Available online at www.sciencedirect.com
SCIENCE DIRECT®
Microbes and Infection 7 (2005) 645–649
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

# The establishment of *Neisseria meningitidis* serogroup W135 of the clonal complex ET-37/ST-11 as an epidemic clone and the persistence of serogroup A isolates in Burkina Faso

Berthe-Marie Njanpop-Lafourcade [a], Isabelle Parent du Châtelet [a,1], Oumar Sanou [b], Jean-Michel Alonso [c], Muhamed-Kheir Taha [c,*]

[a] *Association pour l'Aide à la Médecine Préventive, Paris, France*
[b] *Centre Muraz, Bobo-Dioulasso, Burkina Faso*
[c] *Neisseria Unit, National Reference Centre for Meningococci, 28, rue du Dr Roux, Institut Pasteur, 75724 Paris cedex 15, France*

Received 27 August 2004; accepted 4 January 2005

Available online 17 March 2005

**Abstract**

We analyzed 48 invasive isolates of *Neisseria meningitidis* that were isolated from meningitis cases in Burkina Faso (April 2002 to April 2003). Thirty-nine of these isolates had the phenotype (serogroup:serotype:serosubtype) W135:2a:P1.5,2, eight isolates were A:4:P1.9 and one isolate was nongroupable:nonserotypable:nonserosubtypable. Genotyping of meningococcal isolates showed that W135 isolates belonged to the sequence type (ST)-11. The nongroupable isolate was of genogroup W135 and belonged to ST-192. Isolates of serogroup A belonged to ST-2859 (a member of the subgroup III/ST-5 clonal complex). W135 (ST-11) isolates involved in meningitis outbreaks in Burkina Faso differed from those involved in the Hajj-2000 associated outbreak by their pulsed-field gel electrophoresis profile. These data confirm the changing epidemiology of meningococcal infection in Burkina Faso with the establishment and expansion of serogroup W135 *N. meningitidis* strains of the ET-37/ST-11 clonal complex, as well as the emergence of a new clone within the subgroup III/ST-5 clonal complex.


*Keywords:* *Neisseria meningitidis* serogoup W135; Meningitis; Epidemiology; Molecular typing; Burkina Faso

## 1. Introduction

*Neisseria meningitidis* is a human pathogenic bacterium showing a high level of genetic diversity due to frequent genetic exchanges [1,2]. Strains of *N. meningitidis* have been classified into 13 serogroups on the basis of the immune specificity of the capsular polysaccharide [3,4]. Invasive infections are most frequently due to serogroups A, B, C, Y or W135. Strains of serogroup A are responsible for major periodic epidemics in the African meningitis belt (AMB) [1,5,6]. This belt spans a band of several sub-saharan countries from Ethiopia to Senegal [5]. The epidemic season usually extends between December and April and coincides with the dry season and the Harmattan sand wind [5]. The introduction of genetic typing approaches such as multilocus enzyme electrophoresis (MLEE) and multilocus sequence typing (MLST) permit fine molecular typing of clinical isolates and assessing epidemiological links. Closely related isolates are clustered into clonal complexes (genotypes or genetic lineages) that are also named subgroups or electrotype (ET) by MLEE or sequence type (ST) by MLST [7,8].

Several clonal complexes of *N. meningitidis* serogroup A were successively incriminated in the epidemics that occurred in the AMB [6]. During the 1960s–1970s strains belonging to subgroups I/II were predominant and were later replaced by strains of the subgroup IV-1 in the 1980s. By the late 1980s a new replacement occurred by strains belonging to the subgroup III. A worldwide spread of strains of the subgroup III was reported after the 1987 Hajj pilgrimage to Mecca in Saudi

*Abbreviations:* AMB, African meningitis belt; MLEE, Multilocus enzyme electrophoresis; MLST, Multilocus sequence typing.

* Corresponding author. Tel.: +33 1 44 38 95 90; fax: +33 1 40 61 30 34.
*E-mail address:* mktaha@pasteur.fr (M.-K. Taha).
[1] Present address: Infectious diseases Department. Institut de Veille Sanitaire, Saint Maurice 94415, France.

1286-4579/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.micinf.2005.01.003

Arabia [6]. Several epidemic waves spanned the AMB in 1990s and were provoked by strains of the subgroup III [9]. Moreover, clonal replacement within the subgroup III was also observed. Indeed, isolates from this subgroup belonged to the sequence type ST-5 (the major sequence type of subgroup III). Since the mid 1990s, an increasing proportion of meningococcal isolates from countries of the AMB and from China, Mongolia and Russia are ST-7 (another sequence type of the subgroup III [10,11]. However, serogroup was not affected and outbreaks can be prevented by A and C polysaccharide vaccine.

