

Neisseria meningitidis: analysis of pili and LPS in emerging Brazilian strains

Amanda Izeli Portilho, Gabriela Trzewikowski de Lima and Elizabeth De Gaspari 

Therapeutic Advances in
Vaccines and Immunotherapy

2020, Vol. 8: 1–10

DOI: 10.1177/
2515135520919195

© The Author(s), 2020.
Article reuse guidelines:
sagepub.com/journals-
permissions

Abstract

Background: *Neisseria meningitidis* is the main cause of bacterial meningitis in Brazil, where the main serogroups isolated are B and C; however, the serogroup W has recently emerged.

LPS and type IV pili are important virulence factors that increase meningococci pathogenicity.

Methods: The characterization of Lipopolysaccharide (LPS) and type IV pili in 19 meningococci strains of serogroup B, 21 of serogroup C, 45 of serogroup W and 28 of serogroup Y, isolated in Brazil between 2011 and 2017, was conducted using the Enzyme-linked Immunosorbent Assay (Dot-ELISA) technique and monoclonal antibodies.

Results: We would like to emphasize the importance of characterizing relevant antigens, such as pili and LPS, the use of monoclonal antibodies to support it, and how such studies improve vaccine development and monitoring. Most of the strains studied presented L3,7,9 LPS and type IV pili; both antigens are associated with the capacity to cause invasive disease.

Conclusion: Due to the impact of meningococcal disease, it is important to maintain and improve vaccine studies. Epitopes characterization provides data about the virulence of circulating strains. The use of monoclonal antibodies and serological techniques are relevant and support vaccine development.

Keywords: invasive meningococcal disease, LPS, *Neisseria meningitidis*, pili, serotyping

Received: 21 November 2019; revised manuscript accepted: 23 March 2020.

Introduction

Neisseria meningitidis or meningococci (MC) is a Gram-negative, aerobic, immobile, and non-sporulating bacteria that can colonize the oropharynx of healthy individuals or provoke invasive meningococcal disease (IMD), causing meningitis and/or meningococemia. Its virulence has been attributed to several factors, as the polysaccharide capsule, outer membrane proteins, such as pili, the lipopolysaccharide (LPS), mechanisms of horizontal gene transfer and antigenic variation.^{1–3}

Serological typing of *N. meningitidis* strains by Dot-ELISA and a panel of monoclonal antibodies is well established.⁴ The characterization of meningococci antigens by monoclonal antibodies uses the capsule, Porin B, Porin A and LPS to characterize the strains, respectively, in serogroup, serotype, subtype and immunotype.^{5,6}

IMD is considered a public health problem, especially in developing countries. It is estimated that every year, there are 1,2 million new cases and 135 thousand deaths.⁷ The distribution of serogroups and the impact of diseases caused by MC in the world are heterogeneous; however, the impact of IMD is greater in developing countries. Adequate surveillance has great importance in estimating the impact of IMD, outlining strategies of prevention, which is the better strategy for control, and evaluating the available diagnostic and monitoring systems.^{8,9} Although there are molecular tools to classify the pathogen,¹⁰ serological techniques present lower costs, are easy to perform and reproducible, being suitable especially in low-income countries.^{4–6}

We performed this study to verify the expression of L3,7,9 immunotype and type IV pili (T4P) among circulating strains of *N. meningitidis* in

Correspondence to:

Elizabeth De Gaspari
Department of
Immunology, Adolfo Lutz
Institute, Dr Arnaldo
Avenue 355, 11 floor, São
Paulo, SP 01246-902,
Brazil

Post-Graduate
Program Interunity in
Biotechnology, Biomedical
Sciences Institute, São
Paulo University, São
Paulo, Brazil
elizabeth.gaspari@ial.sp.gov.br

**Amanda Izeli Portilho
Gabriela Trzewikowski
de Lima**
Department of
Immunology, Adolfo Lutz
Institute, São Paulo,
Brazil Post-Graduate
Program Interunity in
Biotechnology, Biomedical
Sciences Institute, São
Paulo University, São
Paulo, Brazil



