

Carriage of *Neisseria lactamica* in 1- to 29-Year-Old People in Burkina Faso: Epidemiology and Molecular Characterization

Paul A. Kristiansen,^a Fabien Diomandé,^{b,c} Rasmata Ouédraogo,^d Idrissa Sanou,^{e,f} Lassana Sangaré,^e Abdoul-Salam Ouédraogo,^f Absatou Ky Ba,^g Denis Kandolo,^{b,h} Jennifer Dolan Thomas,^c Thomas A. Clark,^c Marie-Pierre Préziosi,^{i,j} F. Marc LaForce,ⁱ and Dominique A. Caugant^{a,k}

Norwegian Institute of Public Health, Oslo, Norway^a; WHO Inter Country Support Team, Ouagadougou, Burkina Faso^b; Centers for Disease Control and Prevention, Atlanta, Georgia, USA^c; Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle^d and Centre Hospitalier Universitaire Yalgado,^e Ouagadougou, Burkina Faso; Centre Hospitalier Universitaire Souro Sanou, Bobo-Dioulasso, Burkina Faso^f; Laboratoire National de Santé Publique, Ministry of Health, Ouagadougou, Burkina Faso^g; WHO Multi Disease Surveillance Center, Ouagadougou, Burkina Faso^h; Meningitis Vaccine Project, Ferney, Franceⁱ; WHO Initiative for Vaccine Research, Geneva, Switzerland^l; and Faculty of Medicine, University of Oslo, Oslo, Norway^k

Neisseria lactamica is a true commensal bacterium occupying the same ecological niche as the pathogenic Neisseria meningitidis, which is responsible for outbreaks and large epidemics, especially in sub-Saharan Africa. To better understand the epidemiology of N. lactamica in Africa and its relationship to N. meningitidis, we studied N. lactamica carriage in 1- to 29-year-old people living in three districts of Burkina Faso from 2009 to 2011. N. lactamica was detected in 18.2% of 45,847 oropharyngeal samples. Carriage prevalence was highest among the 2-year-olds (40.1%) and decreased with age. Overall prevalence was higher for males (19.1%) than females (17.5%) (odds ratio [OR], 1.11; 95% confidence interval [CI], 1.04 to 1.18), while among the 18- to 29-year-olds, carriage prevalence was significantly higher in women (9.1%) than in men (3.9%) (OR, 2.49; 95% CI, 1.94 to 3.19). Carriage prevalence of N. lactamica was remarkably homogeneous in the three districts of Burkina Faso and stable over time, in comparison with carriage of N. meningitidis (P. A. Kristiansen et al., Clin. Vaccine Immunol. 18:435–443, 2011). There was no significant seasonal variation of N. lactamica carriage and no significant change in carriage prevalence after introduction of the serogroup A meningococcal conjugate vaccine, MenAfriVac. Multilocus sequence typing was performed on a selection of 142 isolates. The genetic diversity was high, as we identified 62 different genotypes, of which 56 were new. The epidemiology of N. lactamica carriage and the molecular characteristics of carried isolates were similar to those reported from industrialized countries, in contrast to the particularities of N. meningitidis carriage and disease epidemiology in Burkina Faso.

eisseria lactamica is a lactose-fermenting Gram-negative dip-Vlococcus living in a commensal relationship with humans. The bacteria, frequently found in the upper respiratory tract, most commonly in young children, are transmitted between healthy individuals through close contact and are normally not a threat to humans, as only a few cases of clinical significance have been reported (9, 12, 36). Neisseria meningitidis, a closely related species, occupies the same ecological niche but may cause severe disease, such as meningitis and/or septicemia. Large epidemics can occur, with devastating consequences, especially in countries of the meningitis belt, a sub-Saharan area with modest yearly rainfall (17, 26). N. meningitidis possesses a protective polysaccharide capsule, from which the serogroup is determined. N. lactamica does not express a capsule, but subcapsular components, such as outer membrane proteins, are similar in these two species (3, 5, 18, 29). Early studies have shown that N. lactamica carriage is frequent in age groups with low prevalence of N. meningitidis asymptomatic carriage and vice versa (15, 32). It is believed that carriage of N. lactamica can protect against meningococcal infection by crossreactive immunity (11), and meningococcal vaccines based on N. lactamica have been considered (14, 16).

