AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

RESEARCH ARTICLE

Neisseria meningitidis Serogroup C Clonal Complex 10217 Outbreak in West Kpendjal Prefecture, Togo 2019

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ABSTRACT Togo has reported seasonal meningitis outbreaks caused by non-*Neisseria meningitidis* serogroup A (NmA) pathogens since the introduction of meningococcal serogroup A conjugate vaccine (MACV, MenAfriVac) in 2014. From 2016 to 2017, NmW caused several outbreaks. In early 2019, a NmC outbreak was detected in the Savanes region of Togo and its investigation is described here. Under case-based surveillance, epidemiological and clinical data, and cerebrospinal fluid specimens were collected for every suspected case of meningitis. Specimens were tested for meningitis pathogens using confirmatory microbiological and molecular methods. During epidemic weeks 9 to 15, 199 cases were reported, with 179 specimens being available for testing and 174 specimens (97.2%) were tested by at least one confirmatory method. The NmC was the predominant pathogen confirmed (93.9%), belonging to sequence type (ST)-9367 of clonal complex (CC) 10217. All NmC cases were localized to the West Kpendjal district of the Savanes region with attack rates ranging from 4.1 to 18.8 per 100,000 population and case fatality rates ranging up to 2.2% during weeks 9 to 15. Of the 93 NmC confirmed cases, 63.4% were males and 88.2% were in the 5 to 29 age group. This is the first report of a NmC meningitis outbreak in Togo. The changing epidemiology of bacterial meningitis in the meningitis belt post-MACV highlights the importance of monitoring of emerging strain and country preparedness for outbreaks in the region.

IMPORTANCE The recent emergence of an invasive NmC strain in Togo is an example of the changing bacterial meningitis epidemiology in the meningitis belt post-MACV. The current epidemiology includes the regional circulation of various non-NmA serogroups, which emphasizes the need for effective molecular surveillance, laboratory diagnosis, and a multivalent vaccine that is effective against all serogroups in circulation.

KEYWORDS Togo, outbreak, meningitis, *Neisseria meningitidis*, NmC, Togo

The African meningitis belt, a region of sub-Saharan Africa stretching from Senegal to Ethiopia, has the highest incidence of meningitis globally to date (1–3). In this region, countries experience major epidemics every 7 to 12 years as well as a seasonal increase in cases, usually during the dry seasons (2, 3). Historically, most epidemics in the region were caused by *Neisseria meningitidis* serogroup A (NmA) until the introduction of meningococcal serogroup A conjugate vaccine (MACV, MenAfriVac) beginning in 2010 (4). Since then, NmA epidemics have been eliminated, while non-NmA epidemics remain a significant burden in the meningitis belt countries (4, 5).

Editor John M. Atack, Griffith University

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The authors declare no conflict of interest.

