluated. This study validated the use of MTRT PCR as a diagnostic method for the detection of MG and should facilitate large-scale screening for this microorganism. In conclusion, this new PCR assay could serve as a model for future assay development not only in MG detection, but for other sexually transmitted diseases (STDs).

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