World Journal of Medical Sciences 9 (3): 142-146, 2013
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2013.9.3.76220

Detection of Some Sexually Transmitted Bacterial Infection Using Molecular Genetic Technique

[1]Nabil S. Awad, [2]Manal M. Said, [1]Alaa A. Mohamed and [1]Adel E El-Tarras

[1]Biotechnology and Genetic Engineering unit,
Scientific Research Deanship, Taif University, KSA
[2]Department of Biotechnology, Faculty of Science, Taif University, KSA

Abstract: Worldwide *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are two of the most prevalent sexually transmitted pathogens. The present study aimed to estimate the prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection in women from Taif, western region, KSA. A polymerase chain reaction (PCR) method was carried using DNA extracted from 144 female urine samples. Primer pairs specific for Orf8 and 16S rRNA was used to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae,* respectively. The prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was 11.1 and 2.1% respectively. Further larger studies more focused on other sexual transmitted infections (STIs) are needed.

Key words: Sexual transmitted disease · KSA · PCR · Orf8 · 16rRNA

INTRODUCTION

Sexually transmitted infections (STIs) are one of the most important health problems worldwide. There are more than 30 different sexually transmissible agents [1]. Of the "top ten" sexually transmitted infections, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* ranked second and fifth, respectively, worldwide [2].

Chlamydia are small gram-negative eubacteria that grow intracellular. It is responsible for high levels of morbidity [3-5]. It has been reported that, the most serious sequel occurring in women are pelvic inflammatory disease (infection in the fallopian tubes) and ectopic pregnancy (pregnancy in the tubes) [6]. Moreover, untreated maternal cervical chlamydial infection increases the risk of preterm delivery, premature rupture of membranes (PROM) and prenatal mortality or stillbirth [7]. In newborns, infection can occur as a result of prenatal exposure; approximately 65% of babies born from infected mothers become infected during vaginal delivery. Infections caused by *C. trachomatis* are particularly difficult to confine as a high proportion of these infections are asymptomatic, thus making part of the population (those not tested) a reservoir for further transmission [8].

Neisseria gonorrhoeae is a gram-negative diplococcus that causes a disease called "Gonorrhea". *Neisseria gonorrhoeae* is an exclusive human pathogen that primarily infects the urogenital epithelia. *N. gonorrhoeae* is transmitted by sexual contact and usually causes infection in cells of the mucous membrane of the male urethra or the endocervix and urethra in females. Urogenital disease due to gonorrheal infection occurs as asymptomatic (50%) or mild urethelial and cervicitis [9-11]. Based on culture method and immunoassay, several studies were conducted to detect *Chlamydia trachomatis* as well as *Neisseria gonorrhoeae* in different Saudi Arabia localities [2, 5, 12-15].

The sensitivity of culture method is estimated to be only 80% [16]. Moreover; it requires up to 72 h for incubation and is costly [17]. On the other hand, the Enzyme linked immunoassay method is widely used in detecting sexually transmitted infections. These tests are less expensive and can be performed more rapidly than cell culture; however, they are less sensitive and specific [18].

The Polymerase Chain Reaction (PCR) proved to be the more sensitive than these culture and immunoassay methods and it could be used for the rapid diagnosis of sexual transmitted infections [19-21].

Corresponding Author: Alaa Mohammed, Biotechnology and genetic engineering unit, Scientific Deanship, Taif University, KSA.

To obtain reliable and accurate data regarding prevalence and incidence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, it is important to utilize nucleic acid based methods such as PCR, which has high sensitivity and specificity. In addition, it provides an alternative method for screening both symptomatic and asymptomatic individuals for *C. trachomatis* and *N. gonorrhoeae* [22, 23].

So the aim of the present work was to detect and estimate the prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection in the general population of women in Taif, western region, KSA using molecular genetics technique as a molecular diagnostic tool.

MATERIALS AND METHODS

Urine Sample Collection: Urine samples were collected from 200 females attended to perform pregnancy test at Prince Sultan hospital at Taif, KSA.

Twelve to forty five ml of urine from each woman was collected into sterile cups and stored immediately at 4°C until transferred and processed in biotechnology and genetic engineering unit laboratory at scientific research deanship at Taif University. The urine samples were centrifuged at (3,000 rpm) for 15 min and the supernatant was discarded. For three times the urine pellets were washed in 10 ml of sterile water and then centrifuged again at 5,000 rpm for 10 min.

DNA Extraction from Urine Pellets: The DNA was extracted from only 180 urine pellets according to urine samples properties. DNA was extracted according to instruction of Bacterial DNA preparation kit (Jena Bioscience GmbH, Germany). Briefly, urine pellets were subjected to the cell lysis, RNase treatment, protein precipitation, DNA perception and finally to DNA hydration.

