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Epidemiology and molecular characterization of *Neisseria lactamica* carried in 11–19 years old students in Salvador, Brazil

Ana Rafaela Silva Simões Moura[a], Cécilia Batmalle Kretz[b], Ítalo Eustáquio Ferreira[a], Amélia Maria Pithon Borges Nunes[a], Ivano de Filippis[c], José Cássio de Moraes[d], Mitermayer Galvão Reis[a], Alan John Alexander McBride[a,e], Xin Wang[b], Leila Carvalho Campos[a,*]

[a] *Laboratório de Patologia e Biologia Molecular, Instituto Gonçalo Moniz, FIOCRUZ-BAHIA, Rua Waldemar Falcão 121, 40296-710, Salvador BA, Brazil*
[b] *Meningitis and Vaccine Preventable Diseases Branch, Division of Bacterial Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta GA 30333, USA*
[c] *Instituto Nacional de Controle de Qualidade em Saúde – INCQS, FIOCRUZ, 21040-900, Rio de Janeiro RJ, Brazil*
[d] *Faculdade de Ciências Médicas da Santa Casa de São Paulo, 01220200, São Paulo SP, Brazil*
[e] *Núcleo de Biotecnologia, Centro de Desenvolvimento Tecnológico, Universidade Federal de Pelotas, Campus Universitário s/n, 96160-000, Pelotas RS, Brazil*

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ABSTRACT

Neisseria lactamica is a nonpathogenic commensal bacterium that is potentially associated with the development of natural immunity against *N. meningitidis*. However, the genetic variation present in natural populations of *N. lactamica* has not been fully investigated. To better understand its epidemiology and genetic variation, we studied *N. lactamica* carriage in 1200 students aged 11–19 years old in Salvador, Brazil. The carriage prevalence was 4.5% (54/1200), with no statistical difference among sex and age, although we observed a trend towards higher carriage prevalence among 11-year-old individuals. Whole genome sequence analysis revealed a high genetic diversity among the isolates, with the presence of 32 different STs, 28 (87.5%) of which were new. A total of 21/50 (42%) isolates belonged to three different clonal complexes. While none of the isolates contained *nadA* or *fHpb* alleles, we detected 21 FetA variants, 20 NhbA variants and two variants of PorB. The data provide detailed information on circulating *N. lactamica* isolates in adolescents in Brazil and are complementary to studies in other countries.

1. Introduction

Neisseria lactamica is a lactose fermenting diplococcus closely related to *N. meningitidis*, which lives in a commensal relationship with humans. This bacterium is frequently isolated from the nasopharynx of children, and is rarely associated with invasive disease as it lacks several virulence factors usually found in *Neisseria meningitidis* (Changal et al., 2016; Everts et al., 2010).

N. lactamica is of interest as it has been implicated in the age-related development of natural immunity against *N. meningitidis* (Gold et al., 1978). Although poorly understood, the prevalence of *N. lactamica* carriage in young children (< 5 years of age) is significantly higher compared to *N. meningitidis*. Furthermore, these children developed significant IgG responses that were cross-reactive with serogroup A, B, and C meningococci soon after colonization with *N. lactamica* (Gold et al., 1978).

N. lactamica does not express the meningococcus protective capsule (Kim et al., 1989) and the outer-membrane protein PorA (Ward et al., 1992). However, there is similar relatedness among some outer membrane proteins, including porin B (PorB) (Bennett et al., 2008), iron-regulated enterobactin (FetA) (Bennett et al., 2009) and neisserial heparin-binding antigen (NhbA), although the variants are mainly not overlapping in the two species (Lucidarme et al., 2013).

The evidence of cross-reactivity responses against common antigens (Cann and Rogers, 1989; Troncoso et al., 2002) encouraged the development of anti-meningococcal vaccines based on *N. lactamica* (Griffiss et al., 1991; Finney et al., 2008; Gorringe et al., 2009).

Some of the subcapsular antigens common to *N. lactamica* and *N. meningitidis* are included in the multiple component serogroup B meningococcal vaccine, 4CMenB (Serruto et al., 2012). The use of vaccines containing surface proteins shared with *N. lactamica* could interfere in the colonization of the nasopharynx by *N. lactamica*, potentially hampering the acquisition of natural immunity (Lucidarme et al., 2013; Troncoso et al., 2002). The impact of meningococcal vaccines on

* Corresponding author.
E-mail address: lccampos@bahia.fiocruz.br (L.C. Campos).

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neisserial species with similar surface proteins warrants further investigation (Toneatto et al., 2017).

Although there is a degree of relatedness between some of the surface antigens, the commensal *N. lactamica* has not been prioritized to the same degree as *N. meningitidis*, especially with regard to studies on epidemiology and genetic variation (Alber et al., 2001; Bennett et al., 2005; Kristiansen et al., 2012; Lucidarme et al., 2013). Furthermore, there is no information regarding the circulation and genetic diversity of *N. lactamica* isolates in Brazil.

Previously, we conducted a cross-sectional study to assess the meningococcal carriage status of 11–19-year-old student's resident in Salvador (Nunes et al., 2016; Moura et al., 2017). Although the laboratory methodology was primarily designed for meningococcus isolation, all lactose-fermenting Gram-negative diplococci were registered and stored for further investigation. In the present study, we describe the epidemiology and the genetic profiles of *N. lactamica* isolates recovered from 11 to 19-year-old carriers in Salvador, Brazil.

2. Material and methods

2.1. Ethical considerations

This study was approved by the Ethics Committee of the Instituto Gonçalo Moniz, FIOCRUZ-BA (CAAE #16099713.1.0000.0040). Written informed consent from all study participants (or guardians) was obtained before sample and data collection.

