Institut Pasteur
Microbes and Infection 20 (2018) 19–24
www.elsevier.com/locate/micinf

Original article

Dissemination of the ST-103 clonal complex serogroup C meningococci in Salvador, Brazil

Soraia Machado Cordeiro [a,e], Cristiane Wanderley Cardoso [a,b], Lorena Galvão de Araújo [a], Luis Eduardo Ribeiro [e], Jailton Azevedo [a], Rita de Cassia Vilasboas Silva [c], Mitermayer Galvão dos Reis [a], Albert Icksang Ko [a,d], Joice Neves Reis [a,e,*]

[a] *Gonçalo Moniz Research Institute, Oswaldo Cruz Foundation, Brazil Ministry of Health, Rua Waldemar Falcão, 141, Candeal, Salvador 40296-710, Brazil*
[b] *Municipal Secretary of Health of Salvador, Rua da Grécia, 3, Comercio, Salvador 40010-010, Brazil*
[c] *Public Health Central Laboratory (LACEN/BA), Rua Waldemar Falcão, 123, Horto Florestal, Salvador 40295-010, Brazil*
[d] *Department of Epidemiology of Microbial Diseases, Yale School of Public Health, 60 College Street, New Haven, CT 06520-8034, USA*
[e] *School of Pharmacy, Federal University of Bahia, Rua Barão do Jeremoabo, n° 147, Ondina, Salvador, 40170-115, Brazil*

Received 13 June 2017; accepted 21 September 2017
Available online 27 September 2017

Abstract

Invasive meningococcal disease (IMD) is a major public health problem worldwide. An epidemic of serogroup C (NmC) IMD occurred in 2010 in the city of Salvador. In this study, we describe the antigenic and genetic characterization of meningococcal isolates collected from meningitis cases in Salvador from 2001 to 2012. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were performed for the analysis of IMD isolates. A total of 733 cases were identified, and the serogroup was determined for 391 (53.0%) of these. Most cases were caused by NmC (53%) or B (47%). The most prevalent strains were B:4,7:P1.19,15 (32.9%; 129/391) and C:23:P1.14–6 (28.6%; 112/391). Based on PFGE/MLST analysis, 71.3% (77/108 PFGE-tested isolates) clustered as two clones of sequence type ST-3779 and ST-3780, both belonging to the ST-103 clonal complex. ST-3779 has been detected in Salvador since 1996 and together with ST-3780 became predominant after 2005. There was a predominance of C:23:P1.14–6, ST-3779/3780 in Salvador during the period of 2007–2012, establishing a major clonal lineage, which remained in the community for a long time; this has serious implications for public health, particularly in terms of prevention and control strategies of IMD.

Keywords: Meningococcal disease; *Neisseria meningitidis*; Meningococcal serogroup C

1. Introduction

Invasive meningococcal disease (IMD) is an infection caused by *Neisseria meningitidis*. After dramatic reductions in the incidence of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b infections through the use of conjugate vaccines, IMD is considered a leading cause of bacterial meningitis [1–4]. The epidemiology of the disease varies widely around the world [5]. These variations are due to several factors, including the pathogenic characteristics of the prevalent strains of *N. meningitidis* [3,5].

In Brazil, *N. meningitidis* serogroup B (NmB) was associated with most cases during the 1980s and 1990s, with a peak in 1996. However, since 2001, the number and proportion of cases due to serogroup C (NmC) have been increasing markedly; this was followed by a reduction in the number of cases due to serogroup B [6,7]. In 2008, a NmC IMD epidemic started, which peaked in 2010, in the city of Salvador (estimated population 2,676,606, 21% of the state population), Bahia's state capital and the fourth most populous city of Brazil. To

* Corresponding author. Faculdade de Farmácia, Universidade Federal da Bahia, Rua Barão de Jeremoabo s/n, Ondina, 40170-115 Salvador, Bahia, Brazil.
E-mail address: joice@ufba.br (J.N. Reis).

https://doi.org/10.1016/j.micinf.2017.09.010
1286-4579/

combat the epidemic, in February 2010, the state government introduced meningococcal serogroup C conjugate (MCC) vaccine for children <5 years and also included mass vaccination for individuals 10–24 years of age, before the national introduction of the MCC vaccination in Brazil's National Immunization Program, initiated in August 2010 [8]. In total, >611,673 doses of MenC vaccine were administered during the campaigns, with an estimated coverage of 92% among the target population of children aged <5 years, 80% among 10–14 year olds, 67% among 15–19 year olds, and 41% among adults aged 20–24 years; the MenC vaccines administered during the meningococcal epidemic were highly effective [9].

