

Screening of Antenatal Lower Genital Tract Infection in Women of Saudi Arabia for Preventing Preterm Birth and Low Birth Weight using PCR Techniques

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The aim of this study was to detect bacterial infection of: *Chlamydia trachomatis*, *Ureaplasma* spp. and *Mycoplasma*, from Saudi women samples. Samples were 100 from pregnant women attending the obstetrics and Gynecology clinic at Alymamah Hospital, Riyadh, Saudi Arabia during March 2013 to March 2014. DNA was successfully amplified from 56 samples. The results of this study indicated more reliable method which detected *Chlamydia trachomatis*, *Ureaplasma* spp. and *Mycoplasma* which caused vaginitis in lower genital, showed high frequency of both *Mycoplasma* spp (44.6%) and *Ureaplasma* spp (30.4%) infection among Saudi pregnant women. Association of *Mycoplasma* spp, *Ureaplasma* spp, and *C. trachomatis* has been observed but no association was observed between *M. hominis*, *M. genitalium* and *U.urealyticum*.

Keywords: *Chlamydia*, *Mycoplasma*; *Ureaplasma*; Vaginitis.

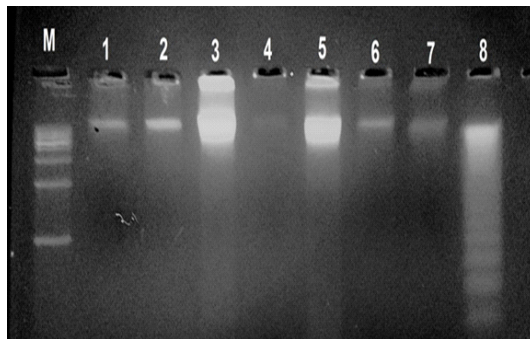
Infection screening of pregnant women before 37 weeks, between conception and birth may help decrease preterm birth and low birth weight and over growth of bacterial species normally present in the vagina along with anaerobic bacteria cause the disease. (Hillier *et al.*, 1993) *M. genitalium* genome very small comprises 580 Kbp, with a capacity to encode only 482 genes. (Fraser *et al.*, 1995). (Antonio *et al.*, 1999). and (May *et al.*, 1999). Though, *M. genitalium* genome being so small it is capable to cause the most frequently reported like pelvic

inflammatory disease (PID). (Glass *et al.*, 2006). *Mycoplasma hominis* is an opportunistic human mycoplasma. Two pathogenic human species, *M. genitalium* and *Ureaplasma parvum*, reside within the same natural niche as *M. hominis*: the urogenital tract. These three species have overlapping, but distinct, pathogenic roles. (Pereyre *et al.*,2009). *Mycoplasma genitalium* is well established as a cause of urethritis and has also been associated with cervicitis, endometritis and pelvic infection. (Ross *et al.*,2009). To estimate the accuracy of the clinical diagnosis of the three most common causes of acute vulvovaginal symptoms (bacterial vaginosis, candidiasis vaginitis, and trichomoniasis vaginalis) using a traditional, standardized clinical diagnostic protocol compared with a DNA probe laboratory

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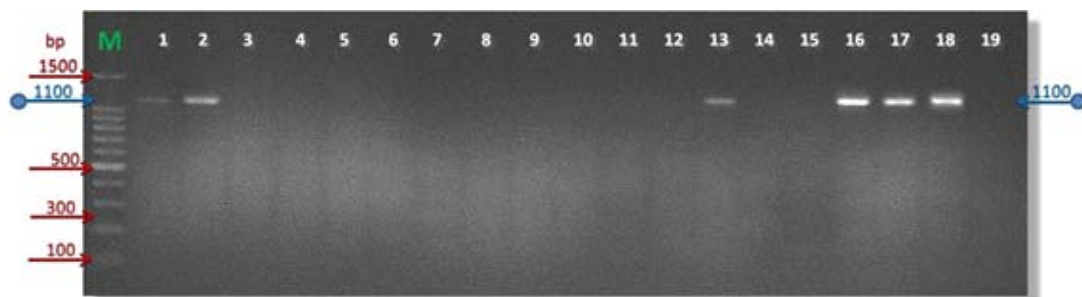
standard had been reported by (Lowe *et al.*,2009). The prevalence of *M. genitalium* and *C. trachomatis* were 2.5% and 2.8%, respectively. The *M. genitalium* was strongly associated with post-termination pelvic inflammatory disease. The increased risk for pelvic inflammatory disease associated with *M. genitalium* infection after termination of pregnancy suggests a causal relationship. (Bjartling, *et al.*, 2010). *Mycoplasma hominis* and *Ureaplasma urealyticum* has historically been the subject of attention, but *Mycoplasma genitalis* which causes male urethritis in addition to colonizing the female genital tract and the division of *Ureaplasma* into two species, *urealyticum* and *parvum*, has also added new taxonomic clarity .

