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Institut Pasteur

Microbes and Infection xx (2017) 1–6

www.elsevier.com/locate/micinf

Original article

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

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

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

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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%)

Please cite this article in press as: Cordeiro SM, et al., Dissemination of the ST-103 clonal complex serogroup C meningococci in Salvador, Brazil, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.09.010