

COMUNICACIÓN BREVE

Phenotypic and genotypic characterization of *Neisseria meningitidis* serogroup B isolates from Cartagena, Colombia, 2012-2014

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Introduction: *Neisseria meningitidis* is one of the leading causes of bacterial meningitis. In 2013, an increase in invasive disease caused by *N. meningitidis* serogroup B occurred in Cartagena, Colombia.

Objective: The aim of this study was to characterize serogroup B isolates recovered from patients with meningococcal meningitis in Cartagena during 2012 and 2014.

Materials and methods: Twenty-five isolates were collected through a national, laboratory-based surveillance, and phenotypic and genotypic characteristics were determined by serosubtyping, serotyping, antimicrobial susceptibility testing, repetitive sequence-based PCR (rep-PCR). MLST data available were also used.

Results: The B:10,15:nt phenotype, associated with clonal complex ST-41/44, was responsible for 68.0% of all cases.

Conclusion: The clinical, epidemiological and molecular characterization of meningococcal disease is necessary for understanding the spread of virulent meningococcal clones and for facilitating the early detection of cases to reduce epidemic risks.

Key words: *Neisseria meningitidis*, serogroup B; meningitis, phenotype.

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Caracterización fenotípica y genotípica de aislamientos de *Neisseria meningitidis*, serogrupo B, procedentes de Cartagena, Colombia, 2012-2014

Introducción. *Neisseria meningitidis* es una de las causas principales de meningitis bacteriana. En el 2013 se evidenció un aumento de la enfermedad invasiva causada por *N. meningitidis*, serogrupo B, en Cartagena, Colombia.

Objetivo. El objetivo de este estudio fue caracterizar los aislamientos de *N. meningitidis*, serogrupo B, recuperados de pacientes con meningitis meningocócica en Cartagena durante el año 2013.

Materiales y métodos. Se recolectaron 25 aislamientos a través de la vigilancia basada en el laboratorio nacional y se determinaron las características fenotípicas y genotípicas mediante serosubtipificación, serotipificación, pruebas de sensibilidad a los antimicrobianos, amplificación de secuencias de ADN repetidas (REP-PCR) y el uso de datos disponibles en el sitio de MLST.

Resultados. El fenotipo B:10,15:nt, asociado con el complejo clonal ST-41/44, fue responsable del 68,0 % de todos los casos.

Conclusión. La caracterización clínica, epidemiológica y molecular de los aislamientos causantes de enfermedad meningocócica es necesaria para el conocimiento de la propagación de clones virulentos y facilita la detección temprana de casos, lo que reduce los riesgos de epidemia.

Palabras clave: *Neisseria meningitidis*, serogrupo B; meningitis, fenotipo.

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Neisseria meningitidis has become the leading cause of bacterial meningitis in children, especially after the introduction of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b routine vaccination (1). There are 12 serogroups of

N. meningitidis; however, the majority of invasive meningococcal infections is caused by serogroups A, B, C, W, X and Y, and the distribution varies with the geographical location and the age group (2). The overall incidence of meningococcal disease differs

Author's contributions:

Carolina Duarte and Jaime Moreno conceived and designed the experiments.

Olga Sanabria, Sandra Yamile Saavedra and Karina Rodríguez performed the experiments.

Carolina Duarte, Jaime Moreno and Sandra Saavedra analyzed the data.

Jaime Moreno wrote the paper.

from one region to another ranging between <2 cases/100,000 population per year in the United States, China and European and Latin American countries, and >10/100,000 population per year in some African countries (3,4).

N. meningitidis serogroup B has emerged as an important cause of invasive meningococcal disease in some regions of Europe, Latin America and North America since the introduction of vaccines against serogroups A and C (5). Despite the genetic heterogeneity of serogroup B strains, two hypervirulent clonal complexes, ST-41/44 3 and ST-32, are responsible for the majority of serogroup B disease cases in several countries (5). In 2012, 33 cases of meningococcal disease were reported in Colombia, of which 69.7% were caused by *N. meningitidis* serogroup B (6). In 2012, an increase in invasive disease caused by *N. meningitidis* serogroup B (25 cases in total) was observed in Cartagena de Indias, Colombia, which has been declared by UNESCO as a world heritage site. In the present study, we characterized *N. meningitidis* serogroup B isolates recovered between 2012 and 2014 from infected patients living in deprived urban areas in Cartagena.

