

ETIOLOGY OF GENITAL DISCHARGE WITH SPECIAL REFERENCE TO UROGENITAL MYCOPLASMA IN A MEDICAL COLLEGE AND HOSPITALSwagnik Roy¹, Kalpana Karak², Barun Sahadlal³**HOW TO CITE THIS ARTICLE:**

Swagnik Roy, Kalpana Karak, Barun Sahadlal. "Etiology of Genital Discharge with special reference to Urogenital Mycoplasma in a Medical College and Hospital". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 86, October 26; Page: 14984-14988, DOI: 10.14260/jemds/2015/2128

ABSTRACT: Mycoplasma species are associated with infection of the genito-urinary tract, reproductive failure, and neonatal morbidity and mortality. Infection with genital mycoplasmas has been linked with infertility. Study was conducted from August 2012 to August 2013 in a Medical College and Hospital, Kolkata. Total 170 patients cervical swab sample were processed among which 120 were symptomatic patients and 50 were Healthy controls among the symptomatic patient's six Mycoplasma hominis were isolated, three Mycoplasma genitalium were isolated and twelve Ureaplasma urealyticum were isolated. Among healthy controls only one Ureaplasma urealyticum were isolated which may be attributed due to urogenital colonization.

KEYWORDS: Mycoplasma, Genital Discharge, PCR.

INTRODUCTION: Mycoplasma genitalium (M. genitalium) is 1 of 15 Mycoplasma species isolated in humans. The most commonest colonization site is the urogenital tract.^[1] The organism adheres to epithelial cells, invades them, and uses the intracellular environment as a survival area where it multiplies and survives.^[2-4] M. Genitalium was first isolated in 1980 from the urethra of 2 men with nongonococcal urethritis.^[5] However, because the organism is difficult to culture, clinical and epidemiological studies only became possible following the development of sensitive and specific polymerase chain reaction (PCR) assays in the early 1990s.^[6,7] Studies investigating transmission of M. Genitalium in couples and DNA sequence typing among sexual partners have concluded that the bacterium is sexually transmitted.^[8-12] M. Genitalium is now considered a main cause of nonchlamydial nongonococcal urethritis (NCNGU) in men. A meta-analysis of 15 case-control studies reported that the prevalence of M. genitalium in men with NCNGU was 22%, compared with 6% in those without urethritis (Pooled odds ratio [OR], 5.15; 95% confidence interval [CI], 3.6-7.4).^[13] Except for 1, all studies were conducted in high income countries.^[14] M. Genitalium is also found in the female reproductive tract, and there is increasing evidence that this bacterium may cause cervicitis and urethritis and may be correlated with upper genital tract infections and its sequelae such as tubal factor infertility.^[15-20] Mycoplasma species are associated with infection of the genitourinary tract, reproductive failure, and neonatal morbidity and mortality. Infection with genital mycoplasmas has been linked with infertility.^[21]

MATERIALS AND METHODS: Present study was conducted from August 2012 to August 2013 in a Medical College and Hospital, Kolkata. For the detection of M. genitalium, the endocervical specimen was inserted in a buffer solution, using Cobas Amplicor specimen transport medium collection tubes (Roche Diagnostic Systems). The samples were stored at 4°C until transport to the Microbiology Dept. laboratories within 12 hours of collection. These specimen were kept at -20°C until sample collection from 30 specimen were completed.

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Culture for genital mycoplasmas. Specimens were inoculated onto A7agar (Becton Dickinson, Cockeysville, Md. 21030) and incubated at 37°C in 5% CO₂ for 5days. For Ureaplasma it is inoculated in 10% urea supplemented broth. Cultures were examined microscopically daily for 5days for the appearance of typical mycoplasma colonies.

A7agar incorporates a direct test for urease that allows the differentiation of urea plasma from the other Mycoplasma tales. Specimens were also inoculated in Urogenital Mycoplasma broth incorporated with yeast extract, Horse Serum, vitamin and mineralo growth supplements and then followed by subculture in to A7agar.

Multiplex PCR assay for genital mycoplasma infection. Bacterial DNA from 100micro litre of specimen or transport media was isolated by lysis in 400 micro litre of lysis buffer, extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), and extracted again with chloroform-isoamyl alcohol. DNA was then precipitated in 100% isopropanol, washed in 70% ethanol, and suspended in 15 micro litre of RNase-DNase free sterile deionized water (Sigma, St. Louis, Mo.).

