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Detection of *Neisseria gonorrhoeae* from Vagimal Swabs of Ewin, Rajaii Shahr, Karaj and Varamin Female Prisoners by PCR and Culture Methods

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Abstract: Isolation of *N. gonorrhoeae* by culture method is currently the gold standard for the definitive diagnosis of gonorrhoea. However, PCR techniques are being used more frequently as sensitivity and specificity of the newer tests are improved. In this study, 500 vaginal swabs from Ewin, Rajaii shahr, Karaj and Varamin female prisoners were used for detection of *N. gonorrhoae* by culture and PCR techniques. Five hundred vaginal swabs from Ewin, Rajaii shahr, Karaj and Varamin female prisoners were cultured in modified Thayer Martin in 37°C with 5% CO_2 for 72 h. Oxidase, catalase tests, biochemical tests such as maltose and glucose oxidation and gram staining, were used to confirm the isolated species. Amplification by PCR using 2 targets which are specific for *N. gonorrhoeae*, *Ngu1* and *Ngu2*, were used to detect the presence of gonococcal specific DNA. Despite of finding some questionable samples as *N. gonorrhoeae* by using biochemical tests, PCR method confirmed that none of them were positive for *N. gonorrhoeae*. This study deals with detection of *N. gonorrhoeae* among woman prisoners in three main prisons in Tehran, Iran. The high specificity and sensitivity coupled with low cost and rapidity of the method (PCR) provided a substantial advantages over the time consuming culture methods currently used in hospitals and laboratories.

Key words: *N. gonorrhoeae*, PCR method, modify thayer martin media

INTRODUCTION

Neisseria gonorrhoeae is an intracellular, kidney shape, gram negative cocci which is a major cause of worldwide sexual transmitted infection (Hjelmevoll *et al*., 2006).

In most of the time, this infection is asymptomatic in women and leads to pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy, neonatal conjunctivitis and infertility.

Patients with *N. gonorrhoeae* are sensitive to Human Immunodeficiency Virus (HIV) infection.

Odds ratio estimates for increased risk of HIV infection due to previous infection with an STD vary from 3.5 to 9.0 for *N. gonorrhoeae*.

The incidence rate of *N. gonorrhoeae* infection with undiagnosed and/or untreated is high Therefore, accurate diagnosis of both symptomatic and asymptomatic infections is essential (Geraats-Peters *et al*., 2005).

However, culturing is still gold standard for *N. gonorrhoeae* diagnosis, but it seems that, the sensitivity of culturing may be quite low and time consuming (Hjelmevoll *et al*., 2006).

Because, fastidious *N. gonorrhoeae* can not survive very long outside, optimizing sample collection, transportation and storage of the specimens as well as adequate culture methods are important (Hjelmevoll *et al*., 2006).

On the other hand, false-positive results can be obtained from biochemical tests of certain strains of *N. subflava*, *N. cinerea*, *N. flavescens*, *N. lactamica*, *N. sicca* and *Lactobacillus* species (Luijt *et al*., 2005).

Consequently, molecular tests such as PCR or real time PCR can be used for *N. gonorrhoeae* diagnosis, because these methods are fast, accurate and can be used for a deal number of samples in different laboratories (Chaudhry *et al*., 2002).

From the other side, control of gonorrhea, with a consequent reduction in morbidity due to its complications, is difficult and needs complex social and behavioral change (Whiley *et al*., 2006).

To provide a more accurate understanding of the hidden epidemics of untreated infection with *N. gonorrhoeae*, we involved female prisoners in our study.

The aim of our study was detection of *N. gonorrhoeae* from 500 vaginal swabs of Karaj, Varamin and Ewin and Rajaiishahr (different states of Iran) female prisoners by using culture and PCR methods.

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MATERIALS AND METHODS

Sample collections: This study spanned over a one year period January 2008 till January 2009, during which, 500 dacron vaginal swabs were collected from Karaj, Rajaii shahr, Varamin and Ewin female prisoners-married, ages between 16 to 45-attending the clinic of prisons.

Any prisoner presenting to the clinic of prison for evaluation of genitourinary symptoms, for therapy as a known contact of an individual with a diagnosed STD, or for routine STD screening was approached to volunteer for study enrollment. If the prisoner agreed to participate and signed the informed consent, she was assigned a unique and confidential study number.

Two Dacron vaginal swabs from each female prisoner were taken with phosphate-buffered saline. One Dacron swab was inoculated directly on to modified Thayer Martin medium (contains the antimicrobial agents, vancomycin 3 mg L^{-1}, colistin 4.5 mg L^{-1}, Nystatin 12.5 mg L^{-1} and trimethoprim 5 mg L^{-1}) and used to make a smear for Gram's-staining.

After incubating at 36°C with 5% CO_2 for 2 days, *N. gonorrhoeae*-like colonies were used to make a smear for Gram's-staining, oxidase and catalase tests. Patterns of acid production from the carbohydrates-glucose, maltose, lactose, sucrose- were also used in this study.

The other swab was also stored frozen in 2 mL of phosphate buffered saline at -20°C, except during transport. *Neisseria gonorrhoeae* which was isolated from a patient was used as positive control.

PCR analysis: DNA of suspected organisms were extracted by using Fermentas DNA purification Kit, the extracted DNA was suspended in TE- buffer and stored at -20°C.

Amplification was performed in 25 μL reaction volumes containing 50 pmol of each oligonucleotide primer and 1 μL of purified chromosomal DNA, 200 μM each of dATP, dCTP, dGTP, dTTP, 1.5 mM $MgCl_2$, 1U of Taq DNA polymerase (Fermentase, Litvania).