Although *N. lactamica* is closely related to *N. meningitidis*, little is known about its epidemiology. A limited number of studies have reported carriage prevalence and distribution (1, 2, 4, 7, 8, 11, 19, 20, 27, 30, 32–34), some of them conducted within the African meningitis belt (7, 27, 30). To date, two studies have used molecular techniques to characterize *N. lactamica* isolates (1, 4), one using multilocus sequence typing (MLST) (4), the same scheme as

currently recommended for *N. meningitidis* genotyping. Research on the relationship between the two commensals and on characteristics of the circulating strains may contribute to a better understanding of meningococcal colonization and virulence factors.

In the meningitis belt of Africa, *N. meningitidis* serogroup A belonging to the sequence type 5 (ST-5) clonal complex has been responsible for the majority of epidemics in the past decades, although serogroups X and W135 have also caused outbreaks (31). To eliminate meningococcal epidemics in the meningitis belt, an affordable serogroup A conjugate vaccine, MenAfriVac, was first introduced in a country-wide mass vaccination campaign in Burkina Faso in December 2010 (10, 24, 25) after a large-scale safety study had been conducted in the whole district of Kaya in September 2010. The impact of MenAfriVac vaccination on meningococcal carriage was evaluated in a large carriage study performed before and up to 13 months after mass vaccination (21, 22). In the course of that study, all lactose-fermenting Gram-negative diplococci were registered and some were kept for further investigation.

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Address correspondence to Dominique A. Caugant, dominique.caugant@fhi.no. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JCM.01717-12

We present here the epidemiology of *N. lactamica* carriage in Burkina Faso before and after MenAfriVac vaccination and the genetic profile of *N. lactamica* isolates circulating in the country.

MATERIALS AND METHODS

Ethics. The study obtained ethical clearance from the Norwegian Regional Committee for Medical Research Ethics, southern Norway (file no. S-08375a), the Ethical Committee for Health Research in Burkina Faso, and the Internal Review Board at Centers for Disease Control and Prevention (CDC), Atlanta, Georgia (file no. 5524).

Study population and data collection. In a repeated cross-sectional study performed between 2009 and 2011, oropharyngeal swabs were collected from 1- to 29-year-old residents of Burkina Faso, as described previously (22). A multistage cluster sampling design ensured the enrollment of a representative proportion of the 1- to 29-year-old population from three health districts: the urban district of Bogodogo and the rural districts of Dandé and Kaya. Nine sampling campaigns, S1 (January and February 2009), S2 (April and May 2009), S3 (July and August 2009), S4 (October and November 2009), S5 (October and November 2010), S6 (February and March 2011), S7 (May 2011), S8 (August 2011), and S9 (October and November 2011), were performed simultaneously in the three districts within a 4-week period. The sampling campaigns covered meningitis epidemic seasons occurring in the dry period of the year (S1, S2, S6, and S7) and nonepidemic (rainy) seasons (S4, S5, S6, S8, and S9). For each sampling campaign, a random selection of households in the rural districts and city blocks in the urban district was made, and all healthy 1- to 29year-olds in the selected households or city block were invited to participate in the study and to be swabbed. Epidemiological data on each participant such as the person's age and gender were collected on handheld computers in the field.

