Scientific Research Publishing

Advances in Infectious Diseases, 2023, 13, 66-76
https://www.scirp.org/journal/aid
ISSN Online: 2164-2656
ISSN Print: 2164-2648

Meningitis Outbreak Caused by *Neisseria meningitidis* Serogroup C ST 10217 in 2019 in Diapaga, Burkina Faso

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How to cite this paper: Dinanibè, K., Issa, T., Gautier, O.W.H., Mahamoudou, S., Wilfrid, O.L.S.L., Mahamoudou, S., Mamadou, T., Moumouni, Z., Oumarou, O., Azaque, Z.A., Rebeca, C., Tani, S., Sylvie, Z., Théophile, S.R.S., Flavien, A., Lassana, S., Isaïe, M., Séni, K. and Rasmata, O.-T. (2023) Meningitis Outbreak Caused by *Neisseria meningitidis* Serogroup C ST 10217 in 2019 in Diapaga, Burkina Faso. *Advances in Infectious Diseases*, **13**, 66-76.
https://doi.org/10.4236/aid.2023.131008

Received: August 4, 2022
Accepted: March 3, 2023
Published: March 6, 2023

Abstract

Introduction: Meningitis caused by *Neisseria meningitidis* constitutes a burden for the countries in the meningitis belt of sub-Saharan in general and particularly for Burkina Faso. In 2019 the Diapaga health district experienced a meningitis epidemic due to *N. meningitidis* serogroup C. **Methods:** This is a cross-sectional study with a descriptive aim in the health district of Diapaga where all cases of meningitis were included in this work. Rapid diagnostic tests (RDTs), culture as well as real-time PCR were used for the biological analysis of cerebral spinal fluid (CSF) samples. **Results:** Of 155 CSF samples analysed, 42% (65/155) were tested positve. Of them, *N. meningitidis* C accounted for 83% of all positive cases. Likewise, all thirteen (13) NmC strains were susceptible to oxacillin, ceftriaxone, penicillin and chloramphenicol. All strains of NmC belonged to the sequence type (ST) 10 217 and to the clonal complex (CC) 10 217. These CCs belonged to the same variant PorA type: P1.21-15.16; FetA type: F1-7; PorB type: 3-463. **Conclusion:** Burkina Faso had known an epidemic of meningitis caused by NmC in 2019 in the health district of Diapaga. This outbreak was contained in time due to the performance of the epidemiological surveillance system which made it possible to investigate on time and introduce the vaccine against the pathogen NmC.

Keywords

Meningitis, *Neisseria meningitidis* VS, Diapaga, Burkina Faso

1. Introduction

Acute purulent bacterial meningitis remains a priority in countries located in the meningitis belt of sub-Saharan Africa [1]. These meningitis were generally caused by three major pathogens, namely *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae* serotype b. *Neisseria meningitidis* is the most involved in meningitis epidemics. These meningitis, which can occur cyclically, are mainly due to *N. meningitidis* serogroup A [2] [3]. However, in 2001 in Burkina Faso, cases of meningitis had been caused by *N. meningitidis* serogroup W (NmW) had been reported with the occurrence in 2002 of an epidemic of NmW with 14,455 suspected cases including 1743 deaths [4] [5]. In addition, in 2010, Burkina Faso recorded a large epidemic of meningitis caused by *N. meningitidis* serogroup X with an annual incidence of 120 cases/100,000 inhabitants [6]. In short, from 2010 to 2015, meningitis caused by *N. meningitidis* W represented 14.39% according to data collected in Burkina Faso population health protection department.