Received 15 October 2021
Accepted 1 February 2022
Published 2 March 2022

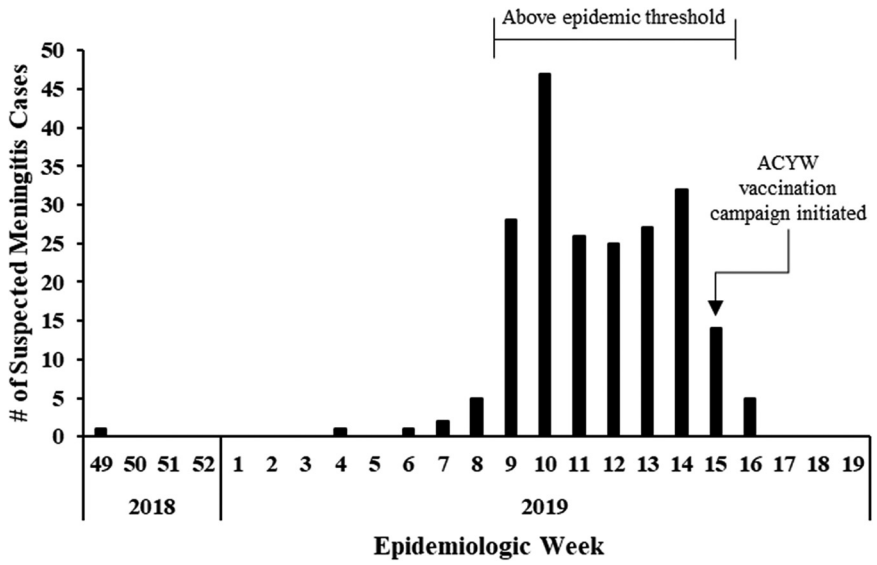

FIG 1 Epidemiologic curve of 2019 meningitis outbreak in the West Kpendjal district of Togo.

In 2013, a new strain of meningococcal serogroup C (NmC), belonging to sequence type ST-10217, emerged in Nigeria (6). Between 2013 and 2017 this unique clone spread to Niger, Liberia (7), and across the Northern States of Nigeria, causing large outbreaks with attack rates comparable to those of historic NmA epidemics (8). In 2015 and 2017, a WHO committee of experts reviewed the emergence of NmC and concluded that risk of NmC epidemics was likely to persist in Nigeria, Niger, and neighboring countries, because of very low level immunity to NmC among children; moreover, NmX and NmY may also pose the ongoing risk of epidemics in the region (9, 10).

Several non-NmA bacterial meningitis outbreaks have occurred in the West African country of Togo since the introduction of MACV in 2014 (11). Three of Togo's northern regions (Savanes, Kara, and Centrale) are located within the meningitis belt and individuals from these regions were included in the country's MACV mass vaccination campaign, in addition to the Plateaux region bordering Centrale (12). The first post-MACV outbreak occurred in 2016, affecting all three northern regions, with nearly 2,000 suspected cases reported. Most laboratory confirmed cases were identified as NmW (4, 12). The following year, two smaller outbreaks occurred, $<$250 suspected cases combined, and NmW was determined to be the predominant pathogen (4). The NmW isolates from the 2016–2017 outbreaks belonged to ST-/clonal complex (CC) 11. More recently, Togo experienced its first NmC outbreak in the Savanes region in 2019 and its investigation is discussed herein.

RESULTS

Meningitis outbreak in the West Kpendjal district. From January, epidemiologic week 1, to late February, epidemiologic week 8 of 2019, the number of suspected cases ranged from 0 to 5 and 1 to 9 per week in the West Kpendjal district and Savanes region, respectively (Fig. 1). During week 9, the West Kpendjal district, one of seven districts in the Savanes region, reported 28 suspected cases surpassing the epidemic threshold (10 per 100,000 population) and marked the beginning of an outbreak. West Kpendjal district remained above the epidemic threshold until epidemiologic week 15. A mass vaccination campaign began with the meningococcal ACYW-polysaccharide vaccine (Menomune) during epidemiologic week 15 and ended during epidemiologic week 16 (April 11 to 15), with over 190,000 doses of vaccine approved for administration by the International Coordinating Group. Cases began to decrease thereafter, and by week 18 the outbreak was declared over. The campaign targeted persons aged 2 to 29 in West Kpendjal district, Kpendjal district, and three cantons of the Oti district

TABLE 1 Laboratory results of specimens received and tested at the regional and national laboratories[a]