(Larsen and Hwang 2010). *Mycoplasma genitalium* is a sexually transmitted organism associated with non-gonococcal urethritis in men and several inflammatory reproductive tract syndromes in women such as cervicitis, pelvic inflammatory disease, and infertility. There was evidence for an association of *M. genitalium* with endometritis and pelvic inflammatory disease (PID), but additional studies are necessary to confirm this. The evidence as to whether *M. genitalium* can cause adverse pregnancy outcomes such as preterm labor is conflicting. But the authors of some studies on *M. genitalium* as a cause of infertility have reported this association. These species are very difficult to culture; thus, nucleic acid amplification testing is the only method available for *M. genitalium* detection (Cazanave *et al.* 2012) and (Sena *et al.*, 2012). Bacterial vaginitis correlates to a decrease or loss of the normally present protective lactobacilli that helps in maintaining vaginal acidic pH through the metabolism of glucose- glycogen. (Ljubomir *et al.*,2014). and (Mirmonsef *et al.*,2014). *Mycoplasma genitalium* is a sexually transmitted organism commonly treated with azithromycin. However, macrolide resistance has been reported and is associated with point mutations in the 23S rRNA gene. (Touati, *et al.*, 2014). In a study of mycoplasma associated with trichomoniasis, vaginal infection, mycoplasma was found almost exclusively in women infected with sexually transmitted pathogen *Trichomonas vaginalis*, but rarely observed in women with no diagnosed disease. (Fettweis, *et al.*, 2014). PCR technology is widely used in the diagnoses of



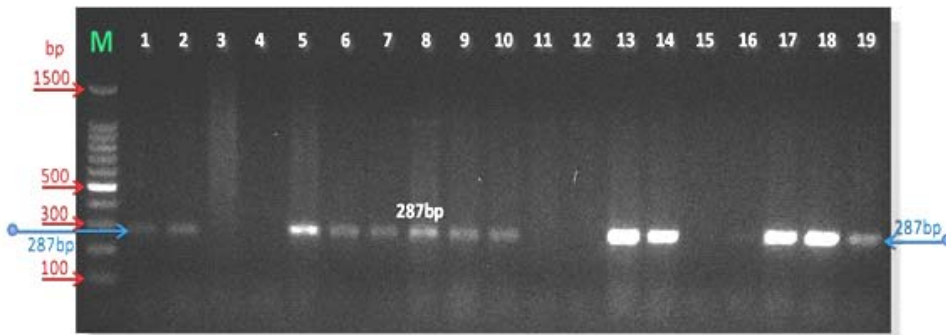
M: is marker 1500bp
Lanes 1—8: are DNA isolated from *Chlamydia trachomatis*, *Ureaplasma* spp. and *Mycoplasma* which was used for PCR template.

Fig. 1. DNA isolated from *Chlamydia trachomatis*, *Ureaplasma* spp. and *Mycoplasma* which was used for PCR template



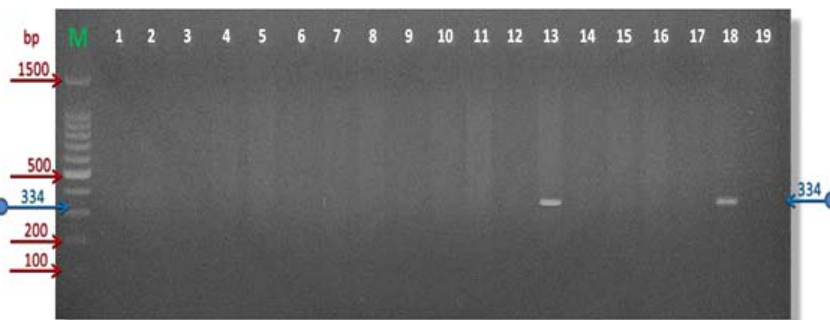
F: ATGAAAAAACTCTTGAAATCG
R: ACTGTAAGTGCATTTGTCTG
M: is marker 1500bp
Lanes 1—19: are samples of PCR product after gel electrophoresis