Estimation of DNA Purity and Quantity: The purity and concentration of extracted DNA samples was estimated using UV spectrophotometer (Bio-RAD-SmartSpec™ plus).

Based on the DNA purity and concentration 36 samples were excluded and only 144 DNA samples were included in the following polymerase chain reaction experiment.

Detection of C. Trachomatis & N. Gonorrhoeae using Polymerase Chain Reaction (PCR): Detection of *Chlamydia trachomatis, Neisseria gonorrhoeae* via PCR assay was carried according to Lee *et al*. [24]. Briefly, PCR reactions were conducted using 2X super-hot PCR Master Mix (Bioron) with 10 Pmol of each used primer. All information of these primers were listed in Table (1). DNA amplification reactions were performed in (Eppendorf, Germany) thermal cycler using the following PCR program: The first cycle, consisted of a 5-min denaturation at 94°C, followed by 35 cycles each of 40 s at 94°C, 30 s at 60°C and 1.5 min, at 72°C, with a final extension for 5 min at 72°C. The PCR products were visualized in 1.5% agarose gels containing 0.5 µg of ethidium bromide/ml against 100bp ladder (Genedirex). All agarose gels were visualized and documented using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA). The digital image files were analyzed using Gene Tools software from Syngene.

RESULTS

Two hundred female urine samples were collected in the present study. Only 180 samples were processed for DNA extraction step according to sample properties. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by polymerase chain reaction (PCR) assay was carried out among 144 samples based on DNA concentration and purity.

The Orf8 forward and reverse primers were used to amplify 200bp specific for *Chlamydia trachomatis* (Figure 1) while 16S rRNA primers were used to amplify 281 bp specific for *Neisseria gonorrhoeae* (Figure, 2).

Table (2) summarizes the total number and infected samples for each studied pathogenic bacteria.

DISCUSSION

STDs are a major global cause of acute illness with severe medical and psychological consequences for millions of men, women and children.

The prevalence of clinical and subclinical infections due to *C. Trachomatis* has been reported as high in both men and women in many countries. The World Health Organization estimated that 89 million cases of *C. trachomatis* infection occurs worldwide [5]. In the present study the prevalence of *Chlamydia*

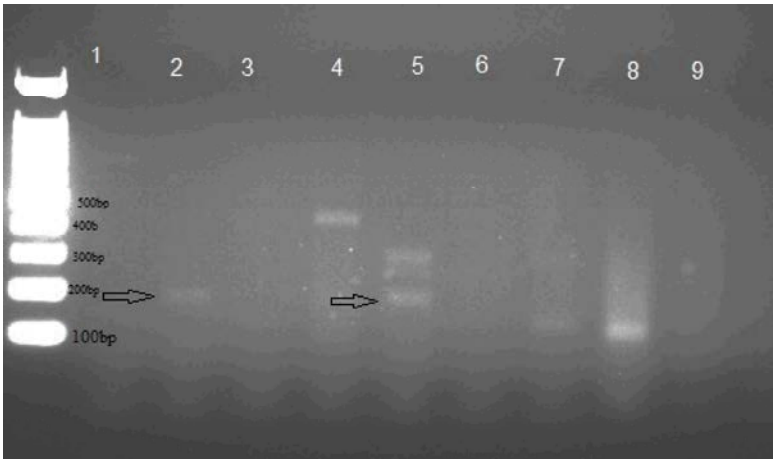

Fig. 1: Electrophoresis of PCR product of *Chlamydia trachomatis* positive samples (200bp) in lane 2 and 5 against 100bp ladder.

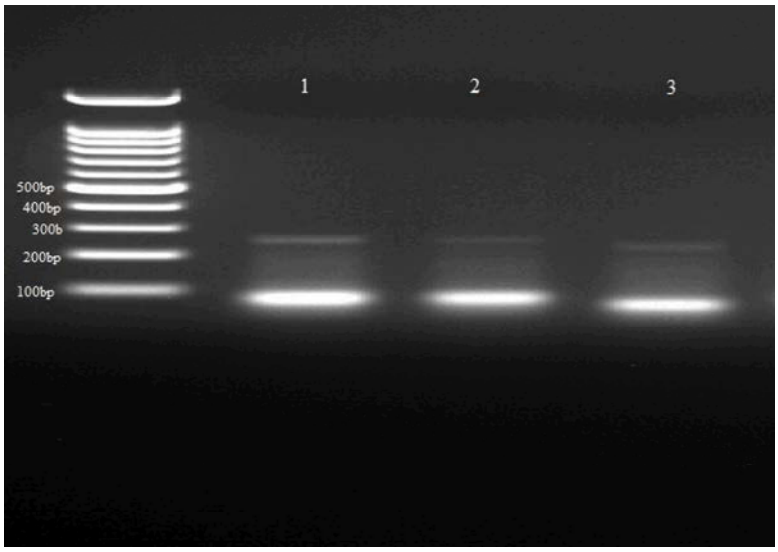

Fig. 2: Electrophoresis of PCR product on *Neisseria gonorrhoeae* positive samples 1, 2 and 3 (280bp) against 100bp ladder

Table 1: Names of pathogenic bacteria and sequence of used primers.