In the year 2000, a clonal outbreak of W135 meningococcal disease occurred among Hajj pilgrims returning from Saudi Arabia and their household contacts [12,13]. Isolates of this outbreak belonged to the ET-37/ST-11 clonal complex. As related strains have been isolated worldwide since 1970 [14], the emergence of such strains in Africa became a great concern. *N. meningitidis* serogroup W135, also belonging to the ET-37/ST11 clonal complex, was detected as epidemic strain for the first time in 2001 in Niger and Burkina Faso [15,16]. During the 2002 outbreak in Burkina Faso, 84% of the isolates characterized were of serogroup W135 [17] demonstrating the epidemic potential of this serogroup. Therefore, prospective surveillance of meningococcal strains became mandatory for controlling epidemics by appropriate vaccination. Between April 2002 and April 2003, we conducted a prospective study to identify the proportion of meningococcal isolates and the distribution of various phenotypes and genotypes of these isolates.

## 2. Materials and methods

### 2.1. Collection of clinical specimens

This prospective surveillance study was conducted in 3 sanitary districts (rural and urban), the rural district of Houndé, and the urban districts 15 and 22 of Bobo-Dioulasso, from April 2002 to April 2003. These districts have a climate typical of the Sub-Saharan meningitis belt. These sites were chosen on the basis of a history of previous meningitis epidemics and the availability of an appropriate reference laboratory [18]. Patients were most frequently examined with meningitis clinical presentation (fever, meningism). Cerebrospinal fluid (CSF) samples were hence the most frequently collected samples from local health centers in accordance with the general guidelines of the Burkina Faso Ministry of Health. Samples were sent to the corresponding district laboratory. The socio-demographic information (age, sex, address) was registered.

### 2.2. Bacteriological identification

At the local reference laboratory in Bobo Dioulasso, CSF samples were cultured on chocolate agar at 37 °C in a candle jar overnight, and suspected colonies were identified by standard bacteriological methods [19]. Serogroup determination was performed using specific antibodies to *N. meningitidis* serogroups A, B, C, and Y/W135 (Pastorex® from BioRad, Marnes-La-Coquette, France). To differentiate between isolates of serogroups Y and W135 specific sera were further used for agglutination (BioRad, Marnes-La-Coquette, France). Meningococcal isolates sampled and transported on Vandekerkove medium [20] were further characterized at the Institut Pasteur. The phenotype, based on the antigenic formula (serogroup: serotype: serosubtype), was determined as previously described [21]. Antibiotic susceptibility testing for penicillin G, amoxicillin, cefotaxime, rifampicin, spiramycin and chloramphenicol, was performed by the disk-diffusion technique and minimal inhibitory concentrations were measured by *E*-test (Solna, Sweden), as previously described [22].

### 2.3. Molecular typing of meningococcal isolates

Genogrouping was performed by using PCR-based prediction of serogroup and the amplification of *siaD* (genogroup B, C, Y and W135) and *mynB* (genogroup A), as previously described [23]. Meningococcal isolates were characterized by three molecular typing techniques, applied sequentially. Multilocus DNA fingerprinting (MLDF) is a rapid DNA restriction fragment-based technique for the characterization of five virulence-associated genes (*pilA*, *pilD*, *crgA*, *regF*, and *iga*) and was performed as previously described [14,24]. This method defines various alleles of these genes that correlate with electrotypes. Strains belonging to the ET-37/ST-11 clonal complex are characterized by the alleles 4, 1, 1a, 1 and 1, of *pilA*, *pilD*, *crgA*, *regF* and *iga*, respectively, while the alleles that characterize strains of the subgroup III/ST-5 clonal complex are 3, 1, 3b, 24 and 16. MLST was performed as previously described [24,25]. Standard pulsed field gel electrophoresis (PFGE) was performed using the restriction enzyme *SpeI* [14,24].

## 3. Results

### 3.1. General characteristics of meningococcal isolates

From April 2002 to April 2003, a total of 439 CSF were tested by culture. Nineteen meningococcal isolates in 2002 and 29 isolates in 2003 were obtained. Patient ages ranged between 3 months and 17 years (mean 5.1 years, median 4 years). Ten cases (21%) occurred among children less than 1 year old and 29 cases (60%) occurred among children 1 to 5 years old. Male to female sex ratio was 33/15 and six fatal cases occurred (12.5%) that were equally distributed in both sexes.

### 3.2. Phenotypic characterization of meningococcal isolates

We determined the phenotypes of all the 48 isolates of this study. All the 19 isolates of the year 2002 were of the pheno-