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Replacement of *Neisseria meningitidis* C cc11/ET-15 variant by a cc103 hypervirulent clone, Brazil 2005–2011

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ABSTRACT

Outbreaks caused by serogroup C meningococci in the northeast region of Brazil from 2005 to 2011 were associated to the emergence of variant ET-15 of cc11, which has been replaced by cc103 from 2006 to date. The increase of cc103 should be closely monitored to prevent the spread of this clone to neighbouring regions.

Neisseria meningitidis is a human upper respiratory tract commensal and is the etiological agent of meningococcal disease (MD), a life-threatening disease causing an estimated 500000 invasive infections worldwide each year (WHO, 2002). *N. meningitidis* is classified into 13 serogroups based on their capsular polysaccharide serologic properties; however, about 90% of invasive infections are caused by serogroups A, B, C, W135, X, and Y. Meningococci can also be genetically classified as sequence types (STs) and grouped into clonal complexes (cc) by multilocus sequence typing (MLST) (Maiden et al., 1998). *N. meningitidis* serogroup C of the ST-11/ET-37 clonal complex (cc11) is a widespread clone that has caused many outbreaks worldwide, including United States in the 1960s and Brazil and South Africa in the 1970s (Caugant, 2002). The variant ET-15 of cc11 first described in 1986 in Canada (Whalen et al., 1995) has been associated with outbreaks and high case fatality rates in different countries of Europe and United States (Garnier et al., 2011; Jackson et al., 1995; Kaczmarski, 1997; Kremastinou et al., 1999; Krizova and Musilek, 1995). In Brazil, isolates from patients with confirmed meningococcal disease are reported through the Brazilian national meningitis surveillance system. Clinical and demographic information on suspected patients with meningitis is routinely collected as part of this surveillance system (de Lemos et al., 2007). Meningococcal disease in Brazil is endemic with a current incidence of 2–3 cases per 100000 inhabitants, an annual average of 2860 cases from 2005 to 2011 and case fatality average rate of 20% according to data of the Ministry of Health (de Filippis et al., 2012a). The most frequent serogroups in the country are B and C accounting for more than 90% of all the serogrouped strains. Since 2005, the prevalence of serogroup C strains increased from 47.5% in 2005 to 80% in 2010, while the prevalence of serogroup B decreased from 46.6% in 2005 to 13% in 2010 (Fig. 1). The rise of serogroup C cases was followed by case fatality rates as high as 22% in 2010. A slight decrease of the number of serogroup C cases has been observed from 2010 to date (75.5%) and increase of serogroup B (16.7%) within the same period. This was in part due to local vaccination campaigns to halt sporadic outbreaks using a conjugated C vaccine. The aim of this study was to assess the prevalence of clone cc11/ET-15 among serogroup C strains isolated from 2 states of the northeast (NE) region of Brazil: Bahia (BA) and Pernambuco (PE), where several outbreaks due to serogroup C meningococci were reported.

A total of 152 serogroup C meningococcal isolates were recovered from 2 states of the NE region of Brazil (BA, n = 84; PE, n = 68) presenting the highest incidence of MD from 1996 to 2011. All strains were confirmed by culture and *nspA*-PCR (de Filippis et al., 2005), and serogroup was confirmed by latex agglutination and siaD-PCR Tzanakaki et al., 2003). STs were determined following the MLST

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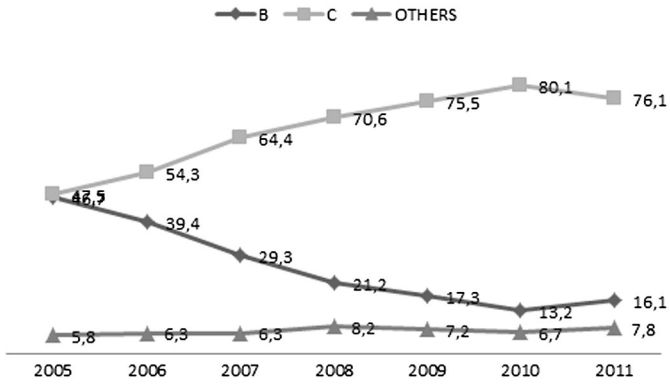

Fig. 1. Distribution of *N. meningitidis* serogroups by years in the NE region of Brazil (%).

protocol described in http://pubmlst.org/neisseria/, and PorA variants were determined using the http://pubmlst.org/neisseria/PorA/ (VR1 and VR2) and the *N. meningitidis* PorA VR3 database http://exon.niaid.nih.gov/meningitidis/index.html.

