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Molecular Typing of *N. gonorrhoeae* Strains Prevalent in the Russian Federation

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Genetic polymorphism of Russian population of *N. gonorrhoeae* was detected and a system for genotyping of its clinical strains was introduced into practice. Comparative analysis of the prevalence of *N. gonorrhoeae* genotypes in Russia and abroad was carried out. For adaptation of the methods of molecular typing of *N. gonorrhoeae* strains and its approbation on clinical strains isolated in Russia 41 clinical strains of *N. gonorrhoeae* were typed. The predominance of PIB serovar (83%) was demonstrated.

Key Words: *N. gonorrhoeae; serovar; typing; resistance*

The prevalence of gonococcal infection on the territory of the Russian Federation remains a pressing problems of practical public health, which is largely due to economic and demographic situation in Russia and CIS countries and the absence of conditions for the use of modern methods for *N. gonorrhoeae* detection and typing. Bacterioscopic and bacteriological methods remain the major methods in the diagnosis of gonorrhea. An important defect of microbiological diagnosis of this disease is the absence of regional monitoring of antibiotic sensitivity of gonococci and strain identification of the agent.

Like the majority of infectious agents persisting in humans, *N. gonorrhoeae* population is characterized by high morphophysiological and genetic polymorphism. The system of typing *N. gonorrhoeae* strains is based on physiological and immunological characteristics of the bacterium (A/S typing).

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The serological method of typing for clinical strains of *N. gonorrhoeae* was developed in 1984 [5, 10]. The modern variant of the test is based on co-agglutination of *N. gonorrhoeae* strains on a panel of monoclonal antibodies specific to different locuses of Por I membranous protein. Por I surface protein is presented in *N. gonorrhoeae* population by two serological variants: PIA and PIB, which, in turn, are subdivided into serotypes. Twenty-six PIA and 31 PIB serotypes of *N. gonorrhoeae* are now described. According to some clinical reports, PIA strains are more often associated with disseminated forms of gonococcal infection [8], while PIB strains are characterized by multiresistance to a wide spectrum of antibiotics (penicillins, tetracyclines, aminoglycosides, fluoroquinolones, macrolides) [12]. Epidemiological screening for different *N. gonorrhoeae* serovars was carried out in the majority of countries. The results indicate that epidemiological situation is different in different geographical zones; in some regions PIA serovar prevails (UK, Sweden) [2], in others PIB serovar is more incident (Germany, Greece) [1]. No studies of this kind were carried out in Russia.

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Typing of *N. gonorrhoeae* strains based on investigation of the bacterial genome nucleotide polymorphism is carried out since 2000. The most informative genotyping method is a combination of evaluation of Por gene mosaicism [4,7,9] (by analogy with serotyping) and restriction analysis of Opa gene polymorphism, which most effectively discriminates between the clinical strains of *N. gonorrhoeae* [11,14,15]. These data help to evaluate the prevalence of gonococcal infection, to compare the heterogeneity of *N. gonorrhoeae* population in different geographic regions, and to follow up the genetic changes in *N. gonorrhoeae* strains.

The purpose of our study was to develop methods for molecular typing of *N. gonorrhoeae* strains and trials of these methods on clinical strains isolated in the Russian Federation.

MATERIALS AND METHODS

Clinical strains of *N. gonorrhoeae* were isolated from urethral and/or cervical discharge of patients with suspected gonococcal infection. Clinical material was transferred into Amies medium and then inoculated into chocolate agar with selective and vitamin additives. The appurtenance of the isolated strains to *N. gonorrhoeae* was confirmed by biochemical methods using Crystal BBL test system including 29 identification parameters. ATCC 49226 strain served as the reference strain.

N. gonorrhoeae cells grown in selective nutrient media were used for DNA isolation by the method developed by R. Boom *et al* [3].

Amplification of Por-1 and Opa genes was carried out with recommended primers [15] in a reaction mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2 mM $MgCl_2$, 250 μM of each of dNTP, 1 U Taq-pol (Promega), and 5 pmol of each primer. The amplification reaction was carried out in a Ternik programmed thermostat (DNK-Tekhnologiya). The amplification protocol included denaturation (30 sec at 94°C), annealing (10 sec at 68°C), and elongation (20 sec at 72°C) stages. The amplification products were fractionated in 2% agarose gel.

In order to detect the nucleotide sequence of Por gene, Por 1 gene amplification products were purified using Wizard PCR Preps DNA Purification System (Promega). Sequencing was carried out by modified Senger's method using Fmol DNA Sequencing System (Promega). Sequencing products were separated in denaturing 6% PAAG (1×TBE buffer, 7 M urea, AA:bisAA ratio 19:1).

Restriction analysis of Opa gene sequence was carried out as follows. Opa gene restriction products were purified using Wizard PCR Preps DNA Purification System (Promega) and cleaved with HpaII and TagI enzymes (Fermentas) in accordance with the restriction protocol for each enzyme. Equal aliquots of purified amplification products of Opa genes were incubated overnight with 10 U HpaII at 37°C and for 1 h with 10 U TagI at 65°C in the corresponding reaction buffers. Restriction products of Opa gene (by HpaII and TagI restriction sites) were analyzed by gel electrophoresis in 12% PAAG at AA:bisAA ratio 49:1. Electrophoretic separation of restriction products was carried out in 1×TBE buffer.