To counter these epidemics, strong decisions had been taken. Thus, in January 2006, the anti-*Haemophilus influenzae* b vaccine had been introduced into the Expanded Program on Immunization (EPI) [2]. In December 2010, the MenAfriVac® conjugate vaccine had been used in mass vaccination against meningococcal serogroup A in subjects aged 1 to 29 years [2] [3]. This vaccination campaign against *N. meningitidis* serogroup A had remarkably changed the etiological landscape of the bacterial agents responsible for the outbreaks of meningitis epidemics in Burkina Faso [3]. From 2011 to 2013, based on laboratory data, pneumococcal meningitis had been accounted for 53% of cases followed by 44% of meningococcal meningitis [7]. This new configuration of the causative agents of meningitis had led to the introduction of the 13-valent pneumococcal conjugate vaccine in children aged 0 to 11 months.

The signs of the occurrence of an epidemic of meningitis caused by *N. meningitidis* serogroup C (NmC) in Burkina Faso were visible especially with the epidemiological situation in Niger and Nigeria. Indeed, in 2015, it had been described in Niger, as an epidemic of meningitis with Nm C [8]. Also, between November 2016 and June 2017, it had been reported as a deadly outbreak in NmC in Nigeria with 12,535 suspected cases and 877 deaths [9].

From January 2019, the first cases of NmC meningitis had been recorded in Diapaga health district and this resulted in an epidemic. The objective of this work is to contribute to a better understanding of the *N. meningitidis* serogroup C meningitis epidemic that occurred in 2019 in Diapaga health district in eastern of Burkina Faso.

2. Materials and Methods

2.1. Study Site and Sampling

From December, 2018 to April, 2019, a descriptive cross-sectional survey that had systematically included 155 cerebro-spinal fluid (CSF) samples taken from patients of Diapaga health district. So, these samples had been stored in cryotubes at −20˚C, in eastern Burkina Faso had been sent to the National Meningitis Reference Laboratory (LNRm) at the Charles De Gaulle Pediatric University Hospital Center (CHU-PDG) for analysis as part of the case-by-case surveillance of meningitis. All patients with suspected meningitis and who had lombar ponction had been included in this survey. The explained variable is the case of menigitis caused by *Neisseria meningitidis* C (NmC). The others variables are age groups, health facilities, weekly NmC cases, NmC sequence and patient meningitis vaccine status. The study population age groups are [0 - 1 year],]1 - 5 years],]5 - 10 years],]10 - 15 years],]15 - 20 years],]20 years and more] and not specified age groups. In fact, the "Not specified age groups" had included all the patients whom the age groups were not filled in the record book. The samples had been routed, either in a cryotube at 4˚C or in a trans-isolate (TI) at room temperature.

2.2. Laboratory Analysis

At LNRm, samples had been analyzed by culture and/or polymerase chain reaction (PCR).

- **Culture:** Of the 155 CSF samples sent to LNRm, only 87 CSF had been inoculated at random into the TI for culture on behalf of lack of TI media at that specific moment. Unfortunately, the rest had not been inoculated as explained above 1) When turbidity or colonies had appeared on the slope of the TI, subculture had been carried out on chocolate agar (Biomérieux, Canada) supplemented with polyvitex. 2) If growing on chocolate agar, fresh state and Gram stain is performed. 3) For Gram-negative cocci evoking meningococci, oxidase (Becton-Dickinson and company, 7 Loveton Circle Sparks, MD21152 USA), ortho-nitrophenyl β-D-galactoside (ONPG) and gamma-glutamyl aminopeptidase (GGT) (Reynolds St., Stamford Texas) have been completed. Strains of *N. meningitidis* are ONPG negative and GGT positive. For the determination of the serogroup, anti-sera were used for *N. meningitidis* A, *N. meningitidis* W135, *N. meningitidis* X, *N. meningitidis* Y and *N. meningitidis* C (Remel Europe Ltd, Dartford/UK). 4) For Gram-positive cocci evoking pneumococci, the optochin test was performed. Pneumococci have an inhibition diameter which is greater than or equal to 14 mm. 5) As for the polymorphic Gram-negative bacilli evoking *H. influenzae* b, a bacterial suspension of the suspect strain is produced which is seeded on HD agar and the discs of factors X, V, XV are deposited separately. There is no growth around factors X and V taken separately, but growth around factor XV is observed, thus highlighting *H. influenzae* b. 6) The antibiotics susceptibility testing had been performed on 13 *N. meningitidis* strains iso-

lates in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM) 2015 which was adopted in Burkina Faso. The antibiotics tested were ceftriaxone (30 µg), chloramphenicol (30 µg), penicillin G (1 IU) and oxacillin (5 µg).

