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Molecular Detection of *Neisseria gonorrhoeae* in Patients attending Different Sexually Transmitted Infections Units in Khartoum State (Sudan)

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Abstract

Background: *Neisseria gonorrhoeae* is a species of Gram-negative coffee bean-shaped diplococcic bacteria which causes gonorrhoea, a sexually transmitted infection. The infection may invade the vagina, cervix, rectum, pharynx, or eyes. In men, *Neisseria gonorrhoeae* infects urethra causing dysuria and acute urethritis with a purulent discharge. Among women, *Neisseria gonorrhoeae* infects the cervix, urethra, vulva and rectum.

Objective: To perform molecular detection of *Neisseria gonorrhoeae* in patients attending different sexually transmitted infections units in Khartoum State (Sudan).

Materials and methods: This was a cross-sectional study conducted during the period from March to August 2017 at the Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, (Sudan). A total of 30 participants aged 18–61 years were enrolled. An interview-administered questionnaire was used to gather data. Each participant was asked to provide a urine specimen. These specimens were examined using urine-based nucleic acid amplification tests.

Results: Two patients (6.7%), were found infected with *Neisseria gonorrhoeae*. Patients investigated were males and females in the active age group (26-40) years.

Conclusion: There was a low detection rate of *Neisseria gonorrhoeae* among teenagers and , female patients in Khartoum State (Sudan).

Key words: **M**olecular detection, *Neisseria gonorrhoeae*, Sexually transmitted infection

Introduction

Rectal infections occur in up to 40% of women infected with gonorrhoeae. Generally, in about 95% of patients, gonococci can be found in urethral discharge or urine sediments. The infection may spread to prostrate, bladder, and epididymis, causing inflammation and swollen epididymis

and may lead to sterility. Symptoms which typically appear 2-14 days after exposure include pain or burning during urination or defecation; a yellowish or greenish (pus-like) penile, vaginal, cervical, or rectal discharge. The infection may progress to develop swelling and tenderness of the vulva, sore throat, and a false urge to urinate or defecate. An estimated 10% of men and up to 80% of women are asymptomatic[1].
Neisseria gonorrhoeae (*N. gonorrhoeae*) is diagnosed using a Gram stain, in which a sample of genital fluid is cultured, stained and examined under a microscope. A urine test for *N. gonorrhoeae* is also available. Untreated *N. gonorrhoeae* can lead to pelvic inflammatory diseases (PID) or epididymitis, as well as damage to the joints. In pregnant women, *N. gonorrhoeae* is associated with premature labour, miscarriage and stillbirth, and it may be transmitted to an infant during birth[2].
Sexually transmitted infections (STIs) such as gonorrhoea are associated in several ways with HIV, which spread by similar types of sexual activity. People who engage in behaviors that transmit HIV are also more likely to contract STIs. Persons who contract an STIs may also have put themselves at risk for HIV and should be tested. Likewise, a person who contracts HIV should be tested for other STIs. In addition to epidemiological pattern of HIV and STI transmission, immune damage related to HIV infection can influence the progression of STIs and vice-versa[3].

Materials and methods

This was a cross-sectional study conducted at different sexually transmitted infections (STIs) units located in Khartoum State (Sudan) to detect *Neisseria gonorrhoeae* by molecular techniques. The study was carried out during the period from March to August 2017.
The study population comprises 30 patients attended the STIs units with various genito-urinary infections. The study participants were well informed about the study objectives which they freely consented.

The age incidence of participants ranged from 18–61 years. An interview-administered questionnaire was used to gather data. 30 first-voided urine specimens (5ml each) were collected from the study participants using a sterile bottle. The specimens bottles were clearly labeled with demographical information such as age and gender.
All the urine specimens collected were allowed to stand for about 2 hours to allow full sedimentation before centrifugation at 3000 rpm for 5 minutes. The supernatants were discarded while the sediments were stored at -20°c until ready to use.
DNA extraction was performed using Magna Medics kit (De Asselen Kuil 12, 6161 RD Geleen - The Netherlands) following manufacturer's instructions. Conventional PCR was used for detection of *N. gonorrhoeae* in urine specimens as described by Mahony and his colleagues[4]. Table (1) shows the primers used in the study were supplied by Macrogen Biotechnology Inc. (South Korea).

Table (1): Primers used for molecular detection of *Neisseria gonorrhoeae*

Organism	Target Gene	Name	Oligonucleotide sequence (5———————————3)	Amplicon size
Neisseria gonorrhoeae	cppB gene	NGF1 NGRI	GCTACGCATACCCGCGTT CGAAGACCTTCGAGCAGACA	390 bp

The PCR reaction was set up as described by Mahony and his colleagues[4] with a modification in the reaction condition.
In brief, the reaction mixture of 25 ul, containing 5 ul of eluted DNA, and 20 ul of a master mix. Amplification was carried out in Techne®Thermal Cycler (Bibby Scientific - UK (Group HQ)) with the following cycling parameters; 10 min at 95°C followed by 40 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C and a final step of 5 min at 72°C. PCR products were analyzed by gel electrophoresis with 1.8% agarose and ethidium bromide staining.
The study was reviewed and approved by the Graduate Collage (Al Neelain University).