Sample collection and bacterial identification. Each volunteer was swabbed at the posterior pharyngeal wall behind the uvula and at one tonsil, using a sterile cotton swab (Copan, Brescia, Italy). The swab was immediately plated onto selective agar (modified Thayer Martin V-C-N-T, containing 3 mg/liter vancomycin, 7.5 mg/liter colistin, 12.5 U/liter nystatin, 5 mg/liter trimethoprim lactate, and Vitox supplement; produced by WHO/Multi Disease Surveillance Centre, Burkina Faso) that supported the growth of both N. meningitidis and N. lactamica. Inoculated plates were placed in sealed jars in the field (Remel, GA) with CO₂ generators (CO₂ GEN; Oxoid, United Kingdom), and the temperature was monitored until the jars were set for incubation at 37°C within a maximum of 6 h after swabbing. In each district, a microbiological laboratory performed the bacterial identification: the Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle, Ouagadougou, for Bogodogo; the Centre Hospitalier Universitaire Souro Sanou, Bobo-Dioulasso, for Dandé; and the Centre Hospitalier Universitaire Yalgado, Ouagadougou, for Kaya. After 24 and 48 h of incubation, one or two colonies growing on the selective agar plate with a typical Neisseria morphology were subcultured on blood agar plates (Reactivos Para Diagnostico, Spain) containing 5% defibrinated sheep blood (Fig. 1). Oxidase positive, Gram-negative diplococci were isolated and further characterized (22). Isolates with β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside [ONPG]) activity (Rosco Diagnostics, Denmark) were categorized as N. lactamica. The technicians working in the national laboratories received extensive training before the start of the study, and the quality of the laboratory analysis was monitored throughout the study period using a quality control system, as described previously (23).

Selection of isolates for molecular characterization. As part of the laboratory quality control system (23), 10% of all the isolates with an enzymatic profile different from that of N. meningitidis were controlled at the Norwegian Institute of Public Health (NIPH). Of these, isolates were confirmed as N. lactamica when the enzymatic profile was ONPG positive and γ -glutamyltransferase (GGT) negative (Rosco Diagnostics, Denmark). A subset of 142 (27.7%) confirmed N. lactamica isolates originating from sampling campaigns S1 to S4 performed in 2009 were randomly

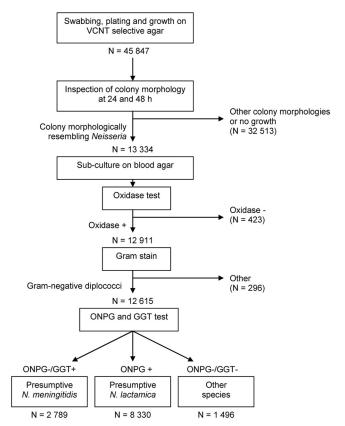


FIG 1 Flow diagram of laboratory analysis performed in Burkina Faso for the identification of *N. lactamica* in oropharyngeal swabs. ONPG, o-nitrophenyl β -galactopyranoside (for β -galactosidase activity); GGT, γ -glutamyltransferase.

selected for molecular characterization to obtain a pool of isolates representative of this period. Isolates were selected from each of the visited villages at all four sampling campaigns. Within each village or city block, isolates were randomly selected from different compounds, both genders, and different age groups when possible.

Molecular characterization. DNA from 142 N. lactamica isolates was isolated by suspending 1 loopful of bacteria in 200 μ l Tris-EDTA (TE) buffer, pH 8.0, heating the mixture at 95°C for 10 min, and subjecting it to centrifugation at 16,000 \times g for 5 min. The supernatants were stored at -20°C until use. MLST using seven housekeeping gene fragments was performed with the primers recommended by Alber et al. (1). Additional primer sets (fumC-P1, fumC-P2, fumC-P1B, and fumC-P2B) (6, 13) were needed for the amplification and sequencing of the fumC locus in some isolates. Each strain was assigned to a specific ST and ST complex (13, 28).