The primers (forward primer 5'- CAACTATTCCCGATTGCG- 3' and reverse primer 5'-GTTATACAGCTTCGCCTGAA-3') amplify the *orf*1 gene.

Therefore, the primer pair set *Ngu1/Ngu2* would generate a 260 bp PCR product.

The PCR cycle involved an initial denaturation at 94°C for 5 min, followed by 45 cycles at 94°C for 30 sec, 52°C for 30 sec and 72°C for 1 min, followed by a final extension at 72°C for 10 min and cooling to 25°C. The 10 μL of the PCR products was analyzed after electrophoresis in 0.5 μg mL^{-1} ethidium bromide-containing 2% agarose gels prepared in 0.5X Tris-borate-EDTA buffer.

RESULTS

In this study any prisoner presenting to the clinic of prison for evaluation of genitourinary symptoms, for therapy as a known contact of an individual with a diagnosed STD, or for routine STD screening was approached to volunteer for study enrollment.

About 187, 100, 90 and 123 prisoners from Ewin, Rajaiishahr, Karaj and Varamin prisons, presented in clinic of prisons. Profile of prisoners is shown in Table 1.

A total of 336 out of 500 specimens were initially positive by gram staining, A total of 243 out of 336 specimens were positive by oxidase and catalase tests but, we did not find any bacteria which produced acid from glucose.

We used PCR method for final diagnostic way (Fig. 1). By using this, it was proved that, none of the prisoners were infected with *N. gonorrhoeae*.

Table 1: Profile of prisoners attending the clinic of prisons

Subjects	Values
Age	Between 16 to 45
Having vaginal discharges	85%
Positive for Aids or Hepatitis	0%
Having vaginal itch	10%
Have taken antibiotics	72%
Contraceptive	
OCP	44%
Condom	30%
IUD	1%
Others	25%

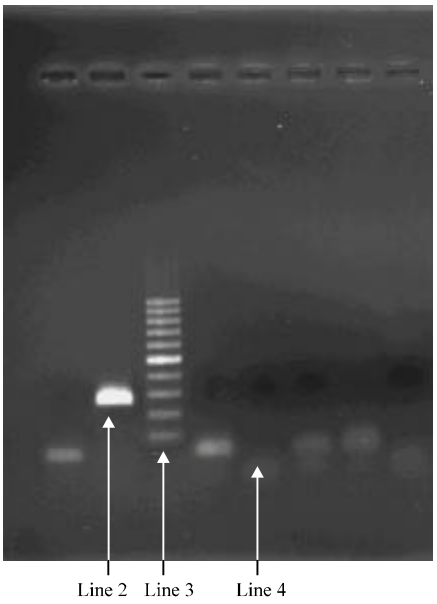

Fig. 1: PCR product of amplified using *orf1* gene-specific primers. Lane 2: Positive control (260 bp), Lane 3: 100 bp DNA ladder, Lane 4: Negative control

DISCUSSION

Gonorrhea, is an important public health problem and is simply transmitted via sexual contact. it can also be transmitted from the mother's genital tract to the new born during birth causing ophthalmia neonatorum and systemic neonatal infection.

Infection with *N. gonorrhoeae* rises the risk for the infection of Human Immunodeficiency Virus (HIV).

However, studies showed that, during coinfection with HIV, *N. gonorrhoeae* peptidoglycan can dramatically enhance HIV replication in human dendritic cells because of activating TLRs.

Due to worldwide high incidence rates, undiagnosed and untreated *N. gonorrhoeae* infection, detection and eradication of this infection is essential (Geraats-Peters *et al*., 2005).

There is very limited data about the epidemiology of *N. gonorrhoea* in Iran and this indicates that the prevalence of this disease is low. It was reported that, the incidence of this infection in women of Kerman and Tehran (two provinces of Iran) is, respectively 1.94% (1.8) and 0.6% (12) and in other studies no cases were reported in Iran.

So, in comparison with other countries, the prevalence of gonorrhea in Iranian females is low (Bkhtiari and Firoozjahi, 2007).

In this study which is performed in Karaj, Rajaii shahr, Varamin and Ewin female prisoners attending the clinic of prisons, the prevalence was less than 0.1% and this result approved other studies which are done by other Iranian researchers.

It seems that, because of taking antibiotics without prescription of doctors, giving antibiotics to prisoners for preventing of contagious infection prevalence (Table 1), using contraceptive methods such as condom and refusing for visiting doctors because of social ashamed of such disease, detection of *N.gonorrhoeae* in prisoners and other people is so difficult.

Despite of other Iranian clinics which are applying classic ways (such as culturing and biochemical tests) for detection of gonococal infection, in this research, we used PCR method for *orf1* gene detecting of *N. gonorrhoeae*.

Neisseria gonorrhoeae PCR is a useful, rapid and repetitive method which can be used for a great number of samples, so *N. gonorrhoeae* culture-positive results should always be confirmed with PCR test (Luijt *et al*., 2005).

As, Chaudhry *et al*. (2002) observed in their researches, the *orf1* gene primer pair (*Ngu1* and *Ngu2*) is useful for *N. gonorrhoeae* detection, because it does not show any sequence homology with the sequences available for other *Neisserial* species.

In conclusion, due to its high sensitivity, capability in amplifying sequences from low amount of DNA, *N. gonorrhoeae* PCR should be replaced with *N. gonorrhoeae* culture, especially when a huge number of samples are tested.

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