Brazil in recent years, because, even though they are important for IMD, being related to high pathogenic levels, they are not routinely tested.^{11,12} This was an important step for the study of the pathogenic potential of most prevalent Brazilian strains, serogroups B and C,¹³ as well as emerging serogroups W and Y, which have recently increased in the Americas and, because of that, got our focus in this study.^{7,8}

Materials and methods

Whole cell suspensions

Whole cell suspensions and its phenotypic characterization were performed at the Bacteriology Center of Adolfo Lutz Institute (São Paulo, Brazil). We used 113 Brazilian strains (19 of serogroup B, 21 of serogroup C, 45 of serogroup W and 28 of serogroup Y), isolated from cases of IMD, between 2011 and 2017. During this period, 12,677 cases of IMD were reported and 6354 of them were caused by the serogroups studied (the difference being caused by 'ignored serogroups'), according to the Brazilian National Notifiable Disease Surveillance System (SINAN).¹⁴

Strains were isolated in chocolate blood agar (5% sheep blood), followed by cultivation in Müller–Hinton agar containing horse serum, incubated at 37°C ± 2°C, in 5% CO₂ atmosphere, during 24 hours. Whole cell suspensions were prepared in PBS/azide solution, with an OD ~2.0, at 620 nm. Following that, the suspensions were inactivated in a water bath 56°C for 30 minutes and maintained under refrigerator temperature. Serogrouping was performed by slide agglutination with polyclonal sera and serosubtyping was performed by Dot-blotting with monoclonal antibodies, serological techniques routinely performed at the Bacteriology Center.¹⁵ Table 1 shows the phenotypic characterization of the strains used.

Monoclonal antibodies (mAbs)

We characterized LPS using 4BE12-C10 mAb (L3,7,9 immunotype),¹⁶ disposable at the *Neisseria*.org database,¹⁷ and pili using 7BE211-E12 mAb (T4P), both obtained by the hybridoma technique,¹⁸ produced in mice as ascitic fluids. They were kindly given to Dr De Gaspari during her post-doctoral fellowship at the Walter Reed Army

Institute of Research, where they were routinely used. Back in Brazil, the mAbs were lyophilized and kept in aliquots at 4°C. For use, they were resuspended, tited and maintained at -20°C.¹⁹

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

To verify specificity against pili, we performed an immunoblotting assay. For that, whole cells of meningococci strain B:4:nt were characterized by electrophoresis in a 10% polyacrylamide gel, in the presence of sodium dodecyl sulfate (SDS), following the protocol described by Laemmli²⁰ and using a molecular weight marker ranging from 11 to 245 kDa (Color Protein Standard Broad Range, New England BioLabs). After the electrophoresis, the gel was stained with Coomassie Blue (PhastGel Blue R; Pharmacia Biotech). Immunotype mAbs were previously tested in our laboratory.²¹

Immunoblotting

A new gel was prepared following the same steps described earlier. After electrophoresis, proteins were transferred to a 0.45 μm nitrocellulose membrane (BioRad Laboratories) at 100 V for 18 hours at 4°C. After that, the membrane was stained with Ponceau-S and discolored with PBS. The membrane was blocked with skimmed milk (La Serenissima) 5% for 2 hours, at room temperature. mAb was diluted at 1:2000 in skimmed milk 2.5% and incubated overnight at 4°C. Then, the membrane was washed five times with PBS pH 7.2 and incubated with goat anti-mouse IgG (whole-molecule)-alkaline phosphatase (Sigma Aldrich) diluted at 1:5000 in skimmed milk 2.5%, for 2 hours, at room temperature. The membrane was washed again and the substrate 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT-plus) (Mabtech) was incubated for 20 minutes, at room temperature and protected from light. The reaction was stopped by adding distilled water and was considered positive by the appearance of color in the membrane.²²

Dot-ELISA

Dot-ELISA was conducted pipetting 1 μL of whole cell suspension in nitrocellulose membrane 0.45 μm (BioRad Laboratories). After that, Ponceau-S staining was proceeded. Membranes were blocked with skimmed milk (La Serenissima) 5% for 2 hours at

Table 1. Serogrouping, serotyping and subtyping characterization of *N. meningitidis* strains used in this study.