However, few studies have been conducted in Brazil to assess the prevalence of *N. meningitidis* isolates and sequence types (STs) during epidemics, and during the pre- and post-vaccine introduction periods. To describe the epidemiological characteristics of IMD, and to assess the molecular epidemiology of the bacterium, we conducted an analysis of IMD-associated serogroups, serotypes, serosubtypes, and STs before and after MCC vaccine introduction in Salvador.

2. Materials and methods

2.1. Surveillance

From 1 January 2001 to 31 December 2012, active hospital-based surveillance of IMD was performed in Couto Maia Hospital, the state reference hospital for infectious diseases in Salvador [10]. Notification of meningitis cases to state health officials is mandatory, and during the study period, Couto Maia Hospital reports represented 86%–90% of such cases among the residents of Salvador. Cases were defined by the isolation of *N. meningitidis* from cerebrospinal fluid specimens and/or by a positive latex agglutination test (BD, Broken Bow, NE, USA) result from a patient with clinical signs and symptoms of meningitis. A study team of physicians and medical students reviewed laboratory records five days a week to identify newly cultured isolates. Demographic and clinical data from patients were collected during interviews and/or from medical chart reviews.

2.2. Laboratory methods

N. meningitidis isolates from patients with IMD were sent to the Molecular Biology Research Laboratory at the Gonçalo Moniz Research Centre at the Oswaldo Cruz Foundation in Salvador for characterisation using serogroup-specific antisera (Difco Laboratories, Detroit, MI, USA), as described previously [7,11].

Serotyping and serosubtyping of *N. meningitidis* isolates were performed at the Medical Biology Division, Bacteriology Department at Adolfo Lutz Institute, Brazil, by dot blot analyses using whole-cell suspensions, as described previously [7].

2.2.1. Pulsed-field gel electrophoresis (PFGE)

Random NmC isolates from each year were examined by PFGE after digestion of bacterial DNA with *NheI* (New England Biolabs, UK), as described previously [12]. The *NheI* fingerprints were analysed using GelCompar II software (Applied Maths, Belgium). Clustering was based on the unweighted pair-group method with arithmetic averages (UPGMA). The Dice correlation coefficient was used to analyse the similarities of the banding patterns with a tolerance of 1.0%. The interpretation of chromosomal DNA restriction patterns was based on the criteria of Tenover et al. for closely related isolates [35]. Briefly, strains showing more than three DNA fragment variations and a similarity of <80% by dendrogram analysis were considered to represent different PFGE types, while one to three-fragment differences and a similarity of >80% upon dendrogram analysis were considered to represent PFGE pattern subtypes.

2.2.2. Multilocus sequence typing (MLST)

Based on the results of the PFGE clustering analysis, a random sample of all isolates showing high relatedness (≥80%) was selected for MLST analysis, according to the methods of Maiden et al. [13]. Primers, the determination of sequence alleles, and the designation of STs are described in the Multi Locus Sequence Typing website (http://pubmlst.org/neisseria).

2.3. Statistical analysis

Patients residing in the city of Salvador, who had laboratory-confirmed IMD were included in this study. Cases were double-entered and validated in Epi-Info v.3.5.1 (CDC/USA). The clinical characteristics of cases were described by absolute and relative frequencies or by means and standard deviations. Statistical significance for the comparison of proportions and means was assessed by performing a χ^2 test or a *t*-test. Differences were considered statistically significant when the two-tailed *P*-value was <0.05. Statistical analyses were performed using Epi Info v.3.5.1 (CDC/USA) and SPSS v.18.0 (IBM Corp., Armonk, NY, USA).