2.2. Isolation and identification of N. lactamica

N. lactamica isolates ($n = 54$) were recovered from the oropharyngeal swabs collected from 1200 students, aged 11–19 years old, attending a total of 134 different public schools in Salvador, Brazil, during September to December 2014 (Nunes et al., 2016). The swabs were immediately used to inoculate selective agar medium (modified Thayer-Martin agar containing vancomycin, colistin, nystatin, and trimethoprim) and transferred to a polystyrene tube containing 1 mL of skim milk-tryptone-glucose-glycerin (STGG) transport medium (O'Brien et al., 2001). After 24–48 h of incubation, the plates were inspected and colonies with the characteristic morphology of *Neisseria* spp. were subcultured on blood agar medium for species identification by Gram staining, oxidase reaction, and carbohydrate utilization tests. The results were confirmed using API-NH1 strips (bioMérieux, Hazelwood, MO, USA), as described previously (Nunes et al., 2016). *N. lactamica* isolates were stored in brain heart infusion (BHI) broth containing 20% (v/v) glycerol at −80 °C.

2.3. Molecular characterization

Of the 54 *N. lactamica* isolates, 50 were characterized by whole genome sequencing (WGS). Genomic DNA was extracted as previously described (Kretz et al., 2016), and sequenced using MiSeq v2 chemistry (Illumina, San Diego, CA, USA). Genome assembly was carried out using CLC Genomics Workbench, ver. 9.0.0 (CLC bio, Aarhus, Denmark) with read trimming and mapping of reads back to contigs. The multilocus sequence typing (MLST) alleles, sequence types (STs) and clonal complexes (cc) were identified by comparison of the assembled genomes with *Neisseria* PubMLST database (http://pubmlst.org/neisseria/), using a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The presence and diversity of *porB*, *fetA* and *nhba* were investigated using sequence analysis as previously described (Kretz et al., 2016). Partial protein sequences were used for PorB, FetA typing, and full-length protein sequences were used for NHBA typing.

2.4. Phylogenetic analysis

Single nucleotide polymorphisms (SNPs) were identified using kSNP version 3 software (Gardner and Hall, 2013) with a kmer length of 25. A maximum likelihood phylogenetic tree was constructed from the core SNPs and the Tamura-Nei model, using MEGA7 (Tamura et al., 2013) and 500 bootstraps interactions.

2.5. Data analysis

Statistical analysis was done using STATA statistical software version 12 (College Station, TX, USA). The prevalence of *N. lactamica* carriage was calculated for the total sample and for subgroups (sex and age). Univariate analysis to identify exposure associated with *N. lactamica* carriage was performed; the chi-square test was used to determine statistical significance.

3. Results

3.1. N. lactamica carriage

Among the 1200 students screened, *N. lactamica* was isolated from 54 (4.5%) individuals (Nunes et al., 2016). There was no significant difference in carriage prevalence based on gender: 31 (57.4%) females and 23 (42.6%) males. Although the *N. lactamica* carriage rate was slightly higher among 11-year-old students (9.7%), it was not statistically significant (Fig. 1). The prevalence of *N. lactamica* carriage across the various age groups was similar to that of *N. meningitidis* in the same population (Fig. 1). Only one participant was co-colonized by both *N. meningitidis* and *N. lactamica*.

3.2. Molecular characterization

A total of 50 *N. lactamica* isolates identified by conventional methods were characterized by WGS. Thirty-two different STs were identified, 28 (87.5%) of which were new. The majority of the isolates (29/50, 58%) lacked association within any known cc in the PubMLST database. A total of 21 (42%) isolates belonged to three different cc: cc613 (13/50; 26%); cc1494 (5/50; 10%); cc624 (3/50; 6%). We were unable to determine the ST of one isolate (M37159) due to a deletion of the *pdhC* housekeeping gene (Table 1). The phylogenetic analysis showed a high level of genetic variability with many different ST identified; and isolates belonging to the same ST and/or cc type to cluster together (Fig. 2). A total of 11329 core SNPs were identified with a difference of 80–5615 SNPs between all isolates analyzed.

Among the outer membrane proteins, two PorB variants were identified: 3–599 (10/50; 20%) and 3–596 (40/50; 80%), the latter being novel and most prevalent among the isolates (Table 1). All but nine of the isolates contained the FetA VR (variable region), with 21 FetA VRs in total. The most prevalent was F1-29 (12/41; 29.3%), and we identified four new variants: F1-143 (2/41; 4.9%), F1-204 (1/41; 2.4%), F4-68 (1/41; 2.4%), and F5-120 (1/41; 2.4%) (Table 1).

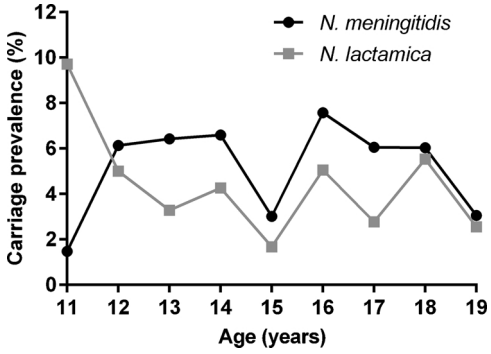

Fig. 1. Carriage prevalence for *N. lactamica* and *N. meningitidis* among adolescents resident in Salvador, Brazil, 2014 by age group. Note, the data for *N. meningitidis* was offset by 0.5% on the y-axis for clarity.