Data collection and analyses. The epidemiological information collected in the field was combined with laboratory results from Burkina Faso and Norway. Of 46,196 oropharyngeal samples collected, 349 were excluded from statistical analysis due to data entry errors. Statistical analysis was done in Stata version 11.1. Odds ratios (OR) were calculated by bivariate logistic regression accounting for the cluster sampling design. Statistical significance was defined as *P* values of <0.05 and as 95% confidence intervals not including null. Phylogenetic analyses based on concatenated gene sequences of different STs were done by the unweighted-pair group method using arithmetic averages (UPGMA) generated in MEGA version 5 (35) with a bootstrap test (1,000 replicates). Bootstrapped UPGMA analysis was also used to compare STs identified in Burkina Faso with those reported to be found in the United Kingdom (4) and with all the *N. lactamica* STs registered in the MLST database (http://pubmlst.org/neisseria) by May 2012.

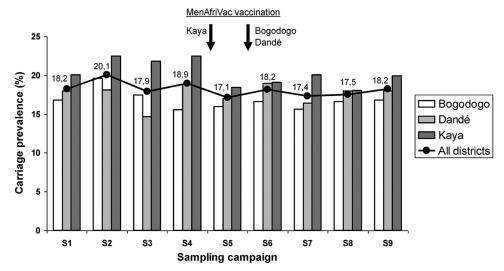


FIG 2 Geographic and temporal variation of N. lactamica carriage in three health districts in Burkina Faso, 2009 to 2011.

RESULTS

N. lactamica carriage prevalence. From 9 sampling campaigns performed between 2009 and 2011, a total of 45,847 participants were included in the analysis. Of these, 43.5% were male and 50.9% <10 years old. A total of 8,330 (18.2%) participants were identified as *N. lactamica* carriers on the basis of colony morphology and biochemical properties of the isolates recovered from the pharyngeal samples collected by the national microbiology laboratories in Burkina Faso.

Accuracy of results. From the sampling campaigns in 2009, 538 isolates classified as *N. lactamica* (ONPG positive) in Burkina Faso were sent to NIPH as part of the external quality control. Of these, 512 (95.2%) were confirmed as such at NIPH, while 11 (2.0%) were identified as *N. meningitidis* (23). Thus, carriage rates of *N. lactamica* presented here are likely to be slightly overestimated. Using the 95% accuracy rate, the overall carriage rate of *N. lactamica* was 17.3%.

Geographic and seasonal variation. A total of 2,587 isolates were categorized as *N. lactamica* in Bogodogo (16.8%), 2,700 in Dandé (17.5%), and 3,043 in Kaya (20.3%). The carriage rate in Kaya was higher than in Bogodogo (OR, 1.26; 95% confidence interval [CI], 1.05 to 1.52), but the difference with Dandé was not significant (OR, 1.20; 95% CI, 0.95 to 1.50). Some fluctuations in carriage prevalence were seen between sampling campaigns (Fig. 2), but the differences between sampling time points were not statistically significant. Overall carriage prevalence in the dry seasons of 2009 and 2011 (S1, S2, S6, and S7) was 18.5%, while in the rainy season, 1.02; 95% CI, 0.94 to 1.10).

Age and gender distribution. *N. lactamica* was carried by 33.0% of the 1-year-olds. *N. lactamica* carriage rate peaked at 2 years (40.1%) and then rapidly decreased with age but remained at around 5 to 10% in the older age groups. This is in contrast to a flatter age distribution curve for *N. meningitidis* carriage (22) in the same population (Fig. 3). This age distribution was similar in all three districts (data not shown). Overall carriage was significantly higher among male (19.1%) than among female (17.5%) participants (OR, 1.11; 95% CI, 1.04 to 1.18). The age distribution was remarkably different between genders, as carriage among

women increased again from the age of 18, while it remained low among men (Fig. 4). In the age group of 18 to 29 years, carriage prevalence in women (9.1%) was significantly higher than in men (3.9%) (OR, 2.49; 95% CI, 1.94 to 3.19).

