PorA typing of *Neisseria meningitidis* isolates from Iranian children for vaccine design

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ABSTRACT

Introduction: As the causative agent of meningitis, *Neisseria meningitidis* has different serogroups. The purpose of this study was to investigate the molecular properties of *N. meningitidis* strains among Iranian cases. **Methods:** 450 samples were collected from children under 5 years of age. Detection of *Neisseria* genus was done by phenotypic and genotypic methods. Multiplex PCR was used to identify the serogroups of N. meningitides. The sequencing of variable regions of *porA* gene was performed for detection of the subserogroups. **Results:** From 137 (30.44%) *Neisseria* isolates, 4 isolates (0.88%) belonged to *N. meningitidis* and 133 isolates (29.55%) belonged to other species. Multiplex PCR results showed that one isolate belonged to serogroup A while 3 belonged to serogroup B. The analysis of amplified VR1 and VR2 variable regions of porA showed 100% identity of the serogroup A strain with strain BZ83N and the serogroup B strains with strain 528 of *N. meningitidis* . In accordance with other findings in Asia, serogroups A and B were the most prevalent serogroups of *N. meningitidis*. Sequencing of variable regions of porA could identify the subserogroups of the isolates. **Conclusion:** sequencing of porA could be a valuable method for identification of *N. meningitidis* strains to be used in epidemiological studies as well as improved vaccine designs.

KEYWORDS: *Neisseria meningitidis*, *porA*, sequencing, PCR, typing.

INTRODUCTION

Neisseria meningitidis (meningococcus) are pathogenic Gram-negative bacteria which cause meningitis, especially in children under the age of 5. Meningococcus consists of 13 different serogroups, based on the differences in the structure of their polysaccharide capsule. The serogroups A, B, C, Y, and W-135 are major pathogens in humans whose geographic distribution is different in various parts of the world [1]. The genetic diversity of isolated *Neisseria* strains is known in many parts of the world as presented in <http://neisseria.org/nm/typing> website, based on *Neisseria* surface molecules, including Porine Class 1 or PorA protein. PorA is an intramembrane cationic protein, expressed in outer membrane of all *N. meningitidis* strains. This protein consists of 16 parallel beta strands with conserved amino acid sequences in the strains having 8 extracellular hydrophilic loops. The differences in PorA protein between the strains, which is the basis for of their typing into the serosubtypes are related to loop 1 and loop 4 which contain variable regions of VR1 and VR2 [2-4]. The current method for the detection of asymptomatic carriers of *N. meningitidis* is culturing of nasopharynx or tonsil samples whereas a PCR-based method is used in epidemiological studies. The purpose of this study was to detect and investigate the molecular properties of *N. meningitidis* strains among kindergarten-age (i.e. under 5-yerars old) Iranian children in Tehran.

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MATERIALS and METHODS

In the present descriptive cross-sectional study, 450 samples were collected from nasopharynx and tonsils of healthy children under 5 years of age with Dacron swab, in Tehran kindergartens from October 2015 to March 2016. Microbiological and biochemical tests were conducted to detect *Neisseria* genus in these samples. After culturing the samples in chocolate agar and Mueller Hinton Agar (MHA), some bacterial colonies were dissolved in 400 µl distilled water and cellular suspensions were prepared. Genomic DNA samples were then extracted using purified DNA kit

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(DNP^{TM}Kit,CINACLON, Iran) and the quality and quantity of the extracted DNA were determined with a NanoDrop device. The 16S rRNA gene sequencing was used to determine *Neisseria* genus and identification of *N. meningitidis* species was done based on *ctrA* gene amplification by PCR. Multiplex PCR was used to identify the serogroups of the isolated *N. meningitidis* (*orf-2* gene for identification of the serogroup A, and *siaD* (capsule polymerase) gene for detection of serogroups B and C). For detection of the strains, the presence of *porA* gene was evaluated by PCR among the serogrouped isolates. The primer sequences used to identify genus, species, serogroups, and meningococcal strains are shown in Table 1.