- **Real-time PCR:** One hundred and fifty-five (155) samples had been analyzed by RT-PCR for the detection of meningococcus and its serogroups; *H. influenzae* b and pneumococcus by targeting *sodC* genes for meningococci, *hpd3* for *H. influenzae* and *lytA* for *S. pneumoniae* as previously described by Xin *et al.*, and Sanou *et al.* [10] [11], and *lytA* by Carvalho *et al.*, Pai *et al.* [12] [13] with some non-major modifications (Table 1). Rt-PCR amplification had been performed on the thermal cycler Stratagene 3005P RT-PCR MxPro from a total volume of 23 µL containing 12.5 µL of Universal Taqman master mix (Applied Biosystems, USA), 2 µL of each primer, 2 µL of DNA (4 ng), 2 µL of probes labeled with FAM fluorochromes (Taqman type (Biosystems, USA)) and 4.5 µL of water. The amplification schedule was: 50˚C for 2 minutes, denaturation at 95˚C for 10 minutes followed by 50 cycles including denaturation at 95˚C for 15 seconds and hybridization at 60˚C for 1 minute. The results are interpreted as follows: Ct ≤ 35: positive results for the bacterial species considered; 35 < Ct ≤ 40: equivocal results and for Ct > 40: negative results.

3. Results

3.1. Socio-Demographic Characteristics of Patients

Within the health facilities of Diapaga sanitary district, 20 health and social promotion centers (CSPS) were affected by the meningitis epidemic. The CSPS of Kogoli de Botou was the most affected with 10 CSF routed. The samples delivery time is between 3 and 21 days. The CSPS of Kogoli of Botou was the most affected health center by the epidemic with 92 cases notified as shown in Figure 1 below.

Table 1. List of primers used for the detection of bacterial species and meningococcal serogroups.

Bacteria	Target gene	Serogroup	Sense primers	Anti primers-sense	Probes
N. meningitidis	*sodC*	(Nm)	F351: GCACACTTAGGTGAT TTACCTGCA	R478: CCACCCGTGTGGATCATA ATAGA	Pb387: CATGATGGCACAGCAA-CAAATC CTGTTT5'FAM, 3'BHQ
	csaB	NmA	F2531: AAAATTCAATGGGTATATCACGAA-GA	R2624: TATGGTGCAAG CTGGTTTCAATAG	Pb2591i: CTAAAAG- "T" AGG AAGGGCACTTTGTGGCATAAT5'FAM; 3'SpC6; "T" BHQ1
	csc	NmC	F478: CCCTGAGTATGCGAAAAAAATT	R551: TGCTAATCCCGCCTGAATG	Pb495i: TTTCAATGC"T" AATGAATACC-ACCGTTTTTTTGC5'FAM; 3'SpC6; "T" BHQ1
	csw	NmW	F857: TATTTATGGAAGGCATGGTGTATG	R964: TTGCCATTCCAGA AATATCACC	Pb907i: AAATATGGAGCGAA "T" GATTACAGTAACTATAATGAA5'FAM, 3'SpC6, "T" BHQ1,
	csxB	NmX	F173: TGTCCCCAAC-CGTTTATTGG	R237: TGCTGCTATCATAGCCGCC	Pb196i: TGTTTGCCC-A CATGAATGGCGGFAM; 3'BHQ
	csy	NmY	F787: TCCGAGCAGGAAATTTATGAGAATAC	R929: TTGCTAAAATCATTCGCTCCATAT	Pb1099i: TATGGTG "T-" ACGATATCCCTAT-CCTTGCCTATAAT5'FAM; 3'SpC6; "T" BHQ1
H. influenzae b	*hpd3* (Hi)	Hi	hpdF822: GGTTAAATATGCCGATGGTGTTG	hpdF952: TGCATCTTTACGCACGGTGTA	Pb896i: TTGTGTACACTCCGT "T" GGTAAAAGAACTTGCAC
	bcsC	Hib	F192: TGATGCATTGAAAGAAGGT-GTAATTT	R359: CCTGCGGTAATAACATGATC-ATAAA	Pb244i: TGTCGTGCAGTAGCAAACCGTAACCTTACTC
S. pneumoniae	*lytA*	(S.pn)	F373: ACGCAATCTAGCAGA TGA -AGC A	R424: TCGTGCGTTTTAATTCCAGCT	Pb400: TGCCGAAAACGC "T" TGATACAGGGAG5'FAM; "T" BHQ; 3'SpC6