Stability of *N. lactamica* carriage after MenAfriVac vaccination. Overall carriage prevalence was 18.8% in 2009, before mass vaccination with MenAfriVac, and 17.8% in 2011, after vaccination, but the reduction was not statistically significant (OR, 0.94; 95% CI, 0.86 to 1.02). Compared to 2009, *N. lactamica* carriage in 2011 was lower in Bogodogo (OR, 0.93; 95% CI, 0.83 to 1.06), higher in Dandé (OR, 1.03; 95% CI, 0.93 to 1.15), and lower in Kaya (OR, 0.86; 95% CI, 0.68 to 1.08), but the differences were not significant in any of the districts. Carriage prevalence for male participants was 19.8% before vaccination and 18.7% after vaccination, while female participants had a carriage prevalence of 18.0% before vaccination and 17.1% after. The age distribution profile of *N. lactamica* carriers after vaccination was similar to the age profile before vaccination, although the prevalence in the 2-year-olds was lower (37.2%, down from 42.8%).

Molecular characterization. A total of 142 confirmed *N. lactamica* isolates from sampling campaigns S1 to S4 in 2009 were

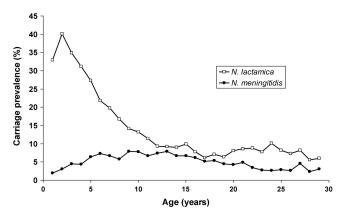


FIG 3 Age distribution for carriers of *N. lactamica* and *N. meningitidis* in Burkina Faso, 2009 to 2011.

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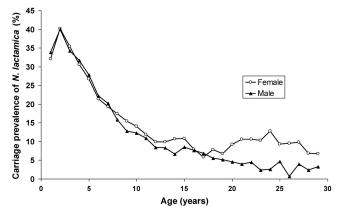


FIG 4 Age distribution of female and male carriers of *N. lactamica* in Burkina Faso, 2009 to 2011.

randomly selected for molecular characterization and included 46 samples from Bogodogo, 51 from Dandé, and 45 from Kaya. None of the persons from which isolates were selected had been swabbed more than once.

In total, 62 STs were identified among the 142 isolates, of which 56 were new. Forty new alleles were registered in the MLST database. The majority of the isolates (103 isolates, 73%) were composed of 36 different genotypes belonging to four clonal complexes: ST-595 complex (n=23), ST-613 complex (n=40), ST-640 complex (n=30), and ST-1494 complex (n=10) (Table 1). The two dominant STs were ST-613 of the ST-613 complex and ST-9060 of the ST-640 complex, each representing 14.1% of the isolates. ST-9099 of the ST-595 complex was represented by 9 isolates (6.3%), and the remaining STs were represented by less than 5% of the isolates.

UPGMA analysis provided a visual representation of the genetic diversity of *N. lactamica* STs and the relative differences between ST complexes (Fig. 5). There were three main groups of STs not yet assigned to a clonal complex, one close to the ST-595 complex, one close to both the ST-613 and the ST-1494 complexes, and a single ST (ST-9100) represented by one isolate, distant from all the other *N. lactamica* STs. All the *N. lactamica* isolates were genetically distant from *N. meningitidis* carriage isolates retrieved from the same population in the same period (22) (Fig. 5).

UPGMA analysis of *N. lactamica* STs identified among infants in the United Kingdom (4) combined with *N. lactamica* STs from Burkina Faso showed a high degree of genetic variation in both countries and no evident clustering of STs from Burkina Faso (Fig. 6).

DISCUSSION

This study presents the epidemiology of *N. lactamica* carriage and the genetic diversity of the isolates recovered from 1- to 29-year-olds in Burkina Faso. The study shows little geographic variation of *N. lactamica* carriage and a stable prevalence over a 3-year period, also observed after mass vaccination with a serogroup A meningococcal conjugate vaccine.