2.4. Ethics statement

During the surveillance, informed consent procedures were applied prospectively to all patients and/or guardians of patients included in this study, which was approved by the National Committee for Research Ethics (CONEP) and the FIOCRUZ Institutional Review Board, Brazilian Ministry of Health. All patients or legal guardians gave written informed consent prior to enrolment of patients in the study, except in situations where the participant was unable to give written informed consent due to illness. In such cases, written informed consent was obtained from the subject's legally authorized representative.

3. Results

During the 12-year study, 733 patients with IMD were admitted to the Bahia's public infectious diseases reference hospital (Couto Maia Hospital). Among these, 461 (62.9%)

were diagnosed by culture. The serogroup was identified in 391/461 (84.8%) of cases that had bacteria available for testing; 185 (47.3%) were serogroup B, 193 (49.4%) were serogroup C, 8 (2%) were serogroup W, and 5 (1.3%) were serogroup Y. There was a male predominance among all cases with a confirmed serogroup, but there was no significant difference in the gender between patients infected with NmB and those infected with NmC (55% vs. 58%, $p = 0.5$; Table 1). Patients infected with NmB were typically younger than those infected with NmC (median: 7 years vs. 14 years, respectively; $p < 0.01$), especially among those under 5 years of age. We also observed a significant difference among patients older than 25 years in that NmC cases were more frequent than NmB cases (26%; $p < 0.01$, Table 1). Case fatality rate was slightly higher in patients infected with NmC than in patients infected with NmB (13% vs. 8%, respectively; $p = 0.09$; Table 1). There was no significant difference between patients infected with NmB and those infected with NmC in terms of intensive care unit admissions (15% vs. 21%, respectively; $p = 0.17$; Table 1). There was no difference in terms of the length of hospital stay, with patients infected with NmC remaining an average of only 1 day longer than patients infected with NmB (Table 1). Based on the results of cerebrospinal fluid laboratory analyses, a small difference was observed; patients infected with NmC had lower glucose (27 ± 17.9, $p < 0.01$) and higher protein levels (369 ± 295, $p = 0.03$), compared with patients infected with NmB (Table 1). From 2001 to 2006, NmB was the prevalent serogroup in the population. After this period, the proportion of cases caused by NmC increased markedly until 2012; this was accompanied by a reduction in cases due to NmB (Fig. 1).

Table 1
Characteristics of subjects included in the study, according to serogroups B and C, Salvador, Brazil, 2001–2012.

Characteristics	N (%) or mean ± SD		*p* Value[a]
	B (N = 185)	C (N = 193)	
Male sex	101 (55)	112 (58)	0.5
Age groups[b]			
<5 years	74 (41)	34 (18)	<0.01
5–9 years	32 (18)	31 (16)	0.73
10–14 years	24 (13)	32 (17)	0.34
15–24 years	34 (19)	45 (24)	0.25
>25 years	18 (10)	49 (26)	<0.01
Clinical evolution			
ICU[c] admission	18 (15)	35 (21)	0.17
Days at ICU	5 ± 7.5	7 ± 8.1	0.20
Outcome			
Days at hospital[d]	12 ± 6.3	13 ± 8.8	0.66
Deaths	14 (8)	25 (13)	0.09
Laboratory analysis (CSF)[e]			
WBC[f] (cells/mm^3)	6901 ± 3854	7029 ± 4000	0.67
Glucose (mg/dl)	33 ± 28.4	27 ± 17.9	<0.01
Protein (mg/dl)	308 ± 162	369 ± 295	0.03

Total of subjects according to serogroups: N = 391. Others serogroups: N = 13; W (N = 8); Y (N = 5).
[a] x^2 and t-test.
[b] Data about age were not available in five patients.
[c] ICU: intensive care unit.
[d] Days at hospital: Mean calculated for 176 cases serogroup B and 185 serogroup C, respectively.
[e] CSF: cerebrospinal fluid.
[f] WBC: White Blood Cells.