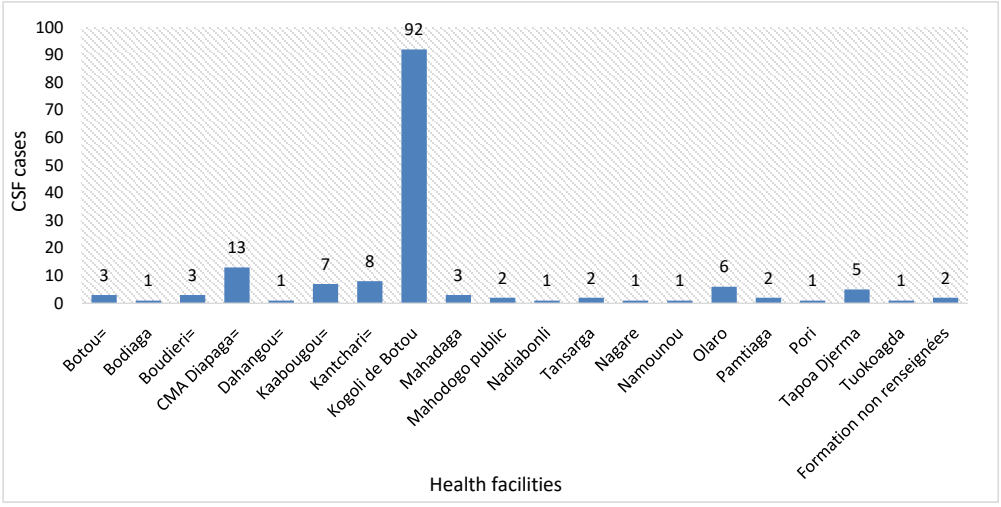

Figure 1. Distribution of cases of CSF collected by CSPS.

At the end of the distribution of the cases taken by CSPS, it is useful to have an idea of the distribution of the same samples by age groups. Indeed, the age group of 20 years and over above has experienced the most notified cases at the level of in the district of Diapaga. **Figure 2** below, gives this breakdown.

Of all the analyzed CSF at the mNLR, 58% (90/155) of them were negative while 42% (65/155) were positive cases. **Figure 2** below gives the frequencies of pathogens identified by RT-PCR. Note that NmC represents 83% of the positive cases identified.

Figure 3 gives the distribution of the results of pathogens taken by age groups. Age group of 5 to 10 years is the most affected by NmC.

The temporal distribution of the results of CSF analyzed by PCR from week 53 (S53) of 2018 to S17 of 2019. The number of positive cases of NmC was most reported from S4 to S9.

3.2. Antibiotics Susceptibility Testing

Thirteen (13) NmC strains underwent antibiotics susceptibility testing.