Materials and methods

We analyzed 25 *N. meningitidis* isolates recovered between March, 2012, and October, 2014, from cerebrospinal fluid (CSF) or blood samples of meningococcal disease patients. The isolates were received at the Colombian Instituto Nacional de Salud through the SIREVA II international surveillance network (6). We confirmed isolates by using standard microbiology techniques, while the serogroup was determined by slide agglutination with commercial antiserum against meningococcal capsular polysaccharides (DIFCO, Beckton Dickinson). Serosubtyping and serotyping were done by dot blot with monoclonal antibodies (RIVM, Bilthoven, the Netherlands, and Institute Adolfo Lutz, São Paulo, Brazil) (7). Agar dilution antimicrobial susceptibility testing for penicillin (P), ceftriaxone (CRO), chloramphenicol (CL),

ciprofloxacin (CIP) and rifampin (RIF) was performed according to the Clinical and Laboratory Standards Institute guidelines (8).

We performed genotyping on the 19 isolates using the bacterial rep-PCR-DiversiLab kit (Bacterial BarCodes, bioMérieux Inc., Houston, TX) following the manufacturer's recommendations. We analyzed patterns by using the Pearson correlation coefficient to create a dendrogram of the genetic relationship among isolates (9). Clusters of rep-PCR patterns exhibiting a similarity of >93% were designated by capital letters. Additionally, we included data from the Multi Locus Sequence Typing (MLST) site for four isolates recovered in 2010 (isolate 858), 2011 (isolates 903 and 905) and 2012 (isolate 926), which were studied as part of a SIREVA II Latin American project. MLST was performed according to the methods of Maiden, *et al.* Primers, as well as the determination of sequence alleles and the designation of sequence types, were retrieved from the MLST website (<http://neisseria.org/nm/typing/mlst>).

Results

The 25 *N. meningitidis* serogroup B isolates were sent by six hospital laboratories, but 50% of them were recovered in only one hospital. All patients came from a geographically defined area in 12 of the 250 city districts characterized by high levels of poverty and low educational levels.

Seventy six per cent of meningococcal disease was diagnosed in men; patients' ages ranged from one month to 55 years: 8 isolates (32.0%) were recovered from patients <5 years of age; 8 (32.0%), from patients 5 to 14 years of age, and 8 (32.0%) from patients >14 years of age; no information was available for one of the patients (4.0%). Isolates were recovered from blood (60.0%) and CSF (40.0%) samples. Patients had been diagnosed with meningitis (60.0%), meningococemia (32.0%) and bacteremia (8.0%). Two (8.0%) were penicillin-non-susceptible isolates (0.250 µg/mL), whereas all of them were susceptible to CRO, CL, CIP and RIF (table 1).

The most prevalent serotype and serosubtype was B:10,15:nt (60.0%), followed by B:1:P1.22-1,14 (16.0%) (figure 1). Isolates were grouped in three rec-PCR clusters (A, B, C) designated by size, of which A was the largest (figure 1). Cluster A, grouping 17 isolates from the study and 2 controls (isolates 903 and 905), was B:10,15:nt and showed genetic relation with the clonal complex ST-41/44.

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Table 1. Patient data and microbiological features of *N. meningitidis* serogroup B isolates collected in Cartagena from 2012 to 2014

Isolate code - INS	Date of isolation	Patient's data			Antimicrobial susceptibility (MIC)*				
		Age (years)	Gender†	Diagnosis	P	CRO	RIF	CL	CIP
926	06/03/2012	5	F	Meningococemia	0.06	0.001	0.06	1	0.016
927	06/03/2012	1	M	Meningococemia	0.125	0.001	0.03	1	0.016
929	14/03/2012	2	M	Bacteremia	0.06	0.001	0.06	1	0.016
941	09/07/2012	ND**	F	Meningitis	0.03	0.001	0.06	1	0.016
953	26/10/2012	54	M	Meningitis	0.06	0.001	0.03	2	0.004
955	30/11/2012	29	M	Meningococemia	0.06	0.001	0.06	2	0.016
958	07/12/2012	32	M	Meningitis	0.06	0.0005	0.03	2	0.016
969	13/03/2013	8	M	Meningococemia	0.25	0.004	<0.016	2	0.016
973	23/05/2013	4	M	Meningococemia	0.06	0.001	0.25	2	0.016
978	05/06/2013	5	M	Meningitis	0.06	0.004	0.125	2	0.016
982	04/07/2013	8	F	Meningitis	0.25	0.004	0.008	1	0.002
987	16/07/2013	7	M	Meningitis	0.03	<0.002	0.008	0.5	0.002
989	25/09/2013	9	M	Meningococemia	0.03	0.002	0.004	0.5	0.004
992	05/10/2013	9 m*	M	Meningitis	0.03	<0.002	0.016	1	0.008
993	11/10/2013	55	M	Meningitis	0.06	<0.002	0.06	2	0.016
994	25/10/2013	51	M	Meningitis	0.03	<0.002	0.06	1	0.008
1002	03/12/2013	8	M	Meningococemia	0.25	0.001	0.06	2	0.016
1004	12/12/2013	14	M	Meningitis	0.06	0.002	0.125	2	0.016
1006	10/01/2014	30	M	Meningitis	0.06	0.002	0.06	1	0.004
1008	04/03/2014	1 m*	M	Meningitis	0.06	0.002	0.03	2	0.016
1015	16/04/2014	4 m*	F	Meningitis	0.03	0.0005	0.125	2	0.016
1017	30/04/2014	4	F	Meningitis	0.016	0.001	0.03	2	0.016
1018	30/04/2014	48	M	Meningitis	0.03	0.005	0.06	2	0.016
1033	01/05/2014	19	F	Bacteremia	0.125	0.0005	0.016	2	0.008
1044	04/10/2014	2	M	Meningococemia	0.06	0.001	0.03	2	0.008