Strain identification	Year	Serogroup	Serotype	Subtype
N.30/11	2011	C	2a	P1.5
N.36/11	2011	C	2b	P1.14-6
N.41/11	2011	B	19,7	P1.14
N.56/11	2011	B	4,7	P1.19
N.60/11	2011	B	4,7	P1.7,1
N.61/11	2011	B	4,7	P1.7,1
N.74/11	2011	W	2a	P1.5,2
N.101/11	2011	W	2b	P1.5,2
N.149/11	2011	C	4,6	P1.19,14
N.180/11	2011	Y	22	P1.3
N.186/11	2011	B	4,7	P1.19,15
N.205/11	2011	Y	17,7	P1.5
N.210/11	2011	W	2b	P1.5,2
N.225/11	2011	W	2a	P1.5
N.267/11	2011	Y	22	P1.3
N.357/11	2011	Y	19	P1.5
N.458/11	2011	W	2b	P1.2
N.554/11	2011	C	2b	P1.14-6
N.601/11	2011	C	23	P1.14-6
N.15/12	2012	W	2a	P1.5,2
N.23/12	2012	C	23	P1.14-6
N.26/12	2012	B	4,7	P1.19,15
N.30/12	2012	B	4,7	P1.19,15
N.33/12	2012	B	4,1	P1.16
N.34/12	2012	B	4,7	P1.19,15
N.37/12	2012	C	23	P1.14-6
N.42/12	2012	C	23	P1.14-6
N.63/12	2012	W	2a	P1.2
N.64/12	2012	W	2a	P1.5,2
N.65/12	2012	B	4,7	P1.19,15
N.72/12	2012	C	2a	P1.5

(Continued)

Table 1. (Continued)

Strain identification	Year	Serogroup	Serotype	Subtype
N.85/12	2012	Y	22	P1.3
N.99/12	2012	W	2b	P1.2
N.119/12	2012	C	23	P1.14-6
N.120	2012	C	23	P1.14-6
N.123/12	2012	W	2b	P1.5,2
N.125/12	2012	Y	22	P1.3
N.03/14	2014	C	23	P1.14-6
N.20/14	2014	B	4,7	P1.19,15
N.143/14	2014	B	19,1	P1.14
N.155/14	2014	C	23	P1.14-6
N.233/14	2014	C	23	P1.14-6
N.278/14	2014	B	19	nt
N.365/14	2014	B	19	P1.4
N.375/14	2014	C	23	P1.14-6
N.39/15	2015	C	23	P1.14-6
N.44/15	2015	B	4,7	P1.14
N.110/15	2015	C	23	P1.14-6
N.135/15	2015	B	4,7	P1.19,15
N.194/15	2015	C	23	P1.14-6
N.21/16	2016	B	4,7	P1.19,15
N.28/16	2016	C	23	P1.14-6
N.101/16	2016	C	2a	P1.5
N.151/16	2016	B	19	P1.19,15
N.221/16	2016	C	23	P1.14-6
N.227/16	2016	B	4,7	P1.19,15
N.13/14	2014	W	2b	P1.5,2
N.111/14	2014	Y	NT	P1.5,2
N.146/14	2014	W	2a	P1.2
N.249/14	2014	Y	22	nt
N.305/14	2014	Y	22	nt
N.327/14	2014	W	2a	nt

(Continued)

Table 1. (Continued)