Pathogenic bacteria	Target gene	Primers
C. trachomatis	Orf8	f5'-CTAGGCGTTTGTACTCCGTCA
		r5'-TCCTCAGGAGTTTATGCACT
N. gonorrhoeae	16S rRNA	f5'-ACTGCGTTCTGAACTGGGTG
		r5'-GGCGGTCAATTTCACGCG

Table 2: Number of examined and infected samples for each studied pathogenic bacteria

Pathogenic bacteria	Total examined samples	No. infected samples	Percentage of infection
C. trachomatis	144	16	11.1
N. gonorrhoeae	144	3	2.1

trachomatis was 11.1%. There are studies conducted in Saudi Arabia that reported the prevalence of *Chlamydia trachomatis* to be from 0.5 to 35% [2, 5, 12, 14, 15, 25, 26]. The prevalence of Chlamydia genital infection in women varies in different groups and communities based on differences in diagnostic test, treatment practices in different areas and subjects health status [1].

There are hardly any data available for the prevalence of *Neisseria gonorrhoeae* infection in Saudi women. Only two reports were published [2, 27]. Madani [27] reported that, the average annual incidence of *N. gonorrhoeae* per 100.000 populations Saudi and non-Saudis is 5.2 and 4.2 respectively. Alzahrani and his colleagues [2] mentioned that, the data on *N. gonorrhoeae* infection in Saudi women are very scanty. They used endocervical and high vaginal swabs to detect *N. gonorrhoeae* via enzyme–linked immunosorbent assay (ELISA) among pregnant women in comparison with female patients with lower genital tract infection. The prevalence of *N. gonorrhoeae* in two groups studied by Alzahrani and his coworkers [2] were (0 & 7.8%).

The prevalence of *N. gonorrhoeae* reported in our study (2.1%) was detected in urine samples by PCR technique while the prevalence of *Chlamydia trachomatis* was 11.1%.

Further larger studies are needed and more focused on other STIs. It is very important to utilize sensitive and specific nucleic acid amplification methods such as PCR for the detection of STIs. There are vast needs to implement a national screening program for early detection and molecular diagnosis of STIs among youth in the marriage age.

REFERENCES

1. Hasanabad, H.M., M. Mohammad Zadeh, A. Bahador, N. Fazel, H. Rakhshani and A. Majnooni, 2011. Prevalence of Chlamydia trachomatis and Mycoplasma genitalium in pregnant women of Sabzevar- Iran. Iranian Journal of Microbiology, 3: 123-128.
2. Alzahrani, A.J., O.E. Obeid, M.I. Hassan and A.A. Almulhim, 2010. Screening of pregnant women attending the antenatal care clinic of a tertiary hospital in eastern Saudi Arabia for Chlamydia trachomatis and Neisseria gonorrhoeae infections. Indian J. Sex. Transm. Dis., 31: 81-86.
3. Kalman, S., W. Mitchell, R. Marathe, C. Lammel, J. Fan, R.W. Hyman, L. Olinger, J. Grimwood, R.W. Davis and R.S. Stephens, 1999. Comparative genomes of Chlamydia pneumoniae and C. trachomatis. Nat. Genet., 21(4): 385-389.
4. Brunham, R.C., 1999. Human immunity to chlamydiae. In: Chlamydia intracellular biology, pathogenesis and immunity. Washington, Ed., Stephens, E.S., DC: ASM Press, pp: 211-238.
5. Ghazi, H.O., M.H. Daghestani and M.F. Mohamed, 2006. Seropositivity of Chlamydia trachomatis among Saudi pregnant women in Makkah family. Community Med., 13: 61-64.
6. Alger, L.S., J.C. Lovchik, J.R. Hebel, L.R Blackmon and M.C. Crenshaw, 1988. The association of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and group B *Streptococci* with preterm rupture of membranes and pregnancy outcome. Am. J. Obstet. Gynecol., 159: 397-404.
7. Martius, J., M.A. Krohn, S.L. Hillier, W.E. Stamm, K.K. Holmes and D.A. Eschenbach, 1988. Relationship of vaginal Lactobacillus species, cervical Chlamydia trachomatis and bacterial vaginosis to preterm birth. Obstet. Gynecol., 71: 89-95.
8. Kelly, M., 2009. Researchers design unique method to induce immunity to certain STDs. In Health & Medicine. University of California, Los Angeles (UCLA).
9. Wasserheit, J. and K.J. Holmes, 1992. Reproductive tract infections: challenges for international health policy, programs and research. In: Germain A, K. Holmes, P. Piot, J. Wasserheit, eds. Reproductive tract infections: global impact and priorities for women's reproductive health. New York: Plenum Press, pp: 10-13.
10. Farraj, M.A., M.A. Gabi, M.S. Abed Alraoof, Y.J. Ayyub, M.R Raba, N. A. Hiba, N.S Israr and A.E. Tamer, 2010. Detection of *Neisseria gonorrhoeae* in Palestinian women using polymerase chain reaction. Asian Biomedicine, 4: 637-640.
11. Maria, R., S. Johanne, V. Manonmany, R. Mafizur, K. Elise and H. Per, 2007. Chlamydia and gonorrhea in pregnant Batswana women: time to discard the syndromic approach?. BMC Infect. Dis., 7: 27-38.
12. Massoud, M., A. Noweir, M. Salah and W.A. Saleh, 1991. Chlamydial infection in Riyadh, Saudi Arabia. J. Egypt Public Health Assoc., 66: 411-9.
13. AL-Gelban, K.S., M.S. AL-Shahrani, M.I. AL-Sunaidi and O.A. Mostafa, 2010. Screening for Chlamydia Genital Infection Among Females Undergoing *in Vitro* Fertilization in Saudi Arabia. Med. J. Cairo Univ., 78: 251-256.
14. Al-Jiffri, O., 2011. Detection of *Chlamydia trachomatis* among infected women. Middle East Journal of Scientific Research, 8: 450-456.
15. Kamel, R.M., 2013. Screening for *Chlamydia trachomatis* infection among infertile women in Saudi Arabia. International Journal of Women's Health, 5: 277-284.