Results:

A total of 30 urine samples were collected randomly from patients attending different sexually transmitted infections (STIs) units in Khartoum State (Sudan). The urine samples were tested for *N. gonorrhoeae* using conventional PCR technique. Positive *N. gonorrhoeae* bands (100 bp) are shown in Fig, (1).

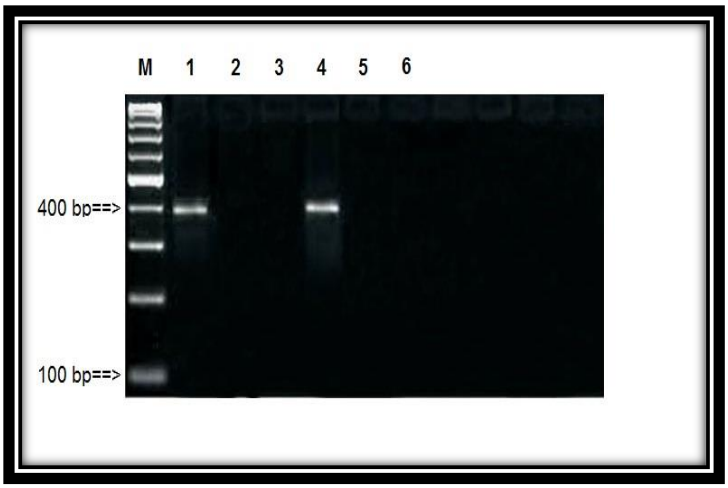

Fig. 1: *N. gonorrhoeae* Ethidium bromide stained agarose gel showing PCR products
* M; molecular weight marker (100 bp ladder).
* Sample 4 showing positive *N. gonorrhoeae*
* Sample 1 showing *N. gonorrhoeae* positive control (390 bp)

From the 30 patients investigated 2 patients were found positive for *N. gonorrhoeae* with an overall prevalence rate of 6.7%. Both positive cases were in the age range (26- 40) years, whereas negative cases were within various age categories (Table 2).

Table (2): Distribution of positive *N. gonorrhoeae* among the patients investigated according to age incidence

Age range (years)	**Patients investigated**	**Positive *N. gonorrhoeae***	**Negative *N. gonorrhoeae***	**Percentage positives**
(15-25)	5	0	5	0%
(26-40)	16	2	14	12.5%
(41-60)	7	0	7	0%
60 and above	2	0	2	0%
Total	30	2	28	6.7%

Discussion

The present study reports a low detection rate of *N. gonorrhoeae* among the patients investigated. Patients from this traditional and conservative society often initiate sexual life with marriage. Pre-marital and extra-marital sexual relation is prohibited; and unmarried individuals usually deny such a practice mainly because of religious constraints.

This study results were in agreement with previous studies conducted in Khartoum State; which reported a low community prevalence rate of gonococcal infections [5, 6].

One study was conducted on pregnant women (2008) at Omdurman Maternity Hospital, Khartoum (Sudan), and revealed a low frequency rate of *Neisseria gonorrhoeae* (1.8%) among the patients investigated[5]. The other study was conducted in Khartoum and reported 0% prevalence rate of gonococcal infection among low socio-economic class women attending antenatal clinics[6].

In the present study, a highly sensitive molecular technique was employed in detecting gonococcal infection in the genitourinary specimens collected[4]. Nucleic acid amplification tests (NAAT) are reliable tools for the diagnosis and screening gonococcal infections in different cervical, urinary and vaginal specimens[7].

Even though invasive specimens such as urethral, endocervical and vaginal swabs show a higher sensitivity for the detection of gonococcal infection, yet urine specimens revealed almost the same diagnostic accuracy[8].

However, as per the meta-analysis by Cook and his co-workers[9], only 56% of gonococcal infections in women were detected from urine specimens as compared to 94% detection rate from cervical specimens. In the present study, first-voided urine specimens were collected to aid in the etiological diagnosis of urethritis. Using NAAT recent techniques, first-voided urine

specimens are as efficacious as mid-stream urine specimens in detecting urethral infection[10]. Privacy, sterile equipment, and trained healthcare personnel are required for investigating urethral, cervical and vaginal swabs for gonococci detection. However, urine samples are considered culturally feasible, socially acceptable and economical for large-scale epidemiological studies.

Urine sampling had a social acceptance for routine screening of patients for STIs. The large scale screening among the population in different regions can provide baseline data for the development and implementation of evidence-based control measures to prevent reproductive morbidity among sexually active individuals. Depending on the study results, it may be assumed that *N. gonorrhoeae* infection is not becoming a public health problem in Khartoum State (Sudan) because of the low frequency rate exhibited in this study.

Conclusion: There was a low detection rate of *Neisseria gonorrhoeae* among teenagers and , female patients in Khartoum State (Sudan).

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