No. specimens received by level (%)	Epidemiologic weeks 9 to 15: *n* = 199 suspected cases with 99 confirmed cases							
	Regional						National	
No. specimens tested by test (%)	179 (89.4) Gram stain[b]		LAT		Culture[c]		168 (93.9) rt-PCR	
	179 (100.0)		121 (68.0)		144 (80.4)		168 (100.0)	
Result by test (%)	DGP	2 (1.1)	NmC	41 (33.9)	NmC	30 (21.0)	NmC	92 (54.8)
	DGN	176 (98.3)	NmX	0 (0.0)	NmX	0 (0.0)	NmX	1 (0.6)
	BGN	1 (0.6)	Sp	3 (2.5)	Sp	1 (0.7)	Sp	4 (2.4)
	Neg	0 (0.0)	Hib	1 (0.8)	Hib	1 (0.7)	Hib	1 (0.6)
			Neg	76 (62.8)	Neg	112 (77.8)	Neg	70 (41.7)
No. specimens confirmed by test (%)	Gram stain[b]		LAT		Culture[c]		rt-PCR	
	179 (100.0)		45 (37.2)		32 (22.2)		98 (58.3)	
Result by test (%)	DGP	2 (1.1)	NmC	41 (91.1)	NmC	30 (93.8)	NmC	92 (93.9)
	DGN	176 (98.3)	NmX	0 (0.0)	NmX	0 (0.0)	NmX	1 (1.0)
	BGN	1 (0.6)	Sp	3 (6.7)	Sp	1 (3.1)	Sp	4 (4.1)
			Hib	1 (2.2)	Hib	1 (3.1)	Hib	1 (1.0)

[a]No., number; Pos., positive; LAT, latex agglutination test; rt-PCR, real-time PCR; DGP, diplococcus Gram positive; DGN, diplococcus Gram negative; BGN, bacillus Gram negative; NmC, *Neisseria meningitidis* serogroup C; NmX, *Neisseria meningitidis* serogroup X; Sp, *Streptococcus pneumoniae*; Hib, *Haemophilus influenzae* type b; Neg, negative.
[b]Gram stain is not a confirmatory method.
[c]Bacterial colonies obtained from culture were tested by slide agglutination.

(Galangashi, Nagbéni, and Kankangou). A total of 162,284 individuals were vaccinated during this campaign.

Laboratory results of specimens received and tested at the regional and national laboratories. From epidemiologic weeks 1 to 19, 213 suspected meningitis cases were reported and 199 (93.4%) occurred during epidemic weeks 9 to 15 (Table 1). The regional laboratory received 179 cerebrospinal fluid (CSF) specimens (89.9%) and tested 179 (100.0%) by Gram stain, 121 (68.0%) by latex agglutination testing (LAT), and 144 (80.4%) by culture. Gram stain identified diplococci Gram negative (DGN) bacteria in 176 (98.3%) of the specimens followed by diplococci Gram positive in two (1.1%) specimens and bacillus Gram negative in one (0.6%) specimen. The LAT identified NmC in 41 (33.9%) specimens, *Streptococcus pneumoniae* (Sp) in three (2.5%) specimens, *Haemophilus influenzae* type b (Hib) in one (0.8%) specimen, and the remaining 76 (62.8%) specimens were negative. Similarly, NmC (30; 21.0%), Sp (1; 0.7%), and Hib (1, 0.7%) were predominantly identified by culture; while 112 (77.8%) were negative. Of the specimens confirmed as positive for at least one meningitis pathogen, the majority were NmC (91.1%) by LAT and NmC (93.8%) by culture. At the national level, 168 (93.9%%) specimens were received and all were tested by rt-PCR. Overall, a total of 174 specimens were tested at least by one confirmatory method. The five remaining specimens were only tested by Gram stain and were all DGN. Among the 174 specimens tested, 93 (53.45%) were NmC, 4 (2.3%) were Sp, 1 (0.6%) was NmX, 1 (0.6%) was Hib, and 75 (43.10%) were negative. Of the specimens confirmed as positive by rt-PCR, 93.9% were NmC.

Characteristics of confirmed cases. During epidemic weeks 9 to 15 a total of 99 cases were laboratory confirmed, all from the West Kpendjal prefecture (Table 2). Among these, NmC was the major pathogen detected and was determined to be the leading cause of the outbreak. The majority of NmC cases during epidemic weeks 9 to 15, were male (59; 63.4%) and in the 5 to 29 year age range (82; 88.2%) with a 93.9% survival outcome. The attack rate for NmC cases during epidemic weeks 9 to 15 ranged from 4.1 to 18.8 per 100,000 population, peaking at week 12 and the case fatality ratio during this period ranged from 0.0% to 2.2% (Table 3).

