DETECTION OF *NEISSERIA GONORRHOEAE* BY POLYMERASE CHAIN REACTION (PCR)

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**ABSTRACT**

Three different sets of primers were designed using FASTA homology search and PRIMERSELECT for the specific detection of *Neisseria gonorrhoeae* using polymerase chain reaction (PCR). These primers amplified the highly conserved regions of genes for Open Reading Frame (ORF), Outer Membrane Protein (OMP) and 23S rRNA sequences of *N.gonorrhoeae*. Each of the PCR primer set was evaluated using the DNA samples isolated from eight different positive isolates of *N.gonorrhoeae* cultured from urethral swabs of patients visiting Maulana Azad Medical College and Safdarjung Hospital. Amplification products were analyzed on agarose gel electrophoresis. Two sets of PCR primers, designated as Ngul/Ngu2 and Ngu5/Ngu6, specific for ORF and OMP gene respectively, amplified four regions of the gene which may help to differentiate the various strains of *N.gonorrhoeae* infecting indigenous population. In contrast, a single, specific PCR product of 650bp was visualized on agarose gel with primers Ngu3/Ngu4, amplifying the 23S rRNA gene. Under optimum conditions, as low as 25ng of DNA isolated from eight different clinical strains of *N.gonorrhoeae* could be detected by PCR using Ngu3/Ngu4 set of primers. Our results suggested that Ngu3/Ngu4 could serve as good primers for the specific, reproducible and sensitive diagnosis of *Neisseria gonorrhoeae* from clinical samples.

**KEY WORDS** : Gonorrhoeae, Polymerase Chain Reaction, 23S rRNA

**INTRODUCTION**

The genus Neisseria includes human-specific pathogens, *Neisseria gonorrhoeae* and *Neisseria meningitis*, which cause gonorrhoeae/pelvic inflammatory disease and bacterial meningitis/sepsis respectively (1). Conventional diagnosis of gonorrhea relies mainly on the demonstration of intracellular Gram negative diplococci in smears of genital discharge or swabs (2). The diagnosis of *N.gonorrhoeae* can be established readily in the acute stage as urethral discharge contains large number of gonococci. However chronic cases often present great difficulties. Definitive diagnosis of gonorrhoea involves culture of clinical specimen on modified Thayer Martin Medium in an atmosphere of 5% CO₂. Growth requires a minimum of 24 hours, and by 48 hours, the organism can be easily identified by Gram staining, enzymatic methods or immunofluorescence (3,4). As cultures are subject to several variable factors, some gonococci fail to grow in culture or are misidentified, making the
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assay less reliable. Several strains of *N. gonorrhoeae* are also susceptible to the concentrations of vancomycin used in the selective medium and hence the recovery rate is less than 100% (5). To eliminate some of these concerns associated with culture, alternative methods such as immunoblotting, ELISA, radioimmunoassay, and DNA hybridization have been developed (6,7). The specificity of these enzyme immunoassays is less than desired (94-100%). When compared with culture, the enzyme immunoassays are even less sensitive (87-98%) (4).

The use of DNA probes as a tool for detection of gonococci in urogenital specimens has been investigated extensively in recent years. Probes using nucleotide sequences of the pilin gene, the IgA gene, protease gene, ribosomal RNA and the cryptic plasmid have been reported (8). However, because of the low sensitivity of these methods, these are not useful as diagnostic tools. One of the reasons for low sensitivity is the low gene copy number. As the number of infectious organisms in clinical samples are usually too few, detection by culture or DNA probe assays or antigen assay does not provide desired sensitivity. Recently this problem has been greatly overcome by the development of the polymerase chain reaction (PCR) where, theoretically, even a single gene copy would be sufficient for amplification (5,9). The practical use of PCR methods developed in West would clearly be dependent on stability of the gene sequence during transmission over a period of time and geographical location.

In the present study, we have developed a polymerase chain reaction-based assay for the detection of *N. gonorrhoeae* using primers specific for 23S rRNA gene. Though 23S rRNA gene is conserved among several bacteria, there are regions which are highly specific for *N. gonorrhoeae*. Use of rRNA gene provides a diagnostic advantage as rRNA gene is present in high copy number per cell. Presence of large number of target molecules increases the likelihood of initiating amplification even when the organism is present in low number. This will be particularly important in asymptomatic patients and for using urine samples instead of endocervical specimens.

**MATERIALS AND METHODS**

**Bacterial Strains**

The *Neisseria gonorrhoeae* strains used were all clinical isolates recovered from urethral or cervical swabs by inoculation onto modified Thayer Martin medium and subsequently identified. Clinical specimens were collected from men and women with acute gonococcal urethritis attending a sexually transmitted disease clinic in Safdarjung Hospital and Maulana Azad Medical College, Delhi. Two urethral swabs from the male and two cervical swabs from the female were taken with phosphate buffered saline pre wet swabs. One swab was inoculated directly onto modified Thayer Martin Medium, incubated in an atmosphere of 5% CO₂ for 48 hrs (3,4) and any suspected organisms were identified by Gram stain, colony morphology, positive oxidase, and rapid carbohydrate utilization tests following standard methods (2). The other swab was stored frozen at -20°C except during transport.