Strain identification	Year	Serogroup	Serotype	Subtype
N.341/14	2014	W	2a	P1.5
N.385/14	2014	Y	19,10	nt
N.51/15	2015	Y	22	nt
N.78/15	2015	W	23	P1.3
N.127/15	2015	W	2b	nt
N.170/15	2015	W	2a	P1.2
N.262/15	2015	Y	19	P1.10
N.268/15	2015	Y	22	P1.3
N.15/16	2016	W	2a	P1.5,2
N.25/16	2016	Y	2b	P1.5,2
N.71/16	2016	W	2a	P1.5,2
N.226/16	2016	Y	4	nt
N.239/16	2016	W	2a	P1.5,2
N.250/16	2016	Y	2b	P1.5,2
N.5/17	2017	W	2a	P1.5,2
N.21/17	2017	W	2a	P1.5,2
N.29/17	2017	Y	4	nt
N.38/17	2017	Y	NT	nt
N.52/17	2017	Y	4	nt
N.55/17	2017	W	2a	nt
N.57/17	2017	W	2a	P1.2
N.58/17	2017	W	2a	P1.5,2
N.73/17	2017	W	2a	P1.5,2
N.85/17	2017	Y	19,10	nt
N.87/17	2017	W	2a	P1.5,2
N.100/17	2017	W	2a	P1.5,2
N.129/17	2017	Y	19	nt
N.132/17	2017	W	2a	P1.2
N.136/17	2017	W	NT	P1.2
N.140/17	2017	W	NT	P1.2
N.147/17	2017	W	2b	P1.2

(Continued)

Table 1. (Continued)

Strain identification	Year	Serogroup	Serotype	Subtype
N.149/17	2017	Y	2a	nt
N.151/17	2017	Y	2a	nt
N.158/17	2017	W	2a	P1.2
N.159/17	2017	W	2a	P1.2
N.160/17	2017	W	2a	P1.2
N.168/17	2017	W	2b	P1.2
N.169/17	2017	W	2a	P1.2
N.170/17	2017	Y	19,10	P1.3
N.172/17	2017	W	2b	P1.2
N.178/17	2017	W	2a	P1.2
N.190/17	2017	W	2b	nt
N.200/17	2017	Y	4	nt
N.205/17	2017	W	2a	P1.2
N.206/17	2017	Y	2c	P1.5
N.208/17	2017	W	NT	P1.3
N.236/17	2017	Y	22	nt
N.243/17	2017	W	2a	P1.2
N.250/17	2017	W	2a	P1.2
N.262/17	2017	Y	22	nt
N.263/17	2017	W	NT	P1.2

room temperature. mAbs, diluted at 1:2000 in skimmed milk 2.5%, were incubated overnight at 4°C. Membranes were washed five times with PBS pH 7.2 and incubated for 2 hours, at room temperature, with goat anti-mouse IgG (whole-molecule)-alkaline phosphatase (Sigma Aldrich) diluted at 1:5000 in skimmed milk 2.5%. After washing, membranes were incubated with the substrate BCIP/NBT-plus (Mabtech) for 20 minutes, at room temperature and protected from light. We added distilled water to stop the reaction, which was considered positive by the appearance of color in the membrane.⁴

This project was performed at the Immunology Center of Adolfo Lutz Institute and was approved

by the Technical and Scientific Committee of this institution (CTC number 41D-2011).

Results

Assessing 7BE211-E12 specificity, Figure 1 shows meningococci strain B:4:nt electrophoretic profile in SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and immunoblotting reaction with the referred mAb, recognizing only one band in immunoblotting. LPS mAb specificity was previously tested in our laboratory.²¹

The results of Dot-ELISA show a high expression of the L3,7,9 immunotype and type IV pili. From 19 MenB strains, all were reactive to L3,7,9

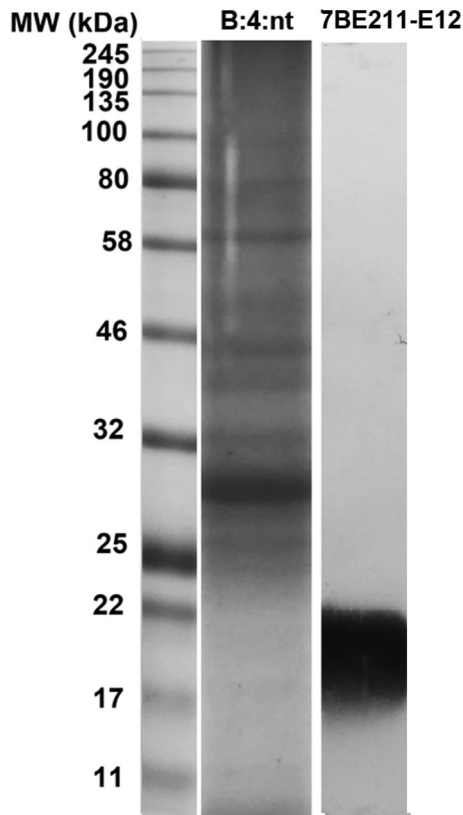


Figure 1. Immunoblotting of mAb 7BE211-E12. On the left, molecular weight and electrophoretic profile of strain B:4:nt, on the right, mAb Immunoblotting, mAb, monoclonal antibody.