Multiplex PCR was performed with primers specific for highly conserved regions in the urease gene of Ureaplasma spp, the 140-kDa adhesion protein gene of *M. genitalium*, and the 16Sr RNA gene of *M. Hominis*.^(4,5,3,6) Hot-start PCR was performed on the equivalent of 25micro litre of sample in 50microlitre reactions containing a 0.2 mM concentration of de-oxynucleoside triphosphate mixture, 10mM Tris, 3mM MgCl₂, 25pmol of each unlabeled forward primers, and 25pmol of biotin-labeled reverse primer (Table1) and 1.25U of GoldTaq (Applied Biosystems.). All reactions were performed in a Thermocycler under the:

FOLLOWING CONDITIONS: 1cycle of 10min at 95°C, followed by 35, two-step cycles of 95°C for 15s and 60°C for 60s, followed by 5min at 72°C PCR product detection. Urea plasma and *M. genitalium* PCR products were detected by enzyme-linked oligosorbent assay (ELOSA).

M. hominis, were further evaluated by digestion with NarI, which results in the digestion of *M. hominis* PCR product to fragments of 62 and 272bp Analytical sensitivity. The analytical sensitivity was determined by amplification of twofold serial dilutions of bacterial DNA, either individually or as a mixture of all three organisms. Dilutions ranged from 3.13 to 100CFU. The lower limit of detection (LOD) was the CFU equivalent of DNA in the last sample positive in the dilution series.

RESULTS OBTAINED: Total 170patients cervical swab sample were processed among which 120 were symptomatic patients and 50were Healthy controls among the symptomatic patient's six *Mycoplasma hominis* were isolated, three *Mycoplasma genitalium* were isolated and twelve Urea plasma urealyticum were isolated.

Among healthy controls only controls only one Urea plasma urealyticum were isolated which may be attributed due to urogenital colonization. 2X2 Contingency table showing association between presence of total genital mycoplasmas and type of patient (pt Vs. Control) is shown in the below table.

Total Genital Mycoplasma Isolated	Patient	Control	Total
Mycoplasma found	21	1	22
Mycoplasma not found	99	49	148
Total	120	50	170

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Exposure rate among patients were found to be 17.5% and among controls it is found to be 2%. The P value is found to be <0.001.

DISCUSSION: In our assay we standardised a multiplex PCR assay for the simultaneous detection of Urea plasma spp. *M. genitalium*, and *M. hominis* in clinical specimens. This assay appeared to be very sensitive, with an analytical LOD of 12.5CFU for all three organisms. Furthermore, this assay demonstrated high analytical specificity. DNA from other closely related *Mycoplasma* species, as well as from a variety of other common urogenital organisms did not amplify in this multiplex PCR.

In addition, the multiplex PCR was more sensitive than culture. In clinical specimens, PCR enhanced the detection rate of genital mycoplasma by using specific primers. This increased sensitivity was seen primarily in female specimens, where more true-positive samples were detected by PCR than by culture. Though further study is required to reach in to this hypothesis that PCR is more specific than culture where Urogenital *Mycoplasma* tales infection is concerned.

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Analysis, Organism, and Primer or Probe	Target or DNA Sequence (5'-3')	Length (bp)
Mycoplasma hominis	RNAH1 CAATGGTAATGCCGGATACGC	334bp
Mycoplasma hominis	RNAH2 GGTCCGTCAGTCTGCAAT	334bp
Mycoplasma genitalium	MG16-45 F TACATGCAGTCGATCGGAAGTAGC	282bp
Mycoplasma genitalium	MG16-447R AAACCTCCGCCATTGCCTGCCTGCTAG	282bp
Ureaplasma urealyticum	U4 primer ACGACGTCTAAGCACT	429bp
Ureaplasma urealyticum	U5 primer CAATCTGCTCGTGGTATTAC	429bp
Table 1: Nucleotide Sequences of Primers and Probes Used		

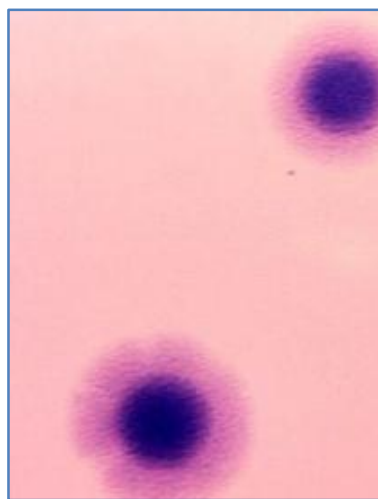


Fig. 1: Dienes's Stain of Mycoplasma colonies on Urogenital Mycoplasma Agar



Fig. 2: Urogenital Mycoplasma Broth

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