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## ARTIGO

# The 2010 Meningococcal outbreak in Bahia, Brazil, was caused by 2 different STs belonging to Clonal Complex ST-103

## O surto de meningite meningocócica de 2010 na Bahia foi causado por dois ST diferentes pertencentes ao Complexo Clonal ST-103

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## ABSTRACT

An outbreak of meningococcal disease in the North-East (NE) region of Brazil with eleven cases and seven deaths was reported in 2009. From the eleven cases, five serogroup C meningococcal strains were isolated, four were classified as belonging to the hypervirulent clonal complex ST-103 (cc103) and one strain was not associated with any clonal complex. DNA sequencing of the genes encoding outer membrane proteins PorA and FetA showed genotype P1.5-1,10-1,36-2;F3-9 for all five strains. cc103 was first detected in the southern region of Brazil in 2007, but not associated with outbreaks. MLST analysis detected three new STs among the isolates, showing the ongoing evolution of cc103 and the need of monitoring its spread in the population.

**KEYWORDS:** *Neisseria meningitidis*; Meningococcal disease; Hypervirulent clone; Clonal complex; Meningococcal outbreak

## RESUMO

Um surto de doença meningocócica foi detectado em 2009 no estado da Bahia (NE) com onze casos confirmados e sete óbitos. A partir do material coletado dos onze casos foi possível isolar cinco cepas de meningococos do sorogrupo C, sendo quatro pertencentes ao complexo clonal hipervirulento ST-103 (cc103), enquanto que uma cepa não foi associada a nenhum complexo clonal. O sequenciamento dos genes codificantes das proteínas de membrana PorA e FetA resultou no genótipo P1.5-1,10-1,36-2;F3-9 para todas as cepas. O cc103 foi isolado pela primeira vez na região sul do Brasil em 2007, mas não de um surto. A análise por MLST detectou a presença de três novos STs entre os isolados, o que mostra a contínua evolução do clone cc103 e a necessidade do monitoramento de seu avanço na população susceptível.

**PALAVRAS-CHAVE:** *Neisseria meningitidis*; Doença Meningocócica; Clone Hipervirulento; Complexo Clonal; Surto de meningite meningocócica



## Introduction

*Neisseria meningitidis* exists in endemic-epidemic cycles in Brazil with an annual average of 3,500 cases, an incidence of 2.2 cases per 100,000 population, and cycles of localized outbreaks every 2-5 years, with smaller peaks. National case fatality ratios (CFR) vary from 15% to 20%. In 2009, 2,514 cases and 514 deaths were reported in Brazil through the national meningitis surveillance system. From 1990 to 2006, serogroup B was the most prevalent in Brazil. However, during the last four years, the number of cases caused by serogroup C has increased and surpassed the number of serogroup B cases in some Brazilian states<sup>1-5</sup>.

The second geographical region most affected by meningococcal disease (MD) in Brazil, after the Southeast, is the Northeast, where 479 cases were reported in 2009, with 99 deaths. One of the most affected states of this region is Bahia (BA) where an outbreak of MD was detected in December 2009. In this state, the number of deaths caused by MD increased 52% during the first months of 2010, compared with the same period of 2009, when 193 cases with 49 deaths were reported. During this period, more than 68% of MD cases were caused by serogroup C meningococci. According to the Ministry of Health (MoH) a confirmed case of MD is defined as a suspected case with positive culture or positive antigen detection, clinically compatible illness with or close contact with a laboratory-confirmed case.

Our study documents a localized outbreak in Porto Seguro, a city in Bahia, visited by thousands of young adults and teens during its hot summer season (December – March). In 2009, eleven cases of MD were confirmed, with nine cases in two weeks of October. A total of seven deaths were reported (CFR=63.6%) caused by serogroup C meningococci with an incidence of 9.8 cases per 100,000 inhabitants. It is noteworthy that the extremely high CFR reported here may also be caused in part by inaccurate medical procedures, particularly when managing acute disease such as MD – which requires medical staff to take precise and rapid actions.