Of all the samples of CSF inoculated into the trans-isolales (TI), 13% (11/87) gave a positive culture for *N. meningitidis* serogroup C. All the strains were susceptible to chloramphenicol, to ceftriaxone, oxacillin and penicillin G. However, 26% (23/87) of the TIs were contaminated and 61% (53/87) were negative.

3.3. Description of the Typical Sequences of the Strains

Table 2 gives the description of the standard sequences of the strains of *N. meningitidis* C determined by Multi Locus Sequence Typing (MLST) at the Center for Diseases Control and Prevention (CDC) in Atlanta in the United States of America (USA) from six (6) strains and five (5) CSF. **Table 2** indicates that most of the NmC strains sequenced belong to the Sequence Type (ST) 10 217. All these STs belong to the clonal complex (CC) 10 217. These CCs belong to the

same variant PorA type: P1.21-15.16; FetA type: F1-7; PorB type: 3-463.

3.4. Vaccination Status of Patients

In terms of vaccination status, of the 155 patients who underwent CSF sampling, only 39 were vaccinated. Of those vaccinated, 59% (23/39) received *Haemophilus influenzae* b vaccine, 18% (7/39) received tetravalent NmACWY vaccine, 10% (4/39) received MenAfriVac® A conjugate vaccine, 8% (3/39) were vaccinated with MenAfriVac® and *Haemophilus influenzae* b vaccine and 5% (2/39) received PCV13 and *Haemophilus influenzae* b vaccine.

Unfortunately, one vaccinated person with the NmACWY tetravalent vaccine had been infected by NmC pathogen

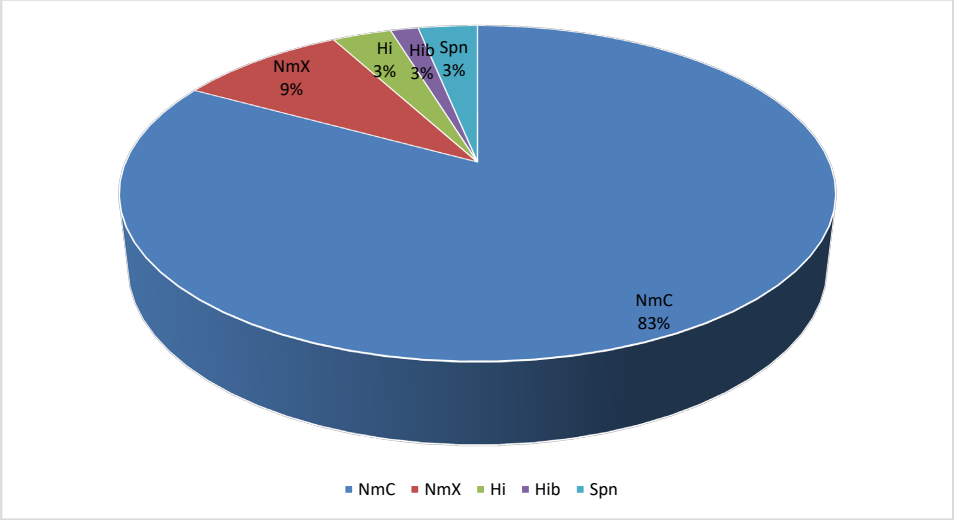

Figure 2. Frequencies of identified pathogens by real-time PCR.

