Hindawi
Advances in Public Health
Volume 2020, Article ID 1932940, 7 pages
https://doi.org/10.1155/2020/1932940

Research Article

Epidemiological Characteristics of Meningococcal Meningitis (2016 to 2018) Four Years after the Introduction of Serogroup A Meningococcal Conjugate Vaccine in Benin

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Received 13 February 2020; Revised 3 August 2020; Accepted 11 August 2020; Published 19 August 2020

Academic Editor: Carol J. Burns

Objectives. This study aims to study the epidemiological and geographic characteristics of the meningococcal serogroups four years after the introduction of serogroup A meningococcal conjugate vaccine. *Methods*. This is a prospective, descriptive, analytical study, and it took place from 2016 to 2018. Cerebrospinal fluid (CSF) samples were taken after the identification of meningitis cases. The samples, thus, taken were sent to the laboratory for culture and identification of *Neisseria meningitidis* in accordance with WHO standards. *Results*. Eight hundred and ninety-nine bacterial strains were identified, of which 219 were strains of *Neisseria meningitidis*. The majority of *N. meningitidis*-positive samples were from male patients (59.8%) with a median age of 4 (IQR: 1–13). Four of *N. meningitidis* serogroups were identified, namely, serogroups C (6.8%), W (19.6%), X (1.8%), and A (0.5%). Geographically, 92.7% of the identified *N. meningitidis* serogroups came from patients who lived in the northern region of the country. The departments most concerned were Alibori (*N. meningitidis* C (66.7%) and *N. meningitidis* W (20.9%)); Atacora (*N. meningitidis* W (41.9%), *N. meningitidis* X (75.0%), and *N. meningitidis* C (13.3%)); and Borgou (*N. meningitidis* W (23.3%)). *Conclusion*. The results of this study showed that there is an emergence of cases of meningococcal of serogroup C four years after the introduction of MenAfricVac in Benin. These results demonstrated the effectiveness of case-by-case surveillance in detecting small changes in the distribution of serogroups that could have important implications for public health strategies in the coming seasons.

1. Introduction

A Gram-negative diplococcus, *Neisseria meningitidis*, is one of the main etiologies of meningitis and sepsis [1], with a mortality history of about 80% among cases registered at the beginning of the first epidemic [2]. Even with the availability of effective antibiotics, meningococcal meningitis is frequently associated with a mortality rate of around 15% [3, 4]. According to the World Health Organization (WHO), approximately 500,000 cases and 50,000 deaths are attributed to *N. meningitidis* each year worldwide [5]. *N. meningitidis* has 12 capsular serogroups, and the majority of serogroups A, B, C, W, Y, and X are responsible for life-threatening invasive meningococcal disease [3, 6].

The burden of meningococcal meningitis is disproportionately higher in the African meningitis belt, with a higher incidence in children under 5 years of age [7]. The most predominant form in Africa is the meningococcus of serogroup A, with 85% of cases, especially in the first thirty years [1]. The disease is serious and can be fatal without adequate antibiotic therapy. Bacterial meningitis can quickly become fatal and cause severe disability in those who survive. Survivors may have complications such as cognitive impairment, behavioral problems, hearing loss, motor weakness, paralysis, incoordination, or seizure disorder; although little data are available in low-resource settings [8], one study found that, up to a quarter of bacterial meningitis survivors had neuropsychological sequelae 3 to 60 months after discharge from hospital [8, 9]. Therefore, prompt treatment is recommended, especially in children [10].

In Benin, the incidence of meningitis was 71 to 619 per 100,000 inhabitants in 2004 with a fatality rate of 3.1% [11]. The epidemic control measures consisted of reactive vaccination campaigns in the epidemic departments, using the polysaccharide vaccines A/C/W or A/C/W/Y combined with the adapted treatment protocol [12]. To detect epidemics, routine surveillance of suspected cases of acute bacterial meningitis is carried out in Benin and in all the other countries of the African meningitis belt according to WHO recommendations [13], although the recommendation to divide large districts of more than 100,000 inhabitants into subdistricts of approximately 30,000–100,000 inhabitants is not always followed. Districts reporting weekly incidences of 3 cases per 100,000 inhabitants for a week are considered to be on alert, and those with incidences of 10 cases per 100,000 inhabitants are considered to be epidemic [14]. In 2003, the World Health Organization and the meningitis vaccination project initiated a project to support Benin and other countries in the establishment of a reinforced meningitis surveillance network [15]. After the introduction of the meningococcal A conjugate vaccine (PsA-TT, MenAfriVac), implemented in Benin in 2012 through mass campaigns targeting people aged 1 to 29, the incidence of meningitis in this serogroup has greatly decreased [12]. Four years after the introduction of MenAfricVac, what about the incidence of meningococcal of *Neisseria meningitidis* A and what is the share of other serogroups in cases of meningococcal meningitis in Benin? It is to answer these questions that this study was initiated. The aim is to study the epidemiological and geographic characteristics of meningococcal meningitis in Benin and to assess the impact of MenAfricVac on the other meningococcal serogroup.