All nine cases detected in October occurred among persons who attended a social event and were, therefore, epidemiologically linked. The incidence of MD among the most affected age groups was as follows: 15-19 and 20-29 years old with 24.9 and 15.7/100.00 respectively (see Table). The average number of cases in Porto Seguro from 2004 to 2008 was five, with one death reported. The high incidence of meningococcal disease among adolescents and young adults observed here is consistent with the age distribution during outbreaks, when incidence is increased in these age groups.

## Methods

Five meningococcal isolates were received at the Laboratory of Reference Microorganisms, INCQS/FIOCRUZ, from the Central Reference Laboratory of Bahia (LACEN-BA), isolated from five of the eleven patients with confirmed MD. Clinical samples from the other six cases failed to recover any bacterial strain and the case was classified as MD based on

clinical symptoms and epidemiological link to the other cases. After growth in GC chocolate agar (BD, Franklin Lakes, New Jersey, USA) at 37°C in a 5% CO<sub>2</sub> atmosphere for 24 hours, cells were picked from agar plates for DNA extraction and purification using the 'Dneasy Tissue Kit' (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Purified DNA samples were stored at 4°C prior to PCR. The isolates were confirmed as *Neisseria meningitidis* by *nspA*-PCR<sup>6</sup>, freeze-dried and deposited in the Meningococcal Culture Collection of the Laboratory of Reference Microorganisms at the INCQS-FIOCRUZ.

PCR amplification and nucleotide sequence determination of the meningococcal *porA* and *fetA* genes were performed as previously described<sup>7,8</sup>. Nucleotide sequences encoding the defined PorA variable epitopes, VR1 and VR2, identified by querying the PorA VR sequence database located at <http://neisseria.org/nm/typing/pora/>. PorA VR3 variants were determined by querying the *Neisseria meningitidis* PorA VR3 database located at <http://exon.niaid.nih.gov/meningitidis/index.html><sup>9</sup>. The amino acid sequence variants determined for the FetA VR were also identified by database interrogation at <http://neisseria.org/nm/typing/feta/>. The amplification and sequencing protocol used for MLST analysis are described in the MLST web site at <http://pubmlst.org/neisseria/>.

The seven genes analyzed were *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate dehydrogenase), *fumC* (fumarate hydratase), *gdh* (G6P-dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit), and *pgm* (phosphoglucomutase). The amplicons were sequenced on both strands using an automatic sequencer, the ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). For MLST analysis, sequences of the seven housekeeping genes mentioned above were determined and properly trimmed to the right length for allele determination. Allele numbers for each locus were assigned by querying each sequence to the PubMLST database. Allelic profile queries were then carried out for ST and clonal complex determination. Strains for which the allelic profile query did not match to a known ST where submitted to the MLST database and a new ST number was assigned by the MLST database curator. A clonal complex is a group containing more than five isolates for which four or more alleles are shared with the alleles from the central founder strain of the complex<sup>10</sup>.

## Results and Discussion

Five strains were recovered from the eleven confirmed cases. These strains were analyzed by MLST and *porA/fetA* genotypes were determined by DNA sequencing. Three new sequence types were identified within the five strains: ST-8435 (n=1), ST-8436 (n=3) and ST-9464 (n=1). Sequence types ST-8435 and ST-8436 were classified within the ST-103 clonal complex, while one strain characterized as ST-9464 was not associated with any known clonal complex; all the isolates showed the same alleles of ST-103 at four loci, but the strain designated



Table: Characteristics of patients and meningococcal isolates of the Porto Seguro outbreak in Bahia, Brazil