The study was part of a large meningococcal carriage study, and the laboratory methodology was primarily aimed at finding *N. meningitidis*. We used an established sampling method, and extensive training and monitoring were an integral part of the

study. To identify N. lactamica, we used results from the analytical steps common for both species. However, as only one or two colonies from the primary agar plate were further examined, cocolonization with *N. meningitidis* was not possible to estimate and the carriage prevalence of N. lactamica might have been underestimated. As meningococcal carriage prevalence (3.98% in 2009 and 6.95% in 2010-2011) (21, 22) was substantially lower than N. lactamica carriage prevalence and because of the difference in age distributions of the individuals colonized by the two species, the underestimation due to cocolonization is probably very small. On the other hand, the N. lactamica carriage rate might have been slightly overestimated, as 5% of the isolates classified as N. lactamica in Burkina Faso were not confirmed as such at NIPH. Among these isolates not confirmed as N. lactamica, one was identified as Moraxella catarrhalis while the remaining ones belonged to other Neisseria species as determined by their enzymatic profile and by 16S rRNA gene sequencing (data not shown).

Our estimate of 18.2% overall carriage, or 17.3% when considering the 95% confirmation rate at NIPH, is within the range of carriage rates reported elsewhere (1, 2, 4, 7, 8, 15, 19, 20, 32–34), and the characteristic age distribution with a maximum prevalence at 2 years of age is consistent with previous findings (4, 32, 34). Although the difference of overall carriage prevalence was calculated as significantly higher among men than women, this estimate might be biased due to the higher participation rate of young children, especially the 3- to 7-year-olds, where higher prevalence among male participants was found. The observation of a higher carriage prevalence among older women is consistent with results from other countries (8, 19), suggesting a closer con-

TABLE 1 Distribution of sequence types among 142 *N. lactamica* strains isolated from 1- to 29-year-old carriers in the districts of Bogodogo, Dandé, and Kaya in Burkina Faso in 2009

			No. (%)
Clonal complex	% of isolates	ST^a	of isolates
ST-595	16.2	595	4 (2.82)
		9099	9 (6.34)
		9146	4 (2.82)
		9271	2 (1.41)
		Other	4 (2.8)
ST-613	28.2	613	20 (14.1)
		9101	6 (4.23)
		9102	2 (1.41)
		9270	4 (2.82)
		9276	2 (1.41)
		Other	6 (4.2)
ST-640	21.1	640	2 (1.41)
		9060	20 (14.1)
		Other	8 (5.6)
ST-1494	7.0	9057	2 (1.41)
		9097	2 (1.41)
		9144	2 (1.41)
		Other	4 (2.8)
UA ^b	27.5	1288	4 (2.82)
		8476	2 (1.41)
		9059	6 (4.23)
		9139	5 (3.52)
		Other	22 (15.4)

 $[^]a$ ST, sequence type; Other, sequence types each representing less than 1% of the isolates.

^b UA, unassigned to any clonal complex.