*Months

†M: Male; F: Female

*Minimum Inhibitory Concentration to: P = penicillin, CL = chloramphenicol, CIP = ciprofloxacin, CRO = ceftriaxone, RIF = rifampin

**ND: No data

Three B:1:P1.22-1,14 isolates were grouped as cluster B, and five isolates with different serotype and serosubtype were grouped in cluster C. Isolate 858 from 2010 was B:4,7:P1.1 and the clonal complex was ST23.

Discussion

Using molecular techniques, we characterized invasive *N. meningitidis* serogroup B isolates that had circulated in Cartagena as part of a meningococcal disease epidemic affecting low socioeconomic population groups considered at high risk for the disease (10). Serogroup B has become a recent threat with sporadic, endemic and epidemic occurrences being recorded in North America, Europe, South America and Australia (5). From 1987 through 2012, 680 *N. meningitidis* isolates were confirmed in Colombia by the Microbiology Group of the Instituto Nacional de Salud, of which 72.2% (n=491) were serogroup B (6).

Overall, isolates were susceptible to most of the antibiotics tested. SIREVA II data showed that between 2006 and 2010, *N. meningitidis* isolates

recovered in Bolivia, Colombia, Ecuador, Perú and Venezuela corresponded predominantly to serogroups B and Y (41.7% and 29.1%, respectively). Decreased sensitivity to P was found in 17.1% of isolates (16.1% intermediate and 1.0% fully resistant isolates) while all of them remained highly susceptible to RIF (99.5%) (11). A systematic review of the effectiveness of different antibiotics for prophylaxis against meningococcal disease found that penicillin and rifampin proved effective for eradicating *N. meningitidis*; however, the use of rifampin may lead to the emergence of resistant strains (12). Therefore, antimicrobial chemoprophylaxis for close contacts of patients with invasive meningococcal disease could be important to prevent further cases (12).

The information on the distribution of *N. meningitidis* serogroup B isolates serosubtypes and serotypes is important in evaluating the possibility of using outer membrane protein (OMP) based vaccines to control endemic meningococcal disease (13). However, the variability in OMP strains causing endemic disease will likely limit their usefulness (10). The main

