



The Brazilian Journal of INFECTIOUS DISEASES

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Letter to the Editor

Molecular diagnosis and genotyping of *Neisseria gonorrhoeae* in urine samples from women at low-income communities in Rio de Janeiro, Brazil

Dear Editor,

Accurate diagnosis of *Neisseria gonorrhoeae* is required to prevent severe complications and to control transmission, especially, among asymptomatic infections.¹ Molecular approaches such as hybridization assays and nucleic acid amplification tests have revolutionized the diagnosis of gonorrheal infections due to their increased accuracy over culture and other tests and their ability to test multiple specimens at the same time.² In addition to diagnosis, molecular approaches have been successfully applied for typing *N. gonorrhoeae* in order to track genetic diversity.

In our study, we aimed at evaluating the usefulness of the 16S rRNA gene in diagnosing and genotyping *N. gonorrhoeae* from urine samples. A cross-sectional study was conducted from June 2009 to April 2010 at outpatient units responsible for the care of people living in two low-income communities in Rio de Janeiro, Brazil. It included women aged 18 to 30 years with either vaginal or urethral discharge, and at least one of the following characteristics: sexually active and no stable partner, more than one partner or a new one in the previous 3 months, partner reporting any sexually transmitted infection (STI) and no use of condom in the last sexual relationship. Asymptomatic women or those who had fulfilled the inclusion criteria were selected as negative controls. Women reporting use of any antibiotics at least 30 days before entering the study were not included. Urine samples were collected and after centrifugation, pellets were washed twice in sterile TE buffer (Tris 10mM, EDTA 0.1mM, pH 7.5) and subjected to bacterial DNA extraction and purification according to the Genomic Prep Cells and Tissue DNA Isolation Kit (Amersham Biosciences). Detection of the 16S rRNA gene from *N. gonorrhoeae* was carried out by PCR.³

The variable region of the 16S rRNA gene was assessed by the low-stringency single specific primer PCR technique (LSSP-PCR).⁴ From the 65 people included in the study, 50 women (78%) reported at least one symptom related to STI for a median time of 60 days (varying from 2 to 1095 days). Fifty-two women (81%) denied using condom in the last sexual intercourse, on average 4 days before the interview (varying from 0 to 120 days) (Table 1). From the examined clinical specimens, 11 women (17%) were diagnosed with *N. gonorrhoeae*. LSSP-PCR assays generated informative reproducible

profiles composed by 2 to 10 polymorphic bands ranging approximately from 50 to 250 bp. Clustering analysis of LSSP-PCR data based on Dice similarity index defined three clonal groups ranging from 49 to 100%.

Clonal group A was composed by two LSSP-PCR types with 84% of similarity and cluster B represented the major type composed by LSSP-PCR profiles closely related with similarity index ranging from 92 to 100%. Cluster C grouped two distinct LSSP-PCR types with similarity index of 78%. The employed methodology was simple, fast and specific for the detection of *N. gonorrhoeae* in urine samples. The polymorphism of the 16S rRNA gene assessed by a simple low-stringency single specific primer PCR technique revealed diversity within the gene.

Recent studies on genetic population of *N. gonorrhoeae* target to housekeeping genes have classified these micro-organisms as effectively panmictic possibly due to the extensive recombination presumably mediated by genetic transformation.⁵ Our results, despite being related to a specific gene target, led us to agree with previous observations and suggest that *N. gonorrhoeae* strains circulating in our community constitute nonclonal

Table 1 - Characteristics of volunteers enrolled in the study (n = 64)*

Characteristic	n (%)	Median (range)
Positive for <i>N. gonorrhoeae</i>	11 (17) **	-
Urethral or vaginal discharge	50 (78)	-
Time since beginning of symptoms (days)	-	60 (2-1,095)
Dysuria	8 (12)	-
Frequency of micturition	19 (30)***	-
Dyspareunia	20 (31)	-
Time since last sexual intercourse (days)	-	4 (0-120)
Use of condom in the last sexual relationship	12 (19)	-

*For one positive volunteer we did not have any clinical data available; **n = 65; ***n = 62

bacterial populations.⁶ In the present study, urine samples were collected from individuals presenting a wide range of clinical signs and symptoms, and living in different geographic areas in the same city, over a relatively long time period.

The observed genetic diversity possibly reflected epidemiologically independent strains and may represent microorganisms exhibiting distinct evolutionary lineages.⁶ Common 16S rRNA genotypes were observed in urine from individuals epidemiologically unrelated suggesting the possible circulation of particular *N. gonorrhoeae* genotypes in the population and geographic area studied. Besides contributing to ensure accurate reporting of infections, these findings are of special value considering that data available on *N. gonorrhoeae* lineages circulating in our community remains scarce.

The precise characterization of *N. gonorrhoeae* is essential for public health surveillance by optimizing standard treatments and control measures as well as for the tracking of microbial circulation and emergence of virulent lineages. Reproducible, sensitive and specific in-house PCR assays are extremely relevant tools in developing areas due their low cost and simplicity.

Acknowledgements

Funding for this study was entirely provided by the Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), grant number E-26/110.239/2009 (Auxílio Instalação 2008/2).

Conflict of interest

All authors declare to have no conflict of interest.

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Received 13 June 2011

Accepted 21 June 2011

1413-8670

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