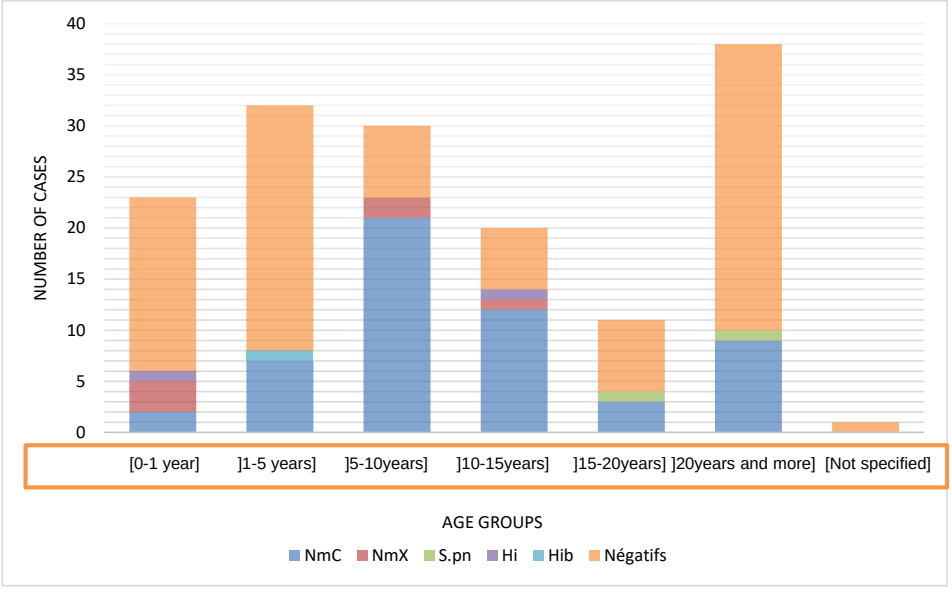

Figure 3. Distribution of CSF pathogens collected by age groups.

Table 2. Results of NmC sequenced with MLST.

CDC ID	Issuer ID	Collection Date	Sample Type	Organism (SG/ST)	ST MLST	CC MLST	Other results
M50549	MEN10055A	01/28/2019	CSF	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50552	MEN10079A	01/29/2019	CSF	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50568	MEN10064A	2/3/2019	CSF	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50576	MEN12170A	2/11/2019	CSF	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50581	MEN12166A	2/7/2019	CSF	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50589	MEN12151A	02/17/2019	Isolate	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50590	MEN12153A	02/19/2019	Isolate	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50591	MEN12155A	02/22/2019	Isolate	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50592	MEN12156A	02/22/2019	Isolate	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50593	MEN12174A	02/24/2019	Isolate	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463
M50594	MEN12190A	02/15/2019	Isolate	*Neisseria meningitidis* serogroup C	10217	CC10217	PorA type: P1.21-15,16FetA type: F1-7PorB type: 3-463

4. Discussions

Burkina Faso had experienced for the second time in 2019, an epidemic of meningitis caused by *N. meningitidis* serogroup C (NmC) after the first one recorded in 1979 which had also touched the Eastern part of the country with 539 cases including 55 deaths [14]. Now, it's particularly Diapaga health district which recorded the NmC outbreak, as mentionned in **Figures 1-4**, a neighboring district of Niger which itself experienced a NmC epidemic in 2015 [8]; as shown in **Figure 5** below.

Also, in 2013 and 2014, Nigeria had respectively experienced an epidemic of NmC meningitis in the state of Sokoto and another of a low amplitude in the state of Kebbi [15]. As we are concerned, within the district of Diapaga, there is a large disparity in the occurrence of the epidemic. Indeed, the CSPS of Kogoli of Botou recorded the highest number of cases unlike many others CSPS which did not record any case. This situation is explained by the fact that this health facility is on the border with the Republic of Niger which had a meningitis epidemic in the past due to the same meningococcal serogroup [8], this is justified by the fact that there is the mixing of populations from both two countries because populations are very closed and have many trading and social activities together like naming ceremonies which potentially increased the risk of contamination.