Of the 391 IMD cases with a defined serogroup, the serotype was determined for 371 (95%) isolates, and serosubtypes were identified for 254 (65%) isolates. The most prevalent strain (serogroup:serotype:serosubtype) from 2001 to 2006 was B:4,7:P1.19,15 (N = 120); however, the incidence of this strain decreased in 2007, despite its continued presence in the study population until 2009 (Fig. 1). From 2005, an increase in the frequency of the strain C:23:P1.14–6 occurred, and this became prevalent after 2007 (N = 91).

A total of 108 NmC isolates were analysed by PFGE, and 36 were assessed by MLST. This analysis identified eight PFGE patterns: three having one isolate each, and five with the number of isolates ranging between 4 and 77 (Supplementary Table 1). PFGE pattern A, with eight subpatterns (A1 to A8) was the largest, harbouring 71.3% of the isolates (77/108), and this group was related to the serotype:serosubtype 23:P1.14-6 (N = 60), 23:nt (N = 7), and NT:nt (N = 10), and to ST-3779 and ST-3780. ST-3779 has been identified since 1996 and together with ST-3780 became predominant after 2005. ST-3779, and the closely-related (single-locus variant) ST-3780, belonged to the ST-103 clonal complex (Fig. 2). The second PFGE group (B) consisted of 11 isolates of serotype:serosubtype 4,7:P1.19,15, belonging to ST-32 clonal complex. PFGE patterns C (nine isolates) and D (four isolates), with phenotypes 2a:P1.2 and 2a:P1.5, respectively, shared the same ST-11 (ST-11 clonal complex). PFGE pattern E (four isolates) shared the phenotypes 2b:P1.3 and 2b:P1.5 with ST-8.

4. Discussion

NmB accounts for a substantial proportion of cases in the U.S.A. and other parts of the world [14–16]. In this study conducted from 2001 to 2012, NmB was found to prevail from 2001 to 2006, when the most affected age group was younger than the age group comprising cases due to NmC. However, in Brazil, an increase in the number and proportion of IMD cases caused by NmC has been observed since 2002 in different regions [17,27]. In Salvador, NmC was responsible for the major proportion of IMD cases in 2007, changing the pattern that was observed in previous years, when NmB was the predominant serogroup [7,8]. In contrast to countries in Europe and North America, an important feature observed in Brazil is the absence of peak incidence of IMD cases in adolescents during endemic periods [17,18]. The high incidence of NmC in teenagers recorded in Salvador in 2010 could be attributed to the occurrence of epidemics. During epidemics and in outbreak situations, IMD incidence tends to increase mainly among young children, older children, and young adults [19,20].

In this active, hospital-based surveillance of IMD, we observed a male predominance among cases when the meningococcal serogroup was identified, and no significant