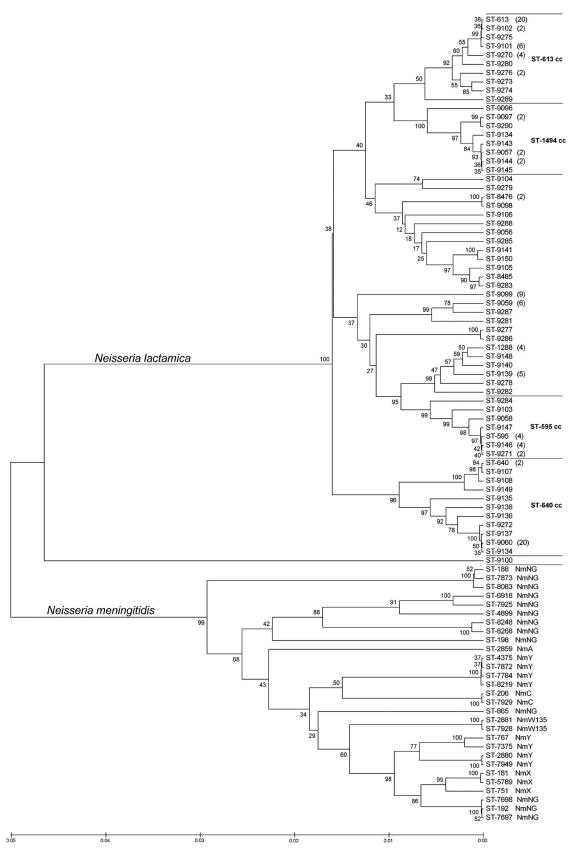


FIG 5 Linkage distance of 62 *N. lactamica* and 29 *N. meningitidis* sequence types (STs) identified among 1- to 29-year-old carriers from Burkina Faso in 2009, as determined by bootstrapped UPGMA analysis. For STs represented by more than one isolate, the number of isolates is given in parentheses. NmA, NmC, NmX, NmY, NmW135, and NmNG are abbreviations for serogroups A, C, X, Y, and W135 and nonserogroupable *N. meningitidis*, respectively. cc, clonal complex.

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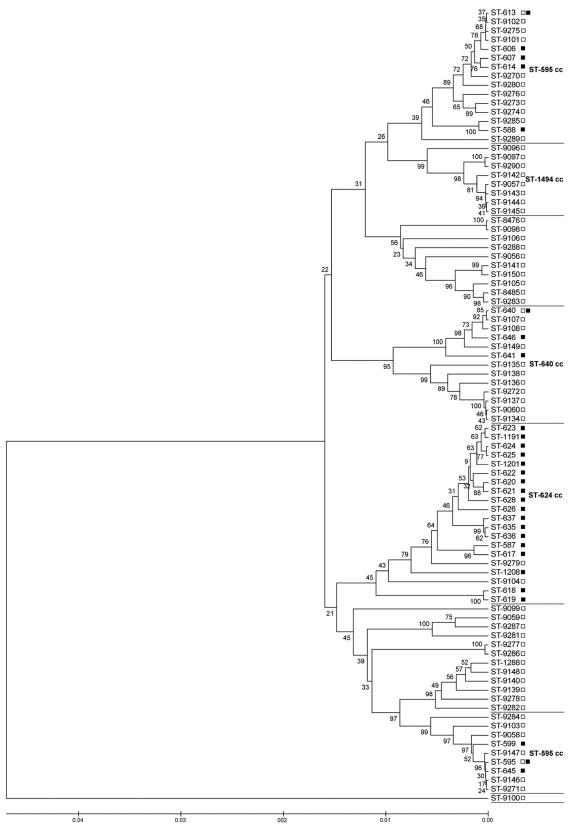


FIG 6 Comparison of sequence types of *N. lactamica* carriage isolates from Burkina Faso and from the United Kingdom, as determined by bootstrapped UPGMA analysis. Sequence types (STs) are followed by an empty square if found in Burkina Faso and a filled square if found in the United Kingdom. cc, clonal complex.

tact between mother and child than between father and child. Interestingly, one study (4) has shown that children did not share the same isolates as their parents, but our results show that they do; in Burkina Faso the two dominant STs were carried by nearly all age groups (1 to 25 years for ST613 and 1 to 28 years for ST9060). In households from which isolates from two participants swabbed the same day were selected for MLST, only a single ST was identified (3 of 3 households; data not shown).

The carriage prevalence of N. lactamica was remarkably homogeneous in Burkina Faso and was stable over time. A study conducted in Northern Ghana (27) also showed relatively stable N. lactamica carriage prevalence over a 6-year period, although the estimated prevalence was lower (4.7 to 9.3%) than that of our study. In Burkina Faso, meningococcal disease epidemiology evolved between 2009 and 2011: in 2009, the majority of invasive cases in Burkina Faso were due to N. meningitidis serogroup A, while in 2011, after MenAfriVac mass vaccination, the total number of cases was historically low; serogroup A disease was almost nonexistent, and serogroup X disease dominated. Carriage of N. meningitidis in the same population varied between 2009 and 2011, as serogroup Y dominated in 2009 (22) and serogroup X dominated in 2010 and 2011 (21). The seasonal and geographic stability of *N. lactamica* carriage in comparison to the significant variations observed for N. meningitidis carriage in the same population (22) and the consistency of N. lactamica carriage before and after MenAfriVac vaccination suggest that it was not affected by changing meningococcal carriage or by the epidemic context.