We have found 17 isolates belonging to cc11 with 15 belonging to ET-15 (14 from Bahia and 1 from Pernambuco states). For all 15 strains, PorA variant was P1.5,2,36 and all strains were submitted to the disk diffusion method and were susceptible to nalidixic acid (30 μg), penicillin G (10 IU), ciprofloxacin (5 μg), and rifampicin (5 μg). Two strains isolated in 1996 were classified as ST-2994 and ST-1026, which were already described in Brazil and Europe according to the MLST database. The remaining 13 strains were characterized as new STs according to the MLST database. Another important clonal complex, cc103, was characterized for the majority of strains. Seventy-three isolates belonged to cc103 with 12 from Bahia and 61 from Pernambuco states. PorA variants for 59 strains was characterized as P1.22,14,36-2, while 2 strains were P1.5,10,36-2. Strains belonging to cc103 showed decreased susceptibility to 3 antibiotics: 3 strains were resistant to nalidixic acid and ciprofloxacin and 1 strain to rifampicin. Temporal distribution of cc11/ET-15 and cc103 suggests that clone ET-15 has probably emerged in the first half of the 90s, with a gradual decrease over the years disappearing after 2001 when clone cc103 started to rise and is now the most prominent clonal complex among serogroup C meningococci in Brazil (Fig. 2). Although strains belonging to the cc11/ET-15 clone have been associated to severe outbreaks with high fatality rates in other countries, in Brazil, this clone did not show to be more aggressive than other circulating clonal complexes. Strains of cc103 clone have been associated to several outbreaks, including an event in the state of Bahia where 11 cases with 7 deaths were reported in 2009 (de Filippis et al., 2012b; Gorla et al., 2012).

Our study describes the recent distribution of meningococcal genotypes among serogroup C strains within 2 states of the NE region of Brazil. The occurrence of the 2 hypervirulent clones cc11 variant ET-15 and cc103 from 2005 to 2011, with the decrease of cc11/ET-15 from 1996 to 2001 followed by the increase of cc103 from 2001 to date, suggests that cc11/ET-15 has been replaced by cc103, which has been associated to several outbreaks within these 2 states. Our data suggest that the use of polysaccharide C–conjugated vaccines during 2010 in several cities of the NE region to halt local outbreaks caused by serogroup C strains has dramatically decreased the circulation of cc103 in that region. Our results also show that from 2006 to 2009, the proportion of cc103 to other cc was of 72/40, while in 2010, this proportion increased to 16/2; therefore, the raise of this clone over others should be closely monitored as well as the antibiotic susceptibility. Additional studies with a larger number of strains from other states of Brazil should be carried out in order to determine the possible association of cc103 with strains with reduced susceptibility to rifampicin and fluoroquinolone antibiotics. We believe that the emergence of new strains belonging to hypervirulent clones that can be prevented by vaccination justifies the introduction of mass vaccination in the whole population to prevent the spread of this clone to neighbouring regions.

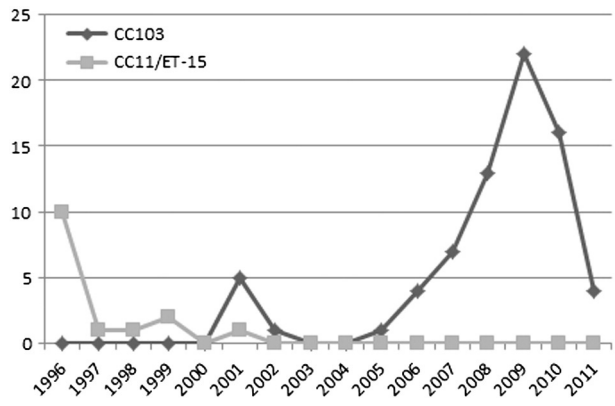

Fig. 2. Distribution of *N. meningitidis* clones by years in the NE region of Brazil.

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