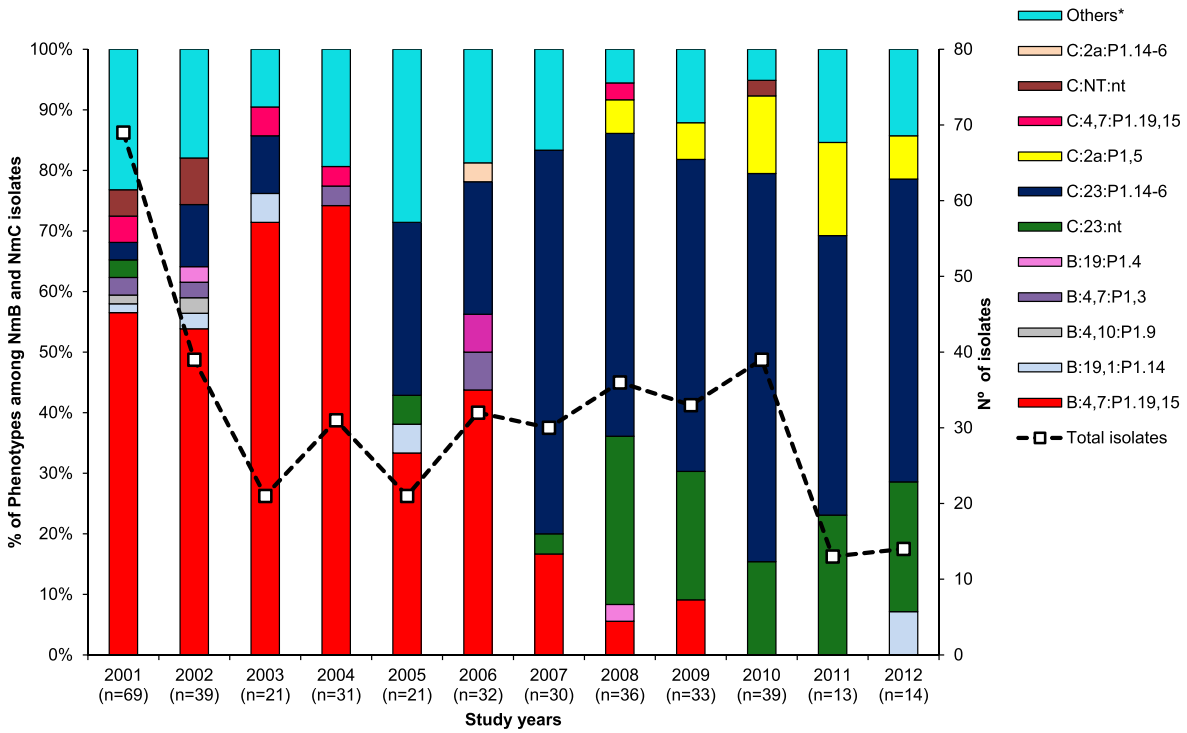
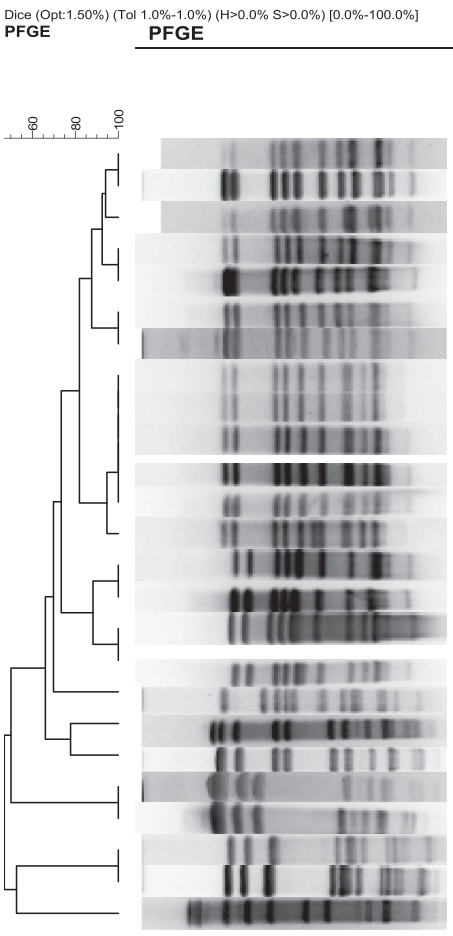


Fig. 1. Frequency of prevalent phenotypes of *Neisseria meningitidis* in Salvador, Brazil, 2001–2012 (N = 391).

gender differences in patients infected by NmB and NmC. For unknown reasons, the risk of IMD is higher among males until approximately the age of 45, at which time the risk becomes higher among women [32,33]. In addition, we found that patients infected by NmC tended to exhibit higher lethality than patients infected by NmB. Added to other risk factors, these results could be attributed to the fact that older individuals were predominant among patients infected with NmC. Older individuals are at a higher risk for complications arising from IMD [21,22]. We also observed differences in terms of cerebrospinal fluid glucose and protein levels in patients infected with NmB and those infected with NmC. Despite these results, we do not know which host or bacterial factors are implicated in the severity of meningococcal disease [15,23,24]. Alterations in the blood cerebrospinal fluid barrier and in the metabolism of glucose by neural cells have been implicated in the genesis of hypoglycorrhachia [25,26]. Diversity in bacterial cell surface area and surface hydrophobicity within *N. meningitidis* species could influence meningococcal pathogenesis [27].