The genetic diversity of *N. lactamica* carriage isolates was consistent with previous findings (1, 4), and STs could be compared with those in a similar study performed in the United Kingdom (4). Three ST complexes were common in both studies, although within each of these common ST complexes, only one ST was represented in both populations (ST-595 for the ST-595 complex, ST-613 for the ST-613 complex, and ST-640 for the ST-640 complex). In both studies, ST-613 was one of the dominant STs. Although some STs from Burkina Faso were clustered, UPGMA analysis of STs for *N. lactamica* isolates from both countries reveals that there is as much genetic diversity within each of the two countries as there is between countries (Fig. 6).

The genetic diversity of *N. lactamica* isolates in Burkina Faso was higher than that of meningococcal carriage isolates retrieved from the same population, as only 29 different STs were identified from 809 meningococcal isolates (22) in comparison with 62 STs from 143 *N. lactamica* isolates. However, the diversity appears lower than in the United Kingdom, where 72 STs of *N. lactamica* were found among only 96 carriers. To date, a total of 340 *N. lactamica* STs have been registered in the MLST database (http://pubmlst.org/neisseria). UPGMA analysis of all 340 STs shows that the STs of isolates from Burkina Faso were not clustered (data not shown).

ST-9100, represented by a single isolate, was genetically distant from all the other *N. lactamica* isolates. Retesting of Gram staining and biochemical properties confirmed the initial classification as *N. lactamica* (oxidase positive, Gram-negative diplococcus with ONPG-positive and GGT-negative activity). The isolate was confirmed as *N. lactamica* by the API NM test (bioMérieux, France), while 16S rRNA sequencing revealed only 95% similarity to an uncultured *Neisseria* species clone (data not shown). Comparison of gene profiles for all the *N. lactamica* STs in the MLST database showed that ST-9100 was genetically close to a group of five STs

registered as *N. lactamica*, the closest being ST-1528 (data not shown). These might represent an as-yet-unidentified species of the genus *Neisseria*.

Four isolates belonged to ST-1288, although in the MLST database, this ST was registered as *N. meningitidis* with an isolate from a carriage study in Ghana in 1999. The assignment of this ST to *N. lactamica* is supported by a genetic resemblance with other *N. lactamica* and not with *N. meningitidis* STs (Fig. 5).

The analysis of the genetic variation of isolates using the genome sequence of seven alleles provides the opportunity to study the variation of each ST within a certain ST complex, between ST complexes, and between species (Fig. 5). Although we found no resemblance between *N. lactamica* and *N. meningitidis* isolates by studying the MLST gene sequences, there might be similarities in other parts of the genome, such as the genes coding for outer membrane proteins. Common FetA variants have been found among *Neisseria* species in the United Kingdom (5), and further studies must be undertaken to verify if this is also true in Burkina Faso. Some STs that were not assigned to a clonal complex should probably be part of one: ST-9142 and ST-9290 should be assigned to the ST-1494 complex, and ST-9138 should be assigned to the ST-640 complex. Furthermore, ST-9279 and ST-9104 seem to be associated with the ST-624 complex (Fig. 6).

In summary, *N. lactamica* carriage in Burkina Faso showed little geographic and seasonal variation in comparison with that seen for carriage of *N. meningitidis*. Carriage of *N. lactamica* remained stable after the MenAfriVac vaccine introduction and was not affected by changing meningococcal carriage or by the epidemic context. The genetic diversity of *N. lactamica* isolates was high, and similarities with carriage isolates from the United Kingdom were documented. In contrast to major differences in the epidemiology of *N. meningitidis* carriage and disease, the epidemiology and the molecular characteristics of carried of *N. lactamica* isolates in Burkina Faso were similar to those reported from industrialized countries.

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