Molecular characterization of the NmC outbreak strain. Whole genome sequencing of 15 NmC isolates revealed all belonged to ST-9367 within clonal complex (CC) 10217. The 15 NmC isolates also had identical PorA, FetA, and PorB types: P1.21-15,16-

TABLE 2 Characteristics of West Kpendjal confirmed cases during epidemic weeks 9 to 15

Epidemiologic weeks 9 to 15: West Kpendjal district confirmed cases (n = 99)[a]			
No. by pathogen (%)			
NmC	93 (93.9)	Sp	4 (4.0)
NmX	1 (1.0)	Hib	1(1.0)
Epidemiologic weeks 9 to 15: West Kpendjal NmC confirmed cases (n = 93)			
No. by week (%)			
9	13 (14.1)	13	12 (13.0)
10	13 (14.1)	14	10 (10.9)
11	17 (18.3)	15	5 (5.4)
12	23 (25.0)		
No. by sex (%) (n = 93 NmC)			
Male	59 (63.4)	Female	34 (36.6)
No. by age group (years) (%) (n = 93 NmC)			
<1	0 (0.0)	10–14	33 (35.5)
1–4	4 (4.3)	15–29	28 (30.1)
5–9	21 (22.6)	≥30	7 (7.5)
No. by outcome (%) (n = 99 confirmed cases)[b]			
Survived	93 (93.9)		Died 4 (4.0) Unknown 2 (2.0%)

[a]Data includes one case confirmed (NmC) by latex agglutination test and culture only (rt-PCR not done).
[b]Data not reported for two confirmed cases.

46, F1-7, and 3-463, respectively. The phylogenetic tree comparing the 15 Togo CC10217 isolates against invasive CC10217 isolates collected from meningitis belt countries is shown in Fig. 2. The Togo isolates were distinct from other CC10217 isolates collected from West Africa over the recent years. The Togo isolates formed their own subclade within a clade containing only an additional single isolate from Niger collected in 2017 (13) which shared the same sequence type. This clade shares a common ancestor with the BL16188 carriage isolate collected from Burkina Faso in 2012 (14). The genetic and phylogenetic characteristics of the isolates agree with those obtained by culture-free sequencing of CSF specimens using selective whole genome amplification from meningitis cases of the same outbreak (15).

DISCUSSION

This is the first report of a non-NmW meningitis outbreak taking place in Togo since the introduction of MACV. Localized to the West Kpendjal district of the Savanes region, the NmC ST-9367 (CC10217) outbreak spanned several weeks and included 199 suspected cases during epidemic weeks 9 to 15. During this period, the majority of confirmed cases fell within the 5 to 29 year age range, similar to other NmC ST-10217 clonal complex outbreaks (6, 16), underscoring the importance of targeting individuals within this age range during vaccination campaigns. There were also a higher number

TABLE 3 West Kpendjal district attack rate and case fatality ratio among NmC confirmed cases during epidemic weeks 9 to 15

Epidemiologic weeks 9 to 15: West Kpendjal district		
Week	**Attack rate[a]**	**Case fatality ratio[b] (%)**
9	10.6	1.1
10	10.6	2.2
11	13.1	0.0
12	18.8	1.1
13	9.8	0.0
14	8.2	0.0
15	4.1	0.0

[a]Attack rate per 100,000 population.
[b]Data not reported for two confirmed cases.