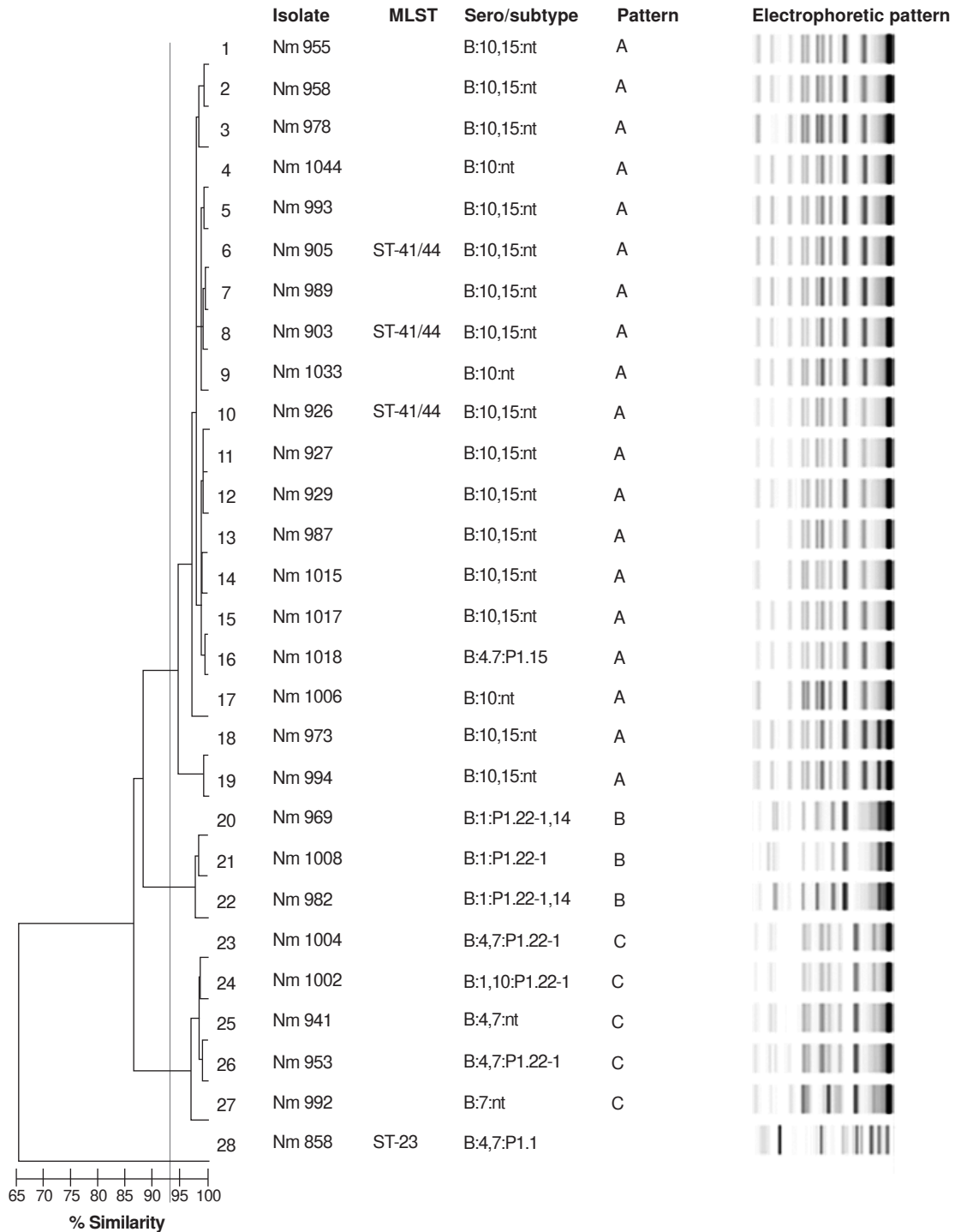


Figure 1. Similarity dendrogram showing the relatedness of rep-PCR profiles and MLST of *N. meningitidis* serogroup B isolates collected in Cartagena from 2012 to 2014

serosubtype and serotype (B:10,15:nt) identified in this study differ from those in the vaccines currently licensed: The Cuban VA-MENGOB-BC vaccine, which was developed from the outer membrane antigens of the epidemic strain type B:4:P1.15

combined with serogroup C polysaccharide (14), and a multicomponent serogroup B meningococcal vaccine (4CMenB), consisting of factor-H binding protein (fHbp), *N. meningitidis* adhesin A (NadA) and *Neisseria* heparin binding antigen (NHBA),

combined with outer membrane vesicles from the New Zealand epidemic strain NZ98/254 (B:4:P1.7b,4) (15). This vaccine was approved for use in the European Union and Australia, and has been found to be safe and immunogenic in a Latin American adolescent population (10,16). The predicted protection of this new vaccine is dependent of vaccine antigens expressed by strains causing disease in different geographical settings, therefore, fHbp, NadA and NHBA genetic typing of invasive *N. meningitidis* serogroup B isolates is required.

A limited number of clonal complexes are responsible for the majority of the disease cases associated with outbreaks or epidemics. In our study, the most common clonal complex was ST-41/44, found in 64.3% of cases. The ST-41/44 is the most diverse clonal complex associated with serogroup B meningococcal disease, and the analysis of available MLST data showed that the most common serotypes in this complex are 41, 44, 42, 40 and 154 (5). This clonal complex has caused epidemics in the Netherlands and New Zealand and was the dominant cause of invasive meningococcal disease in Ireland, Belgium and Italy (5). In Chile, the ST-41/44 complex, mostly associated with B:P1.19,13-1, showed the highest prevalence (29.0%), (17), while in Argentina, only 7% of the serogroup B isolates in 2010 belonged to the ST-41/44 complex (18).

Phenotyping and genotyping methods are important for meningococcal transmission studies and epidemics early detection. Additionally, molecular surveillance is crucial to identify genotypes indicating disease common sources, as well as to determine the antigenic profile and clonal diversity of *N. meningitidis* isolates associated with invasive disease, and to monitor the evolution and distribution of meningococcal serogroups and clones in order to provide information for future vaccine development.

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Conflicts of interest

None declared by the authors.

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