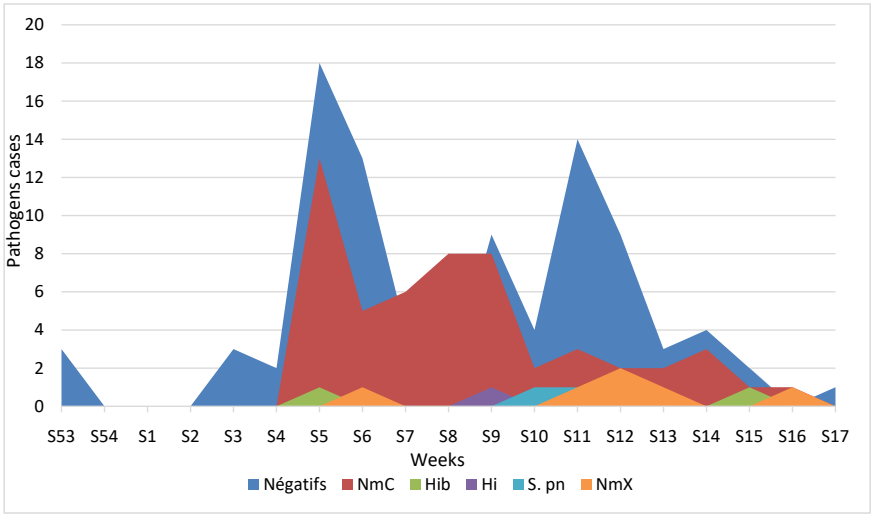

Figure 4. The different pathogens identified according to weekly distribution.

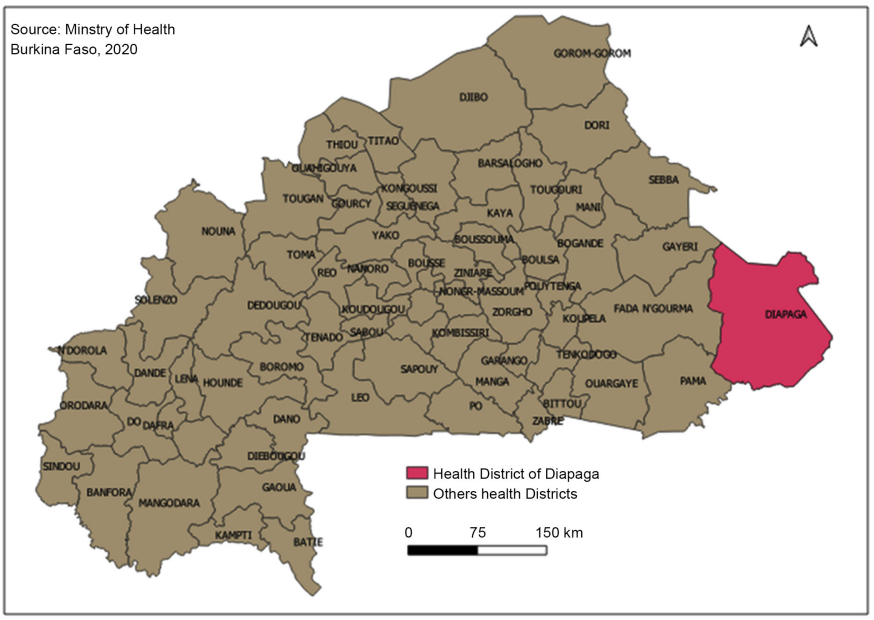

Figure 5. Map of Burkina Faso with the Diapaga health district in red.

By considering the distribution of NmC by age group, our results had shown that the age group of [5 - 10 years] was the most affected followed by that of [10 - 15 years] as mentionned in **Figure 3**. Also, from the cumulative ages, we noticed the same situation as that which had already been observed by Funk and Collaborators in Nigeria in 2013 in Sokoto State during the NmC epidemic [15]. However, we stick to the facts because we had not done any standardization. This is why we reserve the right to make comparisons.