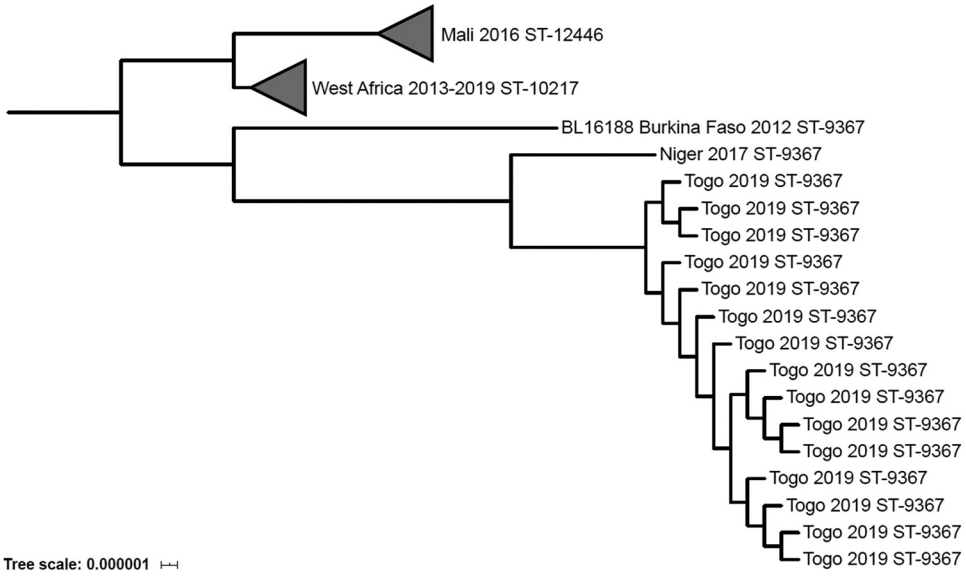

FIG 2 Phylogenetic analysis of the *Neisseria meningitidis* serogroup C outbreak strains collected from Togo and other meningitis belt countries between 2013 and 2019.

of male confirmed cases, outnumbering the female, with the ratio of ~1.7:1. The finding is consistent with the previous report from Niger (17); however, it is unclear whether the males affected during this outbreak had an increased risk of acquiring meningitis and/or were more likely to seek medical care.

Although the NmC outbreak was a novel occurrence for Togo, NmC strains from ST-10217/CC10217 have been the cause of meningitis outbreaks in northwest Nigeria and Niger since 2013 and 2015, respectively (4, 6, 13, 16). Molecular surveillance detected the emergence of ST-9367 in Niger among invasive isolates to monitor regional epidemiology in 2017 (13). The other known ST-9367 strain is a non-groupable isolate identified during a 2012 carriage study in Burkina Faso and thought to be the originating strain of ST-10217 (14, 18). The appearance of CC10217 in Togo is a clear indicator of its expansion in the region.

A very low case fatality rate (0% to 2.2%) was observed during this outbreak compared to other NmC outbreaks in Nigeria (e.g., CFR = 10%). This observation may be explained by the early detection of the epidemic and a well-established patient care strategy in Togo. Sub-districts are divided in three surveillance zones, each comprising specific care centers for identification of complicated cases that may be rapidly, after a first dose of intravenous antibiotic, redirected toward higher-level hospital centers if needed. This approach facilitates timely delivery of appropriate patient care and improves patient outcome.

Once the epidemic threshold has been crossed in a district or sub-district and the Nm genogroup responsible for the outbreak is preventable by vaccination, it is essential that a vaccination campaign is conducted promptly (typically within 4 weeks of crossing the epidemic threshold) in both the population affected and any adjacent district considered to be at risk. Despite the high-quality surveillance conducted in Togo and a rapid laboratory confirmation of the NmC pathogen at the national level during this outbreak, challenges remain. A 6-week lag time was observed between the crossing of epidemic threshold (week 9) and the start of the vaccination campaign (week 15) due to data transmission issues at the district level which delayed the submission of the vaccination request to WHO.

Tracking the emergence and spread of circulating strains within the meningitis belt through molecular surveillance can inform country preparedness efforts by identifying areas proximal to where epidemic strains are detected. Establishing or strengthening national and regional level laboratory capacity building for meningitis diagnosis in these high-risk

areas will be the critical next step for effective laboratory-based outbreak response. Togo's CRL began collaborating with partners in 2015 to strengthen laboratory capacity for the molecular detection of bacterial meningitis pathogens and recently enrolled in an external quality assurance program to ensure the reporting of quality laboratory results (19). The CRL's ability to rapidly test 100% of the specimens received by the laboratory during the outbreak highlights the value of strengthening country laboratory systems in the meningitis belt. Future plans include laboratory capacity building at the regional level within a country, beginning in areas at high risk for bacterial meningitis.