Fig. 2. PCR amplification products of *Chlamydia trachomatis* (1100 bp with primers)



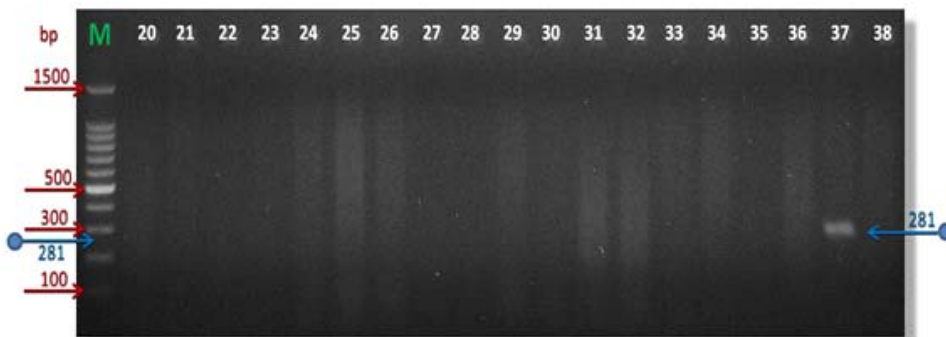
F: GGGAGCAAACAGGATTAGATACCCT
 R: TGCACCATCTGTCACCTCTGTTAACCTC
 M: is marker 1500bp
 Lanes 1—19: are samples of PCR product after gel electrophoresis

Fig. 3. PCR amplification products of *Mycoplasma* spp (287 bp) with primers



F: CAATGGCTAATGCCGGATACGC
 R: GGTACCGTCAGTCTGCAAT
 M: is marker 1500bp
 Lanes 1—19: are samples of PCR product after gel electrophoresis

Fig. 4. PCR amplification products of *M. hominis*(334bp) with primers



F: AGTTGATGAAACCTTAACCCCTTGG
 R: CCGTTGAGGGGTTTCCATTTTTGC
 M: is marker 1500bp
 Lanes: 20—38: are samples of PCR product after r gel electrophoresis

Fig. 5. PCR amplification products of *M. genitalium*(281bp) with primers

bacterial infection such as polyarthritis with axial involvement in a young female and for acute lymphoblastic leukemia. (Balsat *et al.*, 2014). The objectives of the present study are to detect bacterial species causing birth before the time of delivery so that it may minimize consequences of low birth weight and subsequent occurrences of the disease. The objectives include also to establish a reliable PCR based detection of bacterial infectious for routinely application, both symptomatic and asymptomatic women with pregnancy.

MATERIALS AND METHODS

The study population was randomly sampled in duplicate from hundred pregnant women visiting 'Al Yamamah' hospital in Riyadh, Saudi Arabia, who showed both symptomatic and asymptomatic vaginitis and had no antibiotic treatment for 2 months before the date of examination. The study lasted over a period of a year (March 2013–March 2014). The samples were collected by the nurses in obstetrics and Gynecology departments. A written consent was obtained from each of the study participants.

The protective mucous membrane on the exterior of the cervix was first wiped of exudate, a swab was inserted into the endocervical canal, passing the squamo-columnar junction, to permit the acquisition of columnar epithelial cells which are the main reservoir of the organism, firmly

rotated and then withdrawn. The swabs containing the samples were stored in ice and later stored in -20 freezers.

Data collection

An interview questionnaire for clinical and obstetric history was delivered. Data included age, first delivery, previous abortion, use of contraceptives, date and time of sample collection and symptoms of lower genital tract infection (bacterial vaginitis).

DNA Extraction

Total genomic DNA was extracted by using DNA Mini Kit (Qiagen, USA) following the manufacturer's instructions. Using a fluorometer (Hoefer DyNA Quant 200; Pharmacia Biotech, Piscataway, N.J.) the quantity and quality of the DNA was determined. DNA was also run on a 1% agarose gel to determine the consistency and purity. The stock DNA samples were diluted with sterile TE buffer to make a working solution of 10 Kg ml⁻¹ for use in PCR analysis.