Table.1. Primers used to identify genus, species, serogroups, and meningococcal strains

Target	Gene	5'-3'(Sequence)	Ref.
Genus	*16SrRNA*	GTC ATG AAG CAT ACC GTG GT ' CAT AAG AGT TTG ATC CTG GCT	5
Species	*ctrA*	CCA GCG GTA TTG TTT GGT GGT CAG GCG GCC TTT AAT AAT TTC	5
Serogroup	*Nm(A)F* *Nm(A)R*	CGC AAT AGG TGT ATA TAT TCT TCC' CGT AAT AGT TTC GTA TGC CTT CTT	5
	Nm(B)F *Nm(B)R*	GGA TCA TTT CAG TGT TTT CCA CCA GCA TGC TGG AGG AAT AAG CAT TAA	5
	Nm(C)F *Nm(C)R*	TCA AAT GAG TTT GCG AAT AGA GG T CAA TCA CGA TTT GCC CAA TTG AC	5
Strain	*porA*	GTC ATG AAG CAT ACC GTG GGT CAT AAG AGT TTG ATC CTG GCT	5

Finally, the PCR products were purified and sequenced using an ABI Automated Sequencer. The CLC Main Workbench software (CLC Bio, Aarhus, Denmark) was applied to analyze the raw sequencing data.

RESULTS

According to our results, the frequency of collected samples in boys (n = 276, 61.2%) was more than girls (n = 174, 38.8%). After performing phenotypic and molecular tests, 137 (30.44%) of *Neisseria* was isolated from the 450 tested samples and verified as *Neisseria* genus, of which, 81 (59.1%) were from boys and 56 (40.9%) were from girls, including 51 (37.2%) in the age range of 1-3 years and 86 (62.8%) in the age range of 4-5 years. The greatest number of *Neisseria* genus was from Nabi Bagh-e Ghonche kindergarten (15 cases) and the least number was from Shabir, Bagh-e Ghonche and Roghayye kindergartens (each one 3 cases). Of these, 4 (0.88%) were positive for ctrA gene and belonged to *N. meningitidis* and 133 (29.55%) belonged to other species. Multiplex PCR results showed that one isolate out of 4 belonged to serogroup A and 3 to serogroup B. It was found that all 3 strains of the B serogroup were isolated from Amene kindergarten and the serogroup A strain was from Sama kindergarten. Each of the 4 isolates was collected from the tonsils. The PCR results with primers designed based on VR1 and VR2 variable regions of *porA* gene showed the presence of this gene in serogroup A and B. The obtained sequences were analyzed by NCBI BLAST and 100% identity was observed between the serogroup A strain and BZ83N strain as well as the serogroup B strains and strain 528 of *N. meningitidis*.

DISCUSSION

Based on 16s rRNA gene amplification, we detected 137 *Neisseria* genus among the 450 tested isolates. Greisen et al. in Ireland designed the primer sequence of 16S rRNA for detection of *Neisseria* [5]. Various researches in different locations of Iran have reported the prevalence of *Neisseria* genus from 0 to 33%. Also, different rates of *Neisseria* are reported from other countries [6]. The main reason for the discrepancies in these studies could be related to the type of sample, sample size and the geographic area [7-9]. Using a PCR method, we could identify 4 *N. meningitidis* among the 137 *Neisseria* genus (2.9 %) that was close to another report from Spain (5%) [10]. In another study in which the bacterial flora of the nasopharynx was examined, a higher rate of *N. meningitidis* (9%) had been isolated [6]. Although, detailed and comprehensive studies have not been conducted on the prevalence of *N. meningitidis* in Asia, especially in the Middle East, sporadic studies point to increased prevalence of serogroups A and B [6, 11]. We were able to isolate these two serogroups in the present study and achieved the same results. In another study in England [12], the PCR results showed that serogroups B and C were accounted for the most carriers. In addition, a study in Mexico on the prevalence of *Neisseria* has shown that 2.9% of the isolates were positive for *N. meningitidis* while the most frequent of which was related to serotype Y [13]. The sequencing of important genes such as *porA* in different *N. meningitidis* serogroups could identify subserogroups among the isolates [14]. Schuurman, also used the PCR and sequencing of *N. meningitidis* for their typing among 267 CSF specimens [15].

In conclusion, a low rate of *N. meningitidis* was isolated from Iranian children. Sequencing of *porA* could be a valuable method for identification of *N. meningitidis* strains in epidemiological studies. Considering that many studies have used PorA antigen as a vaccine, the collected typing information can potentially be used to design enhanced vaccines against the most prevalent pathogen in different geographical regions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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