16. Mardh, P.A., J. Paavonen and M. Poulakkainen, 1989. Chlamydia. New York: Plenum Press, pp: 79.
17. Wiesenfeld, H.C., M. Uhrin, B.W. Dixon and R.L. Sweet, 1994. Rapid Polymerase Chain Reaction-Based Test for the Detection of Female Urogenital Chlamydial Infections. Infectious Diseases in Obstetrics and Gynecology, 1: 182-187.
18. Barnes, R.C., 1989. Laboratory diagnosis of human chlamydial infections. Clin. Microbiol. Rev., 2: 119-136.
19. Wu, C.H., M.F. Lee, S.C. Yin, D.M. Yang and S.F. Cheng, 1992. Comparison of polymerase chain reaction, monoclonal antibody based enzyme immunoassay and cell culture for detection of *Chlamydia trachomatis* in genital specimens. Sex Transm. Dis., 19: 193-197.
20. Bhalla, P., U.K. Baveja, R. Chawla, S. Saini, P. Khaki, K. Bhalla, S. Mahajan and B.S. Reddy, 2007. Simultaneous detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by PCR in genitourinary specimens from men and women attending an STD clinic. J. Commun. Dis., 39: 1-6.
21. Shim, H.S., S. Noh, A.R. Park, Y.N. Lee, J.K. Kim, H.J. Chung, K.S. Kang and N.H. Cho, 2010. Detection of sexually transmitted infection and human papillomavirus in negative cytology by multiplex-PCR. BMC Infectious Diseases, 10: 284-291.
22. Cotchfelt, K., L. Welsh, D. DeBonville, M. Rosenstraus and T. Quinn, 1997. Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genitourinary specimens from men and women by a co-amplification PCR assay. J. Clin. Microbiol., 35: 1536-1540.
23. Chaudhry, U., K. Ray, M. Bala and D. Saluja, 2002. Multiplex polymerase chain reaction assay for the detection of *Neisseria gonorrhoeae* in urogenital specimens. Curr. Sci.; 83: 634-640.
24. Lee, S.R., J.M. Chung and Y.G. Kim, 2007. Rapid One Step Detection of Pathogenic Bacteria in Urine with Sexually Transmitted Disease (STD) and Prostatitis Patient by Multiplex PCR Assay (mPCR). The Journal of Microbiology, 45: 453-459.
25. Qadri, S.M., J. Akhter and K. Ingacio, 1993. Incidence of *Chlamydia trachomatis* in a large Metropolitan in Saudi Arabia. Saudi Med J., 14: 152-155.
26. Jamjoom, G.A., C.G. Jesua and K. Sayed, 1994. Low rate of isolation of *Chlamydia trachomatis* from Saudi Obstretrics/Gynaecology patients. Saudi Med J., 15: 143-146.
27. Madani, T.A., 2006. Sexually transmitted infections in Saudi Arabia. BMC Infectious Diseases, 6: 3-8.