PFGE analysis showed that the largest group, containing 71.3% of the PFGE-tested isolates, was related to serotype: serosubtype 23:P1.14-6 (N = 60), 23:nt (N = 7), and NT:nt (N = 10), which was further related to ST-3779 and ST-3780. ST-3779 and ST-3780 belong to the ST-103 clonal complex. A marked increase has been observed in the proportion of cases attributed to NmC isolates associated with the ST-103 clonal complex; this clonal complex has been responsible for most disease cases in Brazil since 2002 [28]. In Salvador, since 2007, we observed a predominance of the C:23:P1.14–6 phenotype strain, which contributed to the IMD epidemic in this city in 2010. This epidemic motivated the introduction of the MCC vaccine in Salvador before its introduction in Brazil's National Immunization Program [8]. The predominance of the C:23:P1.14–6 phenotype strain, identified in the current study, is in accordance with the findings of a cross-sectional study during an outbreak of NmC IMD that occurred in 2010 in São Paulo, Brazil. During that outbreak, the C:23:P1.14–6 phenotype strain was predominant, and the most prevalent clonal complex (cc) was cc103, represented by ST-3780 [27]. This reinforces the concept that the predominance of a particular strain is an important marker of epidemic conditions [3,29,30], and that epidemics tend to be caused by the clonal expansion of a single hypervirulent clone.

This study was subject to a number of limitations. First, we could not include septicaemia cases without meningitis, as the main prerogative of the hospital was the management of meningitis cases. Second, cases of NmC IMD might have been missed if they were not referred to the Hospital Couto Maia, and third, isolates were not obtained for all IMD cases for serogrouping. However, considering the fact that in most middle income countries where basic statistics on IMD are available, data regarding the molecular characteristics of dominant strains, such as MLST data [31,32], are lacking, this study enhances our knowledge of the molecular epidemiology of IMD in Brazil. This will help us to better understand IMD during large citywide epidemics. In conclusion, the spread of NmC in Salvador between 2007 and 2010 was caused by the



Sample ID	Phenotype	Year of Isolation	MLST	PFGE profile
GNC742	C:23:P1.14-6	2007	ST3779/CC103	A2
GNC21	C:NT:nt	1996	ST3779/CC103	A2
GNC741	C:23:P1.14-6	2007	ST3779/CC103	A1
GNC968	C:23:nt	2010	ST3780/CC103	A2
GNC920	C:23:nt	2010	ST3780/CC103	A2
GNC924	C:23:P1.14-6	2010	ST3780/CC103	A5
GNC1046	C:23:P1.14-6	2011	ST3780/CC103	A5
GNC932	C:23:nt	2010	ST3780/CC103	A5
GNC934	C:23:P1.14-6	2010	ST3780/CC103	A5
GNC950	C:23:P1.14-6	2010	ST3780/CC103	A5
GNC953	C:23:P1.14-6	2010	ST3780/CC103	A5
GNC919	C:23:P1.14-6	2010	ST3780/CC103	A5
GNC935	C:23:P1.14-6	2010	ST3780/CC103	A7
GNC952	C:23:P1.14-6	2010	ST3780/CC103	A5
GNC923	C:23:P1.14-6	2010	ST3780/CC103	A5
GNC903	C:23:P1.14-6	2009	ST3780/CC103	A5
GNC918	C:23:P1.14-6	2010	ST3780/CC103	A5
GNC120	C:NT:nt	1997	ST3779/CC103	A8
GNC04	C:2a:P1.2	1996	ST11/CC11	C2
GNC02	C:2a:P1.5	1996	ST11/CC11	C1
GNC1032	C:2a:P1.5	2011	ST11/CC11	D2
GNC960	C:2a:P1.5	2010	ST11/CC11	D1
GNC99	C:4,7:P1.19,15	1999	ST33/CC32	B1
GNC225	C:4,7:P1.19,15	1998	ST33/CC32	B1
GNC35	C:2b:P1.3	1996	ST8/CC8	E1

* This analysis contains at least one of the main PFGE clonal group encountered among all samples tested. Scale bar represent the percent (%) of similarity found among PFGE profiles.
Legend: Dice - similarity coefficient; Tol - Position tolerancy; H - minimum height; S - Minimum surface.