immunotype and 16 were reactive to T4P. From 21 MenC strains, L3,7,9 mAb recognized 19 strains and T4P recognized 14 strains. Of 45 MenW strains, all presented L3,7,9 immunotype and 35 presented T4P. Of 28 MenY strains, L3,7,9 mAb recognized 26 strains and T4P recognized 23 strains. Figure 2 shows the results obtained.

Discussion

As expected, mAbs were specific, recognizing only one band in immunoblotting. The pili epitope was approximately 17 kDa according to molecular weight.²³ LPS mAb was previously tested in our laboratory, also recognizing only one band, at 5.9 kDa.²⁴ The Dot-ELISA technique proved to be advantageous due to its technical simplicity, dispensing with the use of specific equipment, using a low volume of monoclonal antibodies and requiring little manipulation of samples, as proposed by Wedge *et al.*⁴ Given

that low-income areas are still linked to a higher burden of IMD, Dot-ELISA characteristics strength that even in the molecular biology era, serological techniques may still be applicable, especially in developing countries.

LPS is considered the main virulence factor of Gram-negative bacteria. It induces an inflammatory response, contributes to adhesion, invasion and inhibits phagocytosis and complement activation, playing roles in infection establishment and progression.^{2,24} The arrangement of the LPS on the cell surface also influences the exposure of outer membrane proteins, which has implications in host–pathogen dynamics.²⁵ Studies showed that some immunotypes, such as L1 and L8, are more often related to carrier strains; however, meningococci can switch its expression during the disease. The L3,7,9 immunotype has already been described between virulent strains^{11,26} and had been associated with a high pro-inflammatory response.²⁷ Detoxified LPS had already been studied as a vaccine compound, given its adjuvanticity qualities, activating Toll-like receptor 4 (TLR-4), being relatively conserved among strains, inducing bactericidal antibodies and probably being able to confer protection against a range of Gram-negative bacteria.^{28,29}

Pili is responsible for the first interaction between the bacteria and host cells, mediates motility, DNA exchange by transformation and allows the meningococci to cross the blood–brain barrier, establishing the infection. T4P is found in several Gram-negative pathogens, such as *Pseudomonas aeruginosa* and *Vibrio cholerae*.^{30–32} Studies showed that pili expression is required for colonization and its removal decreased the level of adherence between the bacterium and the host's cells.^{33,34} A recent study proposed the use of T4P as a target for therapeutics, which reduced the colonization, spread and lethality of the bacterium.³⁵

According to previous studies, antigens of pathogenic species of *Neisseria* usually show great variability among strains.³⁶ However, keeping some antigens is important to maintain the virulence, as pointed out by Urwin *et al.*³⁷ Pili and LPS play important roles in colonization and spread in the host tissue, which are essential steps of invasive disease,³⁸ and several studies, described before, proved its importance to meningococci. Given our results, Brazilian strains express these antigens, suggesting high pathogenic capacity. The