2. Materials and Methods

2.1. Study Framework. This study was carried out in the Republic of Benin, a coastal country in West Africa, with an area of 114,763 km^2, with an average annual temperature ranging from 26°C to 28°C. The population was estimated at 11,496,140 inhabitants in 2018 with 51.2% women. With its pyramidal structure, the health system of Benin is modeled on the administrative division of the country. It is made up of a peripheral level, which is represented by the health zone whose manager is the Coordinating Doctor of the Health Zone of an intermediate level represented by the department with as responsible, the Departmental Director of Health at the central level, and it is represented by the Ministry of Health [16].

2.2. Methods. This study was carried out on the basis of data collected from suspected meningitis cases notified by epidemiological surveillance centers throughout Benin. The study period was from January 1, 2016 to December 31, 2018.

2.2.1. Data Collection. Data collection was carried out according to the method described by Godjedo et al. [16]. Patients with high onset fever, stiff neck and/or altered consciousness, and/or other meningeal signs were included in the study. After the identification of the suspected case, the cerebrospinal fluid (CSF) sample was taken and conveyed to the hospital laboratory for analysis. Each sample taken is accompanied by the Integrated Disease Surveillance and Response (IDSR) notification form. On this sheet, the social, clinical, and epidemiological information of the patient was recorded.

In the event that the zone hospital did not have a bacteriology section within its laboratory, the samples were placed on transport medium (Trans-Isolate) and sent to the departmental laboratories having a bacteriology session within 48 hours. All samples are, then, sent to the National Public Health Laboratory (NPHL) for confirmation and identification of the bacterial species.

2.2.2. Bacteriological Analysis of CSF Samples. The bacteriological analyzes were carried out according to the methodology described by WHO [7] and repeated by Godjedo et al. [16]. After macroscopic examination of the 2,928 samples, the Gram stain followed. The samples were, then, streaked on Columbia blood agar and chocolate agar plates and incubated at 37°C anaerobically for 48 hours to isolate the pure colonies of *N. meningitidis*.

All cocci Gram-negative, catalase, and cytochrome oxidase strains were selected, and serogrouping of *N. meningitidis* species was performed by the latex agglutination test. Latex agglutination was carried out using a Pastorex meningitis kit (Bio-Rad) making it possible to detect the *N. meningitidis* antigens of serogroups A, B, C, Y, and W, and a kit of Directigen meningitis (Becton Dickinson) was used to detect *N. meningitidis* antigens from serogroups X and Z. The kits were used in accordance with the manufacturer's instructions.

2.2.3. Statistical Analysis. The epidemiological data of the patients were recorded in an Excel spreadsheet and were analyzed with the R software. The graphs were produced with the Graphpad Prism 7 software.

3. Results

3.1. General Characteristics of Patients. Bacteriological analysis of CSF samples showed that out of the 2928 samples, 899 bacterial strains were identified, of which 24.4% (219) were strains of *N. meningitidis*. The majority of these *N. meningitidis*-positive CSF samples had a cloudy appearance (83.1%). It should be noted that the patients whose CSF samples were positive for *N. meningitidis* were predominantly male (59.8%) and lived in the northern region (92.7%). The median age of these patients was 4 years with an interquartile range (IQR) from 1 to 13 years. The category of patients (Table 1) aged 0 to 4 years was the most represented (45.4%).

3.2. Proportion of N. meningitidis Serogroups. Four serogroups of *N. meningitidis* have been identified. These are serogroups A, C, W, and X. Serogroup W (Table 1) was the most represented (19.6%), followed by serogroup C (6.8%), serogroup X (1.8), and serogroup A (0.5%). For the study period, the results showed that there were more patients positive for *N. meningitidis* serogroup W in 2017 (44.4%) than in 2018 (33.3%) and 2016 (22, 2%). Serogroups C and W were much more identified throughout the study period. The highest incidence of meningococcal meningitis is that of *N. meningitidis* W (0.14) in 2017. It increased slightly from 2016 to 2017 and decreased slightly from 2017 to 2018. It was in 2017 that the first cases of group C meningococcal meningitis were detected with an incidence of 0.09. This incidence decreased in 2018 (0.04). Also, in 2017, we noted cases of group X meningococcal meningitis with a constant incidence from 2017 to 2018 (0.02). On the other hand, there is a total disappearance of *N. meningitidis* A meningitis from 2017 to 2018 (Figure 1). The age category of patients 6 to 18 years of age is that in which a high proportion (Table 1) of *N. meningitidis* C (46.7%), W (65.1%) and X (75%) with respective median ages of 15 (IQR: 2–25), 9 (IQR: 2–36), and 6 (IQR: 4.5–7.75).

3.3. Geographic Distribution of N. meningitidis Serogroups. The results showed that the majority of *N. meningitidis* serogroups (A, C, W, and X) (92.1%) were identified in patients living in the northern region of the country (Table 1). It should be noted that the region of northern Benin is subdivided into four departments (Alibori, Atacora, Borgou, and Donga). The results from Figure 2 have shown that, in Alibori, there is a predominance of serogroups C ($n = 10$) and W ($n = 9$), in Atacora, the predominance is observed at the level of serogroups W ($n = 18$), X ($n = 3$), and X ($n = 15$), and in the Borgou, serogroup W ($n = 10$) is still the most represented (