Fig. 2. Pulsed-field gel electrophoresis analysis of the main clonal complex found among *Neisseria meningitidis* serogroup C isolates in Salvador, Brazil*.

serotype:serosubtype 23:P1.14−6, ST-3779/3780, which had circulated in the community since 1996.

Conflict of interest

The authors declare that they have no conflicting interests.

Acknowledgements

We thank the clinical, laboratory, and administrative staff of Couto Maia Hospital, especially Neide Oliveira Silva and Ana Paula S. Lemos from the São Paulo Secretary of Health, for providing information regarding serotypes and serosubtypes. This publication made use of the Neisseria Multi Locus Sequence Typing website (http://pubmlst.org/neisseria/) developed by Keith Jolley at the University of Oxford (Jolley & Maiden 2010, *BMC Bioinformatics*, **11**:595) [34]. The development of this site has been funded by the Wellcome Trust and European Union. This work was supported by the Secretaria de Vigilância em Saúde/Ministério da Saúde, TC 283/2013 and Fundação Oswaldo Cruz (PAPES 407551/2012-3).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.micinf.2017.09.010.

References

[1] Rosenstein NE, Perkins BA. Update on *Haemophilus influenzae* serotype b and meningococcal vaccines. Pediatr Clin N Amer 2000;47:337−52.
[2] Ribeiro GS, Reis JN, Cordeiro SM, Lima JB, Gouveia EL, Petersen M, et al. Prevention of *Haemophilus influenzae* type B (HIB) meningitis and emergence of serotype replacement with type A strains after introduction of HIB immunization in Brazil. J Infect Dis 2003;187:109−16.
[3] Harrison LH. Prospects for vaccine prevention of meningococcal infection. Clin Microbiol Rev 2006;19:142−64.
[4] Black SB, Plotkin SA. Meningococcal disease from the public health policy perspective. Vaccine 2012;30S:B37−9.
[5] Tzeng YL, Stephens DS. Epidemiology and pathogenesis of Neisseria meningitidis. Microbes Infect 2000;2:687−700.
[6] Sáfadi MAP, Barros AP. Meningococcal conjugate vaccines: efficacy and new combinations. J Pediatr 2006;82:S35−44.
[7] Cordeiro SM, Neves AB, Ribeiro CT, Petersen ML, Gouveia EL, Ribeiro GS, et al. Hospital-based surveillance of meningococcal meningitis in Salvador, Brazil. Trans R Soc Trop Med Hyg 2007;101:1147−53.