Polymerase chain reaction

Six specific primers for the detection of six different microorganisms (*Chlamydia trachomatis*, *Ureaplasma spp.*, *U. urealyticum*, *Mycoplasma spp.*, *M. hominis*, and *M. genitalium*) were used for PCR amplification (Table 1). The specific primer pair used for the detection of *C. trachomatis* amplified its outer membrane protein A (ompA) gene. To detect *Ureaplasma spp* specific 16S rRNA primer pairs were used and for *U. urealyticum* specific primer pair targeting multiple

Table 1. Specific primer pairs used in this study for detection of *Chlamydia trachomatis*, *Ureaplasma spp.* and *Mycoplasma*, from Saudi women samples

Organism	Primer 5-3	Annealing Temp./°c	Amplicon size	Reference
<i>Mycoplasma spp</i>	F ¹ : GGGAGCAAACAGGATTAGATACCCT R ² :TGCACCATCTGTCACTCTGTAAACCTC	52	287 bp	Choppa <i>et al.</i> , 1998
<i>M. genitalium</i>	F: AGTTGATGAAACCTTAACCCCTTGG R: CCGTTGAGGGGTTTTCCATTTTTGC	62.9	281bp	Jensen <i>et al.</i> , 1991
<i>M. hominis</i>	F: CAATGGCTAATGCCGATACGC R: GGTACCGTCAGTCTGCAAT	54.5	334 bp	Blanchard <i>et al.</i> , 1993
<i>C. trachomatis</i>	F: ATGAAAAAACTCTTGAAATCG R: ACTGTAACCTGCGTATTTGCTG	62	1100 bp	Jurstrand <i>et al.</i> , 2010
<i>Ureaplasma spp</i>	F: GGGTGCACGTATCAGATAGTTG R: TGTGGCTGTTCTACCTCTCAGTACA	58	84 bp	Choppa <i>et al.</i> , 1998
<i>U. urealyticum</i>	F: GTATTTGCAATCTTTATATGTTTTCG R: TTTGTTGTTGCGTTTTCT	55	476 bp	Kong <i>et al.</i> , 2000

banded antigens (MBA) in upstream region was used. For *Mycoplasma* spp primer pair for 16S rRNA for universal region was used. Similarly for *M. hominis* primer pair specific for 16S RNA of *M. hominis*, that amplified 334 bp of amplicon, were used. The primer pair for *M. genitalium* amplified 281 bp adhesion gene fragments. All the six primer pairs were dissolved in sterilized distilled water at a concentration of 10 pmol/μl. Amplification reactions were performed in volumes of 25 μl using 'GoTaq Green Master Mix 2X (Promega, USA). It is optimized for PCR reactions. In each reaction 50 Kg of DNA samples were used along with 20 pmol/μl of primer. PCR amplification was performed in Eppendorf Master Cycler Gradient PCR machine. The following PCR program was used: (1) one cycle for 10 min at 95°C; (2) 35-45 cycles at 95°C for 1 min, 52-62.9°C (depending on the Tm of the primers) for 1.5 min and 72°C for 1 min; (3) one cycle for 10 min at 72°C, followed by a soaking at 4°C.

Electrophoresis

PCR products were separated by electrophoresis according to their molecular weight on 1.5% (w/w) agarose gels submerged in 1x TBE buffer and then stained with ethidium bromide (10 mg ml⁻¹) solution for 20 min. The DNAs were visualized on a UV trans illuminator and documented The amplified fragment was estimated by running 100 pb Ladder (Bio Rad) in the gel as standard size marker.

RESULTS AND DISCUSSION

DNA was successfully amplified from 56 samples out of 100 samples (See fig. 1). Eleven out of 56 samples (19.7%) were detected for *C. trachomatis* with 1100 bp (See fig. 2). Twenty five out of 56 samples (44.6%) for *Mycoplasma* spp were positive with amplicon 287 bp (see fig 3).

By using specific primer targeting MBA genes, two were positive out of 56 samples for *M. hominis* (3.6%) with amplified fragment of size 334 bp (See fig. 4). While one (1.8%) for *M. genitalium* with 281 bp (See fig. 5). Six specific taxon-directed 16S rRNA gene primer pairs were used in PCR assay to detect the presence of vaginal bacteria (see table1). The Prevalence of bacterial vaginitis infection according to the age of the patients (See fig.6).

