Currently, diagnosis of Neisseria meningitidis in nasopharyngeal samples can be made by culture and nucleic acid amplification techniques. In Cuba, molecular diagnosis of meningococcal meningitis was introduced at the National Reference Laboratory for Neisseria (NRLN), in 2010, through a PCR that amplifies a fragment of the ctrA gene using the protocol described by Taha, 2000. This gene codes for a capsule protein that regulates the adherence of N. meningitidis to the host, and 16 to 28% of meningococci isolated usually in the nasopharynx lack this gene. In contrast, the sodC gene, related to the production of superoxide dismutase of this organism, is less sensitive to antigenic variation, hence its importance for molecular diagnosis in patients and asymptomatic carriers. Information on the meningococcal carriage is essential for public health policy. Still, the high number of invasive meningococcal disease (IMD) affecting Cuba during the 1980s and the absence of molecular tools prevented its accurate microbiological diagnosis in carriers. This study aimed to identify and molecular and methods in freeze-dried nasopharyngeal cultures of 50 Neisseria spp., recovered from carriers during 1987-1988, one of the most affected by a large epidemic of IMD in Cuba. Lyophilized material, which was preserved for 50 years, was reconstituted in 2 mL of nuclease-free sterile distilled water (Promega, USA). One milliliter was subcultured onto chocolate agar and incubated at 36.5-37°C for 18-24 hours, with 5-10% CO₂, and the other milliliter was used to perform DNA extraction. Conventional methods used as sugar utilization and Vitek®2 automated systems such as growth medium, cell concentration, freezing and thawing, lyoprotectant, reconstitution medium, and time. In the current investigation, freeze-dried ampules were stored at room temperature and unprotected from light. However, its more prolonged survival of 30 years is noteworthy under these unsuitable conditions. Recently, Swain et al. demonstrated the relatively prolonged survival of the Cuban, New Zealand and Norwegian epidemic N. meningitidis seergroup B strains on inanimate surfaces for up to 8 days, depending on temperature and humidity, in comparison to other meningococci strains belonging to seergroup W135. In addition, carriage isolates appeared to survive better than invasive isolates, with a statistically significant difference (P = 0.002).

Some authors recommend molecular tests for the identification and seergrouping of N. meningitidis in cultures from carriers and lyophilized material. The end point-PCR results that amplify a fragment of ctrA gene detected N. meningitidis in 76% of cell suspensions of Neisseria spp. Investigated (Figure 1). Meanwhile, the rt-PCR that amplified a 127 bp fragment of the sodC gene amplifies a 127 bp fragment of the sodC gene. Serogroup identification of Neisseria meningitidis isolates was developed by slide agglutination using Remel™ Agglutinating Sera (Lenexa, USA). In addition, the main seergroups (A, B, C, W135, Y, X) of meningococci were investigated by rt-PCR, in positive samples identified by both simple and rt-PCR systems. Pharyngeal carriage of N. meningitidis has been considered a prerequisite for the development of IMD and is known to be essential for transmission. In this study, ten isolates (20%) recovered from lyophilized material of nasopharyngeal cultures were identified as meningococci by culture, the standard gold method for detecting bacterial carriage. Nine of ten isolates were seergroup B, which was predominant during the epidemic of IMD in Cuba, and the other isolate was non-seergroupable. Freeze-drying is more practical for the long-term preservation of N. meningitidis cultures and optimal conditions for its conservation are refrigeration (2-8°C) or freezing (-30 to -70°C) temperatures, ampules are protected from the action of light and placed in an environment without humidity. These conditions produce good genetic stability of the product, with a longevity of up to 20 years. Moreira et al., in Cuba, obtained a 46.3% survival of N. meningitidis lyophilized cultures after one year of storage at 4°C. Lyophilization is a very complex physical process affected by many parameters and variables such as growth medium, cell concentration, freezing rate, lyoprotectant, reconstitution medium, and time. In the current investigation, freeze-dried ampules were stored at room temperature and unprotected from light. However, its more prolonged survival of 30 years is noteworthy under these unsuitable conditions. Recently, Swain et al. demonstrated the relatively prolonged survival of the Cuban, New Zealand and Norwegian epidemic N. meningitidis seergroup B strains on inanimate surfaces for up to 8 days, depending on temperature and humidity, in comparison to other meningococci strains belonging to seergroup W135. In addition, carriage isolates appeared to survive better than invasive isolates, with a statistically significant difference (P = 0.002).

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gene was absent in a large percentage (58%, 54/93) of carriage isolates. However, both porA and sodC genes were well represented in the carriage collection (99% and 97%)\(^3\). In addition, Jordens & Heckels reported the development of a porA rt-PCR assay that identified several \(N. meningitidis\) isolates from carriers that were missed by using only the ctrA gene\(^7\).

In the current study, 68.4% (26/38) of cell suspension positive to \(N. meningitidis\) by both PCR belonged to serogroup B, 5.3% was serogroup C, and the remainder (26.3%) were non-groupable. Martínez et al., in a longitudinal study carried out on meningococci strains corresponding to nasopharyngeal carriers of the epidemic (1982-1992) and post-epidemic (1993-2002) stage in Cuba, detected a predominance of serogroup B (67.3%) in the epidemic phase and the non-agglutinable strains during the post-epidemic stage (70.7%)\(^4\). In Colombia, Moreno et al. also report a predominance of \(N. meningitidis\) serogroup B in carriers\(^5\).

The absence of comprehensive information for carriage in developing countries limits clarification of the epidemiology of IMD\(^6\). In the case of the Caribbean region, in particular, there is no previous report about the use of molecular methods for identifying or sero-grouping \(N. meningitidis\) in patients or carriers. The current study supports the usefulness of molecular tools in future studies of nasopharyngeal carriers in the Cuban population. In addition, this genetic material is helpful for further genomic characterization of Cuban meningococci strains by multi-locus sequence typing and/or other sequence methods. Genomic surveillance for \(N. meningitidis\) is fundamental for understanding pathogen evolution and disease epidemiology and can be significantly improved using culture-free methods\(^7\).

**Conclusions**

This study represents the first international report about a more prolonged survival of \(N. meningitidis\) in freeze-dried cultures from nasopharyngeal carriers that were kept under potentially unsuitable temperature, humidity and light...
exposure conditions. The sodC-based rt-PCR assay had an advantage over ctrA gene for detecting meningococci in cell suspensions of *Neisseria* spp. from lyophilized material obtained during the extensive epidemic of IMD in Cuba in the 1980s.

**Acknowledgments**

The authors acknowledged Dr. Yaxier de Armas and Prof. Imti Choonara for comments about the manuscript and Dr. Eldy Machado for English revision.

**Conflicts of Interest**

None.

**Bibliographic references**