Moreover, this situation allows us to perceive that after vaccination with the MenAfriVac® conjugate vaccine in December 2010 against *N. meningitidis serogroup* A, Burkina Faso is now experiencing the phenomenon of replacement in the circulation of meningococci like in Nigeria and in Niger as shown in **Fig-**

ure 2 and Figure 4 [8] [15]. Indeed, since Burkina Faso had introduced the MenAfriVac® vaccine, any case of NmA had been already notified. This situation had showed that how much the country could save money and could protect population by vaccinating the target people. However, these new forms of meningitis caused by meningococcal serogroup C, X and W had showed some low amplitude compared to those formerly caused by meningococcal serogroup A [5]. Also, among the seven people vaccinated with tetravalent NmACWY vaccine and having meningitis sickness, we had the time between vaccination and the onset of the disease in one person with a timeline of 6 days. This implies that vaccination requires some few days to allow the production of antibodies which will be effective with the protective effect in the vaccinated persons. This assumes that the other conditions such as vaccine quality and storage are insured.

By analyzing the data obtained at MLST as shown in Table 2, we found that the meningitis epidemic in the Diapaga health district is caused by ST 10217 of the clonal complex 10217 (CC10217) and the same variant PorA type: P1.21-15.16; FetA type: F1-7; PorB type: 3-463 than one already described in Niger and Nigeria [8] [9]. This is a proof that actions to fight against diseases with potential epidemic risk must be concerted because the occurrence of the disease in one country constitutes a threat for others due to the movement of populations for survival needs, trade or any other similar activities. The silver lining is that the NmC strains that have benefited the antibiotics susceptibility testing show that the antibiotics used in our country remain active. Even we got some appreciate results, some few limitations could be mentioned. Indeed, these samples were collected by the meningitis surveillance system in Diapaga health district during an outbreak in 2019. Then, we only collected systematically samples from all suspected patients of meningitis. Elsewhere, we didn't calculate a sample size because we only collected data for political decision during the outbreak of the meningitis. Then, we didn't have a nationwide data but only for the district of Diapaga.

5. Conclusion

The fight against the occurrence of meningitis due to the different meningococcal serogroups is essentially based on continuity in epidemiological surveillance. This must be done with the effective contribution of the various surveillance actors without whom no response can be effective. These different interactions made it possible to control the expansion of the NmC epidemic in the Diapaga health district in the eastern region of Burkina Faso in 2019. Finally, the fight against meningitis requires, concerted actions between countries and at best, between contiguous districts at the border level.

6. Recommendations

At the end of this work, we make the following recommendations for an effective fight against meningitis. To the Ministry of Health, to provide the decon-

centrated structures of the Ministry with the necessary tools for a good collection and better analysis of data in real time. Also, make available to the region, the necessary inputs for biological diagnosis and management of meningitis. Elsewhere, educate the population on the means of prevention against meningitis and in case of illness, go as quickly as possible to a health centre for appropriate treatment. To the Regional Director of Health for the East, support the districts in the preparedness of response plans to possible meningitis epidemics. Also, ensure that inputs are available in the health facilities in his health area for the efficient management of patients. To the district chief medical officer, ensure the continuous training of nurses on CSF sampling and the management of meningitis cases. Finally, the population should follow the advice of health personal.

Authors Contributions

Conceptualization: KD, ROT, KS, OWHG;
Data curation: KD, TI;
Formal analysis: KD;
Investigation: KD, OLSLW, SM, ZM;
Resources: ROT, MI, AF, SL;
Supervision: ROT IM LM VS RTN CVB;
Validation: ROT, AF, SL, MI, KS;
Visualization: KD, TI, OWHG, TM, SM, OO, ZAA, CR, ST, ZS, SRST;
Writing ± original draft: KD, TI;
Writing ± review & editing: KD, TI, OWHG.

Acknowledgements

We would like to thank the Ministry of health of Burkina Faso Government for all their supports. We also thank the chief of the Population Health Protection Branch, the Governor of the Eastern Region, the Regional Director of Health in the East, the Chief Medical Officer of the Diapaga Health District and his entire team. We also have a special thought for all the victims of this disease. Finally, we express our sympathy to the families who have suffered from the occurrence of meningitis and the team the supervision in Diapaga health district.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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