Mixed infection was observed in this study which may be due to the association of *Mycoplasma* spp, *Ureaplasma* spp, and *C. trachomatis*. No association was observed between *M. hominis*, *M. genitalium* and *U. urealyticum*. These results indicated more reliable method which detected bacteria causing vaginitis in lower genital. The results of this study showed high frequency of both *Mycoplasma* spp (44.6%) and *Ureaplasma* spp (30.4%) infection among Saudi pregnant women. Bacterial vaginitis correlates to a decrease or loss of the normally present protective lactobacilli that helps in

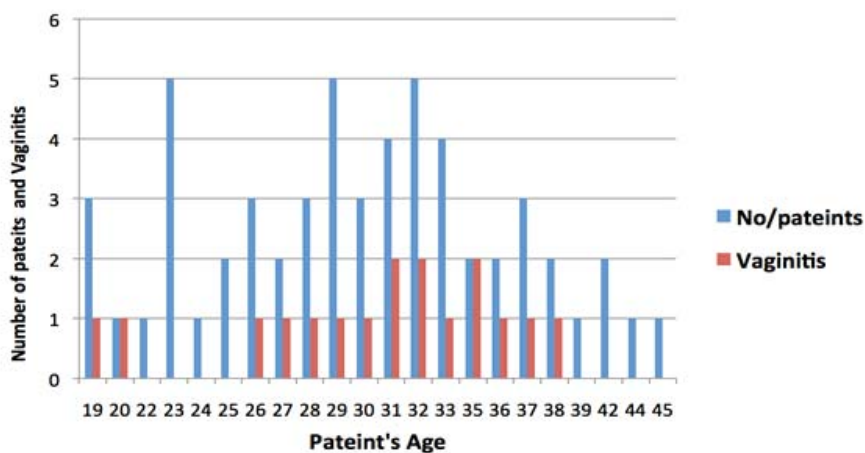


Fig. 6. Prevalence of bacterial vaginitis according to patient's age, and number of individuals

maintaining vaginal acidic pH through metabolism of glucose- glycogen that produce lactic acid. Hydrogen peroxide (H₂O₂) produced by some Lactobacilli spp also helps in maintaining a low pH, which inhibits bacterial over growth. Thus a considerable loss of these protective lactobacilli may lead to vaginitis that could further lead to premature rupture of membranes, HIV, PID, preterm birth and low birth weight (May A. D. *et al.*, 1993) and (Antonio *et al.*, 1999). Diagnoses of vaginitis using traditional method based on clinical symptoms, microscopy, amine odor test and pH test of vaginal fluid, about two third of the cases of bacterial vaginitis were misdiagnosed in women with vaginal complaints. Furthermore, infection may be asymptomatic as high as 50% of cases (Lowe *et al.*, 2009). Cross-sectional samples from rural South Africa women were tested for *Mycoplasma genitalium*. Overall, 10.8% of women were infected with *M. genitalium* either in the vagina or in the rectum. (Hay B *et al.*, 2015). We studied six specific primers for the detection of six different microorganisms (*Chlamydia trachomatis*, *Ureaplasma spp*, *U. urealyticum*, *Mycoplasma spp.*, *M. hominis*, and *M. genitalium*) by using PCR amplification. Their specificity and accuracy were very high and reliable. Lower genital tract infections are the main cause of preterm birth. Infants born preterm are with low birth weight and often develop immediate complications that require significant long term health-care resources. Therefore, we propose screening and treatment of such infections as a preventive measure. Preterm birth is a major public health problem in maternity homes and hospitals.

This study was conducted to determine if PCR can be reliable and useful to detect and quantify several of the lower genital tract bacteria that cause bacterial vaginitis. The results showed that PCR can be reliable to detect *Chlamydia trachomatis*, *Ureaplasma spp*, *U. urealyticum*, *Mycoplasma spp.*, *M. hominis*, and *M. genitalium* in lower genital tract samples. The results of this study showed high frequency of both *Mycoplasma spp* (44.6%) and *Ureaplasma spp* (30.4%) infection among Saudi pregnant women. Mixed infection has also been observed which may be due to the association of *Mycoplasma spp*, *Ureaplasma spp*, and *C. trachomatis*. No association was observed between *M. hominis*, *M. genitalium* and *U.*

urealyticum. PCR assays have advantage over the two most commonly used methods for detecting lower genital tract bacteria; the Amsel criteria and the Gram stain, in that it is more sensitive. In conclusion, this study indicates that it is feasible to use PCR to detect and quantify the numbers of bacterial related vaginitis.

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