RESULTS

A total of 41 clinical strains of *N. gonorrhoeae* isolated during bacteriological studies from patients in Smolensk, Moscow, and Moscow region were typed. The results were pooled, because these areas belong to the same geographical region.

There is no reliable information on specific features of bacterial genome of *N. gonorrhoeae* population in Russia, and therefore we used the combination of Por gene sequencing with Opa typing for typing the clinical strains. Bacterial DNA was isolated; amplification systems for Por and Opa genes were adapted for obtaining the maximum yield of PCR product, and the nucleotide sequences (950 b.p. on average) of the studied *N. gonorrhoeae* strains Por I gene were determined.

The known nucleotide sequences of Por I gene for *N. gonorrhoeae* strains belonging to different serotypes (NCBI, EMBL databases) were analyzed. The genotype (serotype) of the studied clinical strains of *N. gonorrhoeae* was determined by the maximum homology in comparison with the known nucleotide sequences. Comparative analysis of Por I genes nucleotide sequences was carried out using Vector NTI Suite 6 software. The results of typing of *N. gonorrhoeae* clinical strains are presented in Table 1. The very first data proved significant genetic heterogeneity of *N. gonorrhoeae* population. Results of sequencing of Por genes showed considerable genetic polymorphism of the nucleotide sequences. Unique motives different from the nucleotide sequences of the analogous gene of *N. gonorrhoeae* strains described previously and presented in the Gene Bank were detected. Seven (17%) clinical strains of *N. gonorrhoeae* belong to the PIA serovar, while others belong to PIB serovar. It is difficult to determine the predominating serotype among PIA serovar strains. For PIB serovar the predominant serotypes are PIB3 and PIB2 (PIB3=34%, PIB2=20%, PIB5=14%, PIB4=11%, PIB3/5=9%, PIB7=9%, PIB9=3%). It is noteworthy that the genotyping data (predominance of PIB serovar) are in line with the results of microbiological analysis (high percentage of *N. gonorrhoeae* strains resistant to fluoroquinolones).

TABLE 1. Typing of *N. gonorrhoeae* Clinical Strains

Strain	Source	Por type	Opa type
Vlasov	Moscow	IB4	VI
Guliev	Moscow	IB5	VII
Kozlov	Moscow	IA14	X
Petrov	Moscow	IB2	VI
Popov	Moscow	IB3	VI
Smirnov	Moscow	IB2	VI
89	Moscow	IB3	I
34	Moscow	IB4	I
26	Moscow region	IB7	I
30	Moscow region	IB7	I
31	Moscow region	IA8	I
12B	Moscow region	IB3	X
94	Moscow region	IB2	XI
95	Moscow region	IB2	XI
96	Moscow region	IB5	VIII
22	Moscow region	IB3	IX
24	Moscow region	IB4	IX
54	Moscow region	IB3	IV
57	Moscow region	IA8	VII
58	Moscow region	IB3	VIII
59	Moscow region	IB3	IX
37	Moscow region	IB2	VI
39	Moscow region	IB2	VII
41	Moscow region	IB5	IX
45	Moscow region	IB3	III
66	Moscow region	IB7	I
69	Moscow region	IB3	VIII
70	Moscow region	IB3	IX
72	Moscow region	IB5	V
73	Moscow region	IB3	I
75	Moscow region	IB3/5	I
79	Moscow region	IB3/5	I
61M	Smolensk	IB2	XI
62M	Smolensk	IB3	XI
64M	Smolensk	IB4	II
65M	Smolensk	IB3/5	XI
15-5III	Smolensk	IA6	II
15s	Smolensk	IA6	XI
15-B	Smolensk	IA8	I
31s	Smolensk	IB5	I
46s	Smolensk	IA6	XI

Cluster analysis of electrophoretic profiles of restriction products of Opa genes of *N. gonorrhoeae* clinical strains was carried out using Total Lab V.2.01 and TreeconW software, the homology between the studied strains was evaluated, and dendrograms were plotted. Comparative analysis of dendrograms demonstrated higher informative value of restriction by HpaII sites. Opa typing revealed 11 groups of *N. gonorrhoeae* clinical strains, which did not conform to the serotype identified by the Por gene. This considerably increases the resolving capacity of genotyping.

The absence of a universal trend in the distribution of the two main serovars of *N. gonorrhoeae* (PIA and PIB) in different countries is worthy of note. The PIA serovar predominates in British [6], Swedish [2], Philippine, and Singapore [8] populations of *N. gonorrhoeae* (52-53%, the data for 1982-1990). Another picture was observed in Germany, where 82% isolated strains belong to PIB serotype [7] and in Greece, where 65% of 185 strains belong to the PIB serovar (the data of 1989) [13]. Later interregional studies (1999) showed a trend to the predominance of the PIB serovar (64.7%) in the studied sample, with the highest incidence of PIB3 and PIB2 serotypes; for the PIA serovar the most incident was PIA6 serotype [4]. A similar trend was observed among *N. gonorrhoeae* strains isolated in Russia. We cannot make conclusions about correlation of the genotype with antibiotic sensitivity of the strais and disease pattern. Analysis of antibiotic sensitivity of clinical strains of *N. gonorrhoeae* showed high percentage (65%) of multiresistant strains resistant to fluoroquinolones, macrolides, penicillins, and tetracyclines. As was expected, all of them belonged to the PIB serovar. Further collection of facts and comparison of the results of typing with the clinical picture and antibiotic sensitivity of clinical strains will permit us make conclusions about the predictive value of *N. gonorrhoeae* strains genotyping.

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