Case no.	Sex	Age	Disease	Outcome	ST/CC	PorA	FetA
P3475	F	29y	M+M	DE	8435/ST-103	5-1,10-1,36-2	F3-9
P3476	M	18y	M	DI	8436/ST-103	5-1,10-1,36-2	F3-9
P3477	M	26y	M+M	DI	9464/---	5-1,10-1,36-2	F3-9
P3478	F	25y	M+M	DE	8436/ST-103	5-1,10-1,36-2	F3-9
P3479	M	39y	M+M	DE	8436/ST-103	5-1,10-1,36-2	F3-9
2342	M	6y	M	DI	U	U	U
3996	F	3y	M	DE	U	U	U
5051	M	23y	M+M	DE	U	U	U
5052	M	15y	M+M	DE	U	U	U
5322	F	33y	M+M	U	U	U	U
5203	M	17y	M+M	DE	U	U	U

M=meningococcemia; M+M= meningococcemia and meningitis; DE=death; DI=discharged; U=unknown

as ST-9464 had a different allele at the *pdhC* locus of the MLST scheme. All five strains shared the same *porA* and *fetA* genotypes and were designated as P1.5-1,10-1,36-2 and F3-9 respectively (see Table).

Since we could not recover any other strain from the remaining six cases, we can state that at least 36% of the isolates were cc103, but this number could be higher. An ongoing study, with isolates from other cities of the same state, shows that the most prevalent clonal complex among serogroup C strains in BA during the last ten years has been cc103, with 39% of the isolates, followed by cc11, with 15%.

Strains of the ST-103 clonal complex have been isolated in several European countries, USA, Canada and Cuba. In Brazil, strains belonging to cc103 were detected in the southern region<sup>3, 4,11</sup> — at first, associated with an epidemic in 2000<sup>2</sup>. The emergence of MD, associated with cc103, in the southern region in 2008 has contributed to the continued high incidence of the disease in that state as recently reported<sup>4</sup>. A recent study described the same outbreak in Bahia being caused by ST-3780 cc103<sup>12</sup>. This ST was first detected in 2003 and 2004, with three strains in Pernambuco — a state adjacent to Bahia, where this outbreak was reported.

Another strain belonging to ST-3780 was reported in 2005 in the South region of Brazil. These four isolates are described in the MLST database. Although isolates could only be obtained from five of the 11 cases, the identification of a single PorA, FetA and clonal complex from the cases with available data suggests a clonal outbreak. The hypervirulent nature of this clone is demonstrated by the high case fatality rates. Only one strain, out of four belonging to cc103, was not associated with a fatal outcome. The change of the epidemiology of MD observed in Bahia could be associated with the hypervirulent feature of clone ST-103, already documented elsewhere.

An important feature of the strains analyzed is the outer membrane protein (OMP) genotype pattern of *porA* and *fetA*. None of the cc103 strains previously reported in Brazil showed

the same OMP genotypes described here. These findings suggest the introduction of a new clone, with a possible change in its epidemiology with respect to the OMP pattern, reinforcing the importance of molecular characterization of MD cases, in order to monitor the spread of these lineages and to establish control measures for the disease.

In addition, the identification of a strain belonging to a different ST, which could not be associated with any known hypervirulent clonal complex, suggest a possible evolution event of strains associated with cc103. This may be seen as a possible expansion of ST-103, forming a new clone. Several nucleotide substitutions in the *pdhC* gene of isolate P3477 (ST-9464) changed its allele to 247, when compared to *pdhC* of ST-8435 and ST-8436, both classified as allele 18.

To halt the spread of the disease, regional health authorities in Bahia began a mass vaccination campaign against *N. meningitidis* C in February 2010, using a serogroup C polysaccharide conjugate vaccine. After the vaccination campaign in Bahia, the MoH decided to introduce the meningococcal C conjugate vaccine into the National Immunization Program, vaccinating all children aged two and younger.

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### Ethics Statement

This study was granted exemption by the Research Ethics Committee at the Central Laboratory of Bahia, which received the samples from different municipal hospitals throughout the state. Samples were anonymized for purposes of this analysis.



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