MATERIALS AND METHODS

Meningitis surveillance. The Savanes region implemented case-based surveillance (CBS) for bacterial meningitis as a part of MenAfriNet in 2014 (12, 20). For CBS, epidemiological data, clinical data, and CSF specimens are collected for all suspected cases of meningitis (20, 21). A suspected case is defined as any person with sudden onset of fever (>38.5°C rectal or 38.0 axillary) and one of the following signs: neck stiffness, altered consciousness, or other meningeal signs; and any toddler with sudden onset of fever (>38.5 C rectal or 38.0 axillary) and one of the following signs: neck stiffness, or flaccid neck, bulging fontanel, convulsion, or other meningeal signs (22, 23). A confirmed case is defined as a suspected case in which *Neisseria meningitidis*, *Haemophilus influenzae*, or *Streptococcus pneumoniae* has been identified in CSF by culture, real-time PCR (rt-PCR), or latex agglutination test (22). Ethics approval and participant consent was not necessary because specimens were collected for purposes of disease surveillance by TOGO Ministry of Health and were deidentified before shipping to the Centers for Disease Control and Prevention ([CDC] Atlanta, United States).

Laboratory testing. Peripheral health centers collected CSF specimens from each suspected case of meningitis, following WHO guidelines (24), and referred them to the Dapaong Regional Hospital for Gram staining, LAT (Pastorex Meningitis and Wellcogen Bacterial Antigen Kit) and/or culture (Table S1). The latex agglutination testing was performed directly from the fresh CSF specimens while the culture was carried out either from the fresh CSF or inoculated trans-isolate medium. Bacterial colonies obtained from culture were tested by slide agglutination. After testing at regional laboratory, the CSFs were sent to the National institute of Hygiene, Central Reference Laboratory (CRL) for testing by rt-PCR. Capsular groups A, C, W, and Y were tested by latex agglutination and all six invasive serogroups (A, B, C, W, X, and Y) were tested by rt-PCR. Gram stain is not a confirmatory method.

Whole genome sequencing. DNA was extracted from 15 outbreak *N. meningitidis* isolates, using the Gentra Puregene Yeast/Bact kit (Qiagen, USA), processed for library preparation by NEBNext Ultra I DNA Library Prep Kit (New England Biolabs, USA) and sequenced on an Illumina MiSeq system using the 500-cycle V2 kit (Illumina Inc, USA) in accordance with the manufacturer's protocol. The whole procedure was performed at the Centers for Disease Control and Prevention.

Data analysis. The attack rate was calculated using the 2019 estimated population of West Kpendjal prefecture, 122,409 (MOHSP, 2019; unpublished data). For molecular characterization, the PubMLST Genome Comparator tool was used to analyze multilocus sequence typing (MLST) genes. Porin A (PorA), Ferric enterobactin transport (FetA), and Porin B (PorB) types were defined, as previously described (25).

For the phylogenetic analysis, Togo outbreak isolates determined to be CC10217 were compared against 216 invasive CC10217 isolates collected from meningitis belt countries between 2013 and 2019 (Table S2). Each genome was mapped against a reference CC10217 isolate collected from Niger in 2015 to generate a core SNP alignment using Snippy (https://github.com/tseemann/snippy). The core SNP alignment had predicted recombination events masked using Gubbins (26), and the resulting alignment was used as input for RAxML (27) with ascertainment bias correction to generate a maximum-likelihood phylogenetic tree.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

We thank members of the Centers for Disease Control and Prevention Bacterial Meningitis Laboratory, U.S. (particularly Daya Marasini and Christine Lascols for their technical support and assistance) and National Institute of Hygiene, Togo who have contributed to this work.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the United States Centers for Disease Control and Prevention.

This study was funded by CDC and Gavi, the Vaccine Alliance.

All authors have no conflicts of interest to declare.

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