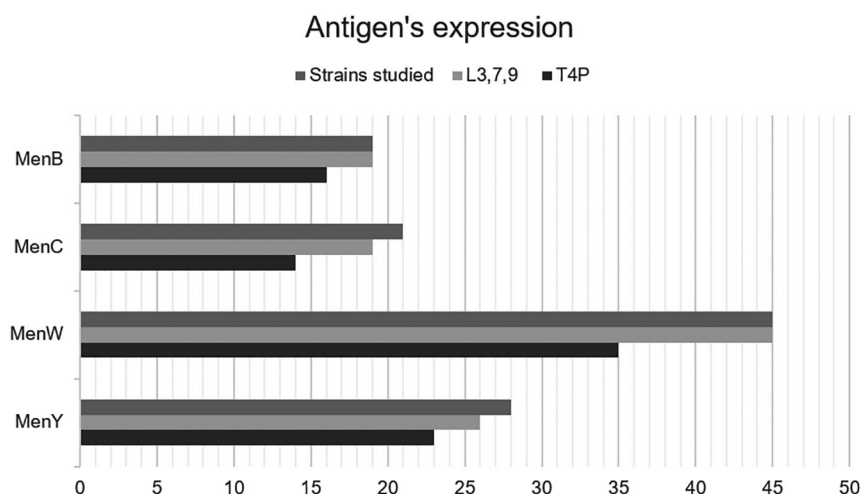


Figure 2. L3,7,9 and T4P expression in Brazilian strains of serogroups B, C, W and Y studied. T4P, type IV pili.

strains were isolated from IMD cases, which agrees with that assumption. The emergence of different serotypes and epidemiological changes, as we have seen with the emergency of serogroups W and Y, are usually connected to hypervirulent strains,³⁹ so it must be important that key factors to pathogenicity are maintained among them and, therefore, are characterized by surveillance programs. Weidlich *et al.*⁴⁰ and Lemos *et al.*⁴¹ studied the hypervirulent strain W:2a:P1.5,2 in Brazilian cases of IMD. That strain was also seen in Argentina⁴² and in Chile, where, in 2010, it became the most prevalent strain and led to an A, C, W and Y quadrivalent vaccine campaign.⁴³

All these data reaffirm how important it is to proceed with antigenic characterization, in order to recognize hypervirulent strains and take control initiatives. Besides the recognition of important antigens between strains, that led us to understand the pathogen better, phenotypic characterization may be applied to study vaccine targets, given that it would be interesting to induce an immune response against antigens that are key factors for virulence. Besides that, characterization allows us to check if current vaccine strains present such antigens, contributing to surveillance over immunization.^{37,44}

Conclusion

Due to the impact of IMD, it is important constantly to maintain and improve vaccine studies.

The characterization of epitopes provides data about meningococci virulence, the prevalence of circulating strains and contributes to immunization research and monitoring. Simple techniques, such as Dot-ELISA, and tools such as mAbs, are important and collaborate with strain selection for vaccine improvement and IMD control.

Acknowledgements

The authors would like to thank Dr Ana Paula Silva Lemos, from the Bacteriology Center of Adolfo Lutz Institute, for providing the whole cell suspensions and information about its serotyping, and Walter Reed Army Institute of Research, for kindly providing the mAbs used in this study.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by São Paulo Research Foundation (FAPESP) (grants 12/15568-0, 13/11147-2, 14/11172-0, 14/07182-0, 18/04202-0) and the National Council for Scientific and Technological Development (CNPq) (132743/2014-6). São Paulo State Secretary of Health and Coordination of Improvement of Post-Graduate Level Personnel (CAPES) provided scholarships.