[8] Cardoso CW, Pinto LL, Reis MG, Flannery B, Reis JN. Impact of vaccination during an epidemic of serogroup C meningococcal disease in Salvador, Brazil. Vaccine 2012;30:5541−6.
[9] Cardoso CW, Ribeiro GS, Reis MG, Flannery B, Reis JN. Effectiveness of meningococcal C conjugate vaccine in Salvador, Brazil: a case-control study. PLoS One 2015;10(4), e0123734.
[10] Brasil. Secretaria de Saúde do Estado da Bahia. Hospital Couto Maia. [online] Available at: www.saude.ba.gov.br. [Accessed 27 July 2014].
[11] Wedege E, Hoiby EA, Rosenqvist E, Froholm LO. Serotyping and subtyping of Neisseria meningitidis isolates by co-agglutination, dot-blotting and ELISA. J Med Microbiol 1990;31:195−201.
[12] Popovic T, Schmink S, Rosenstein NA, Ajello GW. Evaluation of pulsed-field gel electrophoresis in epidemiological investigations of meningococcal disease outbreaks caused by Neisseria meningitidis serogroup C. J Clin Microbiol 2001;39:75−85.
[13] Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA 1998;95:3140−5.
[14] Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and Neisseria meningitidis. Lancet 2007;369:2196−210.
[15] Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. Vaccine 2009;27:B51−63.
[16] Cohn AC, MacNeill JR, Harrison LH, Hatcher C, Theodore J, Schmidt M, et al. Changes in Neisseria meningitidis disease epidemiology in the United States, 1998−2007: implications for prevention of meningococcal disease. Clin Infect Dis 2010;50:184−91.
[17] Brasil. Ministério da Saúde. Secretaria de Vigilância a Saúde. Departamento de Vigilância Epidemiológica. Sistema de Informação de Agravos de Notificação Sinan Net. [online] Available at: http://dtr2004.saude.gov.br/sinanweb/. [Accessed 27 July 2014].
[18] v1 Brasil. Ministério da Saúde. Secretaria de Vigilância a Saúde Saúde Brasil 2012:uma análise da situação de saúde e dos 40 anos do programa de imunizações. 1st ed. 2013. Brasília:536.
[19] Peltola H, Kataja JM, Makela PH. Shift in the age-distribution of meningococcal disease as predictor of an epidemic? Lancet 1982;2:595−7.
[20] Bilukha OO, Rosenstein NE. National center for infectious diseases, centers for disease control and prevention (CDC). Prevention and control of meningococcal disease. Recommendations of the advisory committee on immunization practices (ACIP). MMWR Recomm Rep 2005;54:1−21.
[21] Center for Disease Control and Prevention. Age as a risk factor [online] Available at: http://www.cdc.gov/meningococcal/about/risk-age.html. [Accessed 28 July 2014].
[22] Olivares R, Bouyer J, Hubert B. Risk factors for death in meningococcal disease. Pathol Biol (Paris) 1993;41(2):164−8.
[23] Feavers IM. Bacterial genomics: ABC of meningococcal diversity. Nature 2000;404:451−2.
[24] Manchanda V, Gupta S, Bhalla P. Meningococcal disease: history, epidemiology, pathogenesis, clinical manifestations, diagnosis, antimicrobial susceptibility and prevention. Indian J Med Microbiol 2006;24:7−19.
[25] Hatipoglu M, Ulcay A, Gokce DE, Turhan V. Cerebrospinal fluid/blood glucose should be used as a good diagnostic tool and mortality indicator in bacterial meningitis. Am J Emerg Med 2014;32:470.
[26] Tamune H, Takeya H, Suzuki W, Tagashira Y, Kuki T, Honda H, et al. Cerebrospinal fluid/blood glucose ratio as an indicator for bacterial meningitis. Am J Emerg Med 2014;32:263−6.
[27] Bartley SN, Tzeng YL, Heel K, Lee CW, Mowlaboccus S, Seemann T, et al. Attachment and invasion of Neisseria meningitidis to host cells Is related to surface hydrophobicity, bacterial cell size and capsule. PLoS One 2013;8, e55798.
[28] Sáfadi MAP, Carvalhanas TR, Lemos AP, Gorla MC, Salgado M, Fukasawa LO, et al. Carriage rate and effects of vaccination after outbreaks of serogroup C meningococcal disease, Brazil, 2010. Emerg Infect Dis 2014;20:806−11.
[29] Raghunathan PL, Jones JD, Tiendrebéogo SR, Sanou I, Sangare L, Kouanda S, et al. Predictors of immunity after a major serogroup W-135 meningococcal disease epidemic, Burkina Faso. J Infect Dis 2002;193:607−16.
[30] Trotter CI, Greenwood BM. Meningococcal carriage in the African meningitis belt. Lancet Infect Dis 2007;7:797−803.
[31] Greenwood B, Chiarot E, MacLennan CA, O'Ryan M. Can we defeat meningococcal disease in low and middle income countries? Vaccine 2012;30S:B63−6.
[32] Kaddar M, Schmitt S, Makinen M, Milstien J. Global support for new vaccine implementation in middle-income countries. Vaccine 2013;31S:B81−96.
[33] Rosenstein NE, Perkins BA, Stephens DS, Lefkowitz L, Cartter ML, Danila R, et al. The changing epidemiology of meningococcal disease in the United States, 1992−1996. J Infect Dis 1999;180:1894−901.
[34] Jolley Keith A, Maidem Martin CJ. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinforma 2012;11:595−605.