ORCID iD

Elizabeth De Gaspari  <https://orcid.org/0000-0001-8332-2248>

References

- Pollard AJ and Frasch C. Development of natural immunity to *Neisseria meningitidis*. *Vaccine* 2001; 19: 1327–1346.
- Stephens DS, Greenwood B and Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. *Lancet* 2007; 369: 2196–2210.
- Rouphael NG and Stephens DS. *Neisseria meningitidis*: biology, microbiology and epidemiology. *Methods Mol Biol* 2012; 799: 1–20.
- Wedeg E, Høiby EA, Rosenqvist E, *et al.* Serotyping and subtyping of *Neisseria meningitidis* isolates by co-agglutination, Dot-blotting and ELISA. *J Med Microbiol* 1990; 31: 195–201.
- Frasch CE, Zollinger WD and Poolman JT. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. *Rev Infect Dis* 1985; 7: 504–510.
- Abdillahi H and Poolman JT. Whole-cell ELISA for typing *Neisseria meningitidis* with monoclonal antibodies. *FEMS Microbiol Lett* 1987; 48: 367–371.
- Jafri RZ, Ali A, Messonnier NE, *et al.* Global epidemiology of invasive meningococcal disease. *Popul Health Metr* 2013; 11: 1–9.
- Acevedo R, Bai X, Borrow R, *et al.* The global meningococcal initiative meeting on prevention of meningococcal disease worldwide: epidemiology, surveillance, hypervirulent strains, antibiotic resistance and high-risk populations. *Expert Rev Vaccines* 2018; 18: 15–30.
- Shaker R, Fayad D and Dbaibo G. Challenges and opportunities for Meningococcal vaccination in the developing world. *Hum Vaccin Immunother* 2018; 14: 1084–1097.
- Jolley KA, Brehony C and Maiden MCJ. Molecular typing of meningococci: recommendations for target choice and nomenclature. *FEMS Microbiol Rev* 2007; 31: 89–96.
- Jones DM, Borrow R, Fox AJ, *et al.* The lipooligosaccharide immunotype as a virulence determinant in *Neisseria meningitidis*. *Microb Pathog* 1992; 3: 219–224.
- Pelacic V. Type IV pili: *e pluribus unum?* *Mol Microbiol* 2008; 68: 827–837.
- Ministério da Saúde. *Guia de Vigilância em Saúde*. <http://portalarquivos.saude.gov.br/images/pdf/2017/outubro/06/Volume-Unico-2017.pdf>. (2017, accessed 10 January 2019).
- Ministério da Saúde. *Casos confirmados, óbitos, incidência (por 100.000 habitantes) e letalidade (%) por tipo de meningite*. Brasil, 2010 a 2018. <https://portalarquivos2.saude.gov.br/images/pdf/2019/abril/25/tabela-dados-2010-2018-site.pdf>. (2019, accessed 12 February 2020).
- Gorla MC, Brandão AP, Pinhata JMW, *et al.* Phenotypic characterization of *Neisseria meningitidis* strains isolated from invasive meningococcal disease in Brazil from 2002 to 2017. *Access Microbiol* 2020; 2: 34–43.
- Moran EE, Brandt BL and Zollinger WD. Expression of the L8 lipopolysaccharide determinant increases the sensitivity of *Neisseria meningitidis* to serum bactericidal activity. *Infect Immun* 1994; 62: 5290–5295.
- Neisseria.org* Database. *Information for use of NIBSC monoclonal antibody panel for meningococcal typing*. https://neisseria.org/nm/NIBSC_meningococcal_MAbs.pdf (accessed 12 February 2020).
- Köhler G and Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256: 495–497.
- Johnson M. Antibody storage and antibody shelf life. *Mater Methods* 2012; 2: 120.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature* 1970; 227: 680–685.
- Belo EFT, Farhat CK and De Gaspari EN. Comparison of Dot-ELISA and standart ELISA for detection of *Neisseria meningitidis* outer membrane complex-specific antibodies. *Braz J Infect Dis* 2010; 14: 35–40.
- Trzewikowski de Lima G and De Gaspari E. Individual variability in humoral response of immunized outbred mice and cross-reactivity with prevalent Brazilian *Neisseria meningitidis* strain. *Biologicals* 2018; 55: 19–26.
- Hélaine S, Carbonnelle E, Prouvensier L, *et al.* PilX, a pilus-associated protein essential for bacterial aggregation, is a key to pilus-facilitated attachment of *Neisseria meningitidis* to human cells. *Mol Microbiol* 2005; 55: 65–77.
- Kahler CM and Stephens DS. Genetic basis for biosynthesis, structure, and function of meningococcal lipooligosaccharide (endotoxin). *Crit Rev Microbiol* 1998; 24: 281–334.

25. Stephens DS. Biology and pathogenesis of the evolutionarily successful, obligate human bacterium *Neisseria meningitidis*. *Vaccine* 2009; 27 (Suppl. 2): B71–B77.
26. Poolman JT, Hopman CTP and Zanen HC. Colony variants of *Neisseria meningitidis* strain 2996 (B:2b:P1.2): influence of class-5 outer membrane proteins and lipopolysaccharides. *J Med Microbiol* 1985; 19: 203–209.
27. Braun JM, Blackwell CC, Poxton IR, *et al*. Proinflammatory responses to lipooligosaccharide of *Neisseria meningitidis* immunotype strains in relation to virulence and disease. *J Infect Dis* 2002; 185: 1431–1438.
28. Stoddard MB, Pinto V, Keiser PB, *et al*. Evaluation of a whole-blood cytokine release assay for use in measuring endotoxin activity of Group B *Neisseria meningitidis* vaccines made from Lipid A acylation mutants. *Clin Vaccine Immunol* 2010; 17: 98–107.
29. Bhattacharjee AK, Opal SM, Taylor R, *et al*. A noncovalent complex vaccine prepared with detoxified *Escherichia coli* J5 (Rc chemotype) lipopolysaccharide and *Neisseria meningitidis* group B outer membrane protein produces protective antibodies against gram-negative bacteremia. *J Infect Dis* 1996; 173: 1157–1163.
30. Tzeng YL and Stephens DS. Epidemiology and pathogenesis of *Neisseria meningitidis*. *Microb Infect* 2000; 2: 687–700.
31. Weyand NJ, Wertheimer AM, Hobbs TR, *et al*. *Neisseria* infection of rhesus macaques as a model to study colonization, transmission, persistence, and horizontal gene transfer. *Proc Natl Acad Sci USA* 2013; 110: 3059–3064.
32. Coureuil M, Mikaty G, Miller F, *et al*. Meningococcal type IV pili recruit the polarity complex to cross the brain endothelium. *Science* 2009; 325: 83–87.
33. Stephens DS and McGee ZA. Attachment of *Neisseria meningitidis* to human mucosal surfaces: influence of pili and type of receptor cell. *J Infect Dis* 1981; 143: 525–532.
34. Karupiah V and Derrick JP. Type IV pili – a numbers game. *EMBO J* 2014; 33: 1732–1734.
35. Denis K, Le Bris M, Le Guennec L, *et al*. Targeting type IV pili as an antivirulence strategy against invasive meningococcal disease. *Nat Microbiol* 2019; 4: 972–984.
36. Johnston KH, Holmes KK and Gotschlich EC. The serological classification of *Neisseria gonorrhoeae*. *J Exp Med* 1976; 143: 741–758.
37. Urwin R, Russell JE, Thompson EAL, *et al*. Distribution of surface protein variants among hyperinvasive meningococci: implications for vaccine design. *Infect Immun* 2004; 72: 5955–5962.
38. Hung MC and Christodoulides M. The biology of *Neisseria* adhesins. *Biology (Basel)* 2013; 2: 1054–1109.
39. Hedberg ST, Törös B, Fredlund H, *et al*. Genetic characterization of the emerging invasive *Neisseria meningitidis* serogroup Y in Sweden, 2000 to 2010. *Euro Surveill* 2011; 16: 19885.
40. Weidlich L, Baethgen LF, Mayer LW, *et al*. High prevalence of *Neisseria meningitidis* hypervirulent lineages and emergence of W135:P1.5,2:ST-11 clone in Southern Brazil. *J Infect* 2008; 57: 324–331.
41. Lemos APS, Harrison LH, Lenser M, *et al*. Phenotypic and molecular characterization of invasive serogroup W135 *Neisseria meningitidis* strains from 1990 to 2005 in Brazil. *J Infect* 2012; 60: 209–217.
42. Sorhouet-Pereira C, Efron A and Gagetti P. Phenotypic and genotypic characteristics of *Neisseria meningitidis* disease-causing strains in Argentina. *PLoS One* 2010; 8: e58065.
43. Sáfadi MAP, Berezin EM and Arlant HF. Meningococcal disease: epidemiology and early effects of immunization programs. *J Pediatric Infect Dis Soc* 2014; 3: 91–93.
44. González E, Reyes F, Otero O *et al*. Monoclonal antibodies against the capsular polysaccharides A, C, Y, W, and X of *Neisseria meningitidis*: a platform for the quality control of meningococcal vaccines. *Methods Mol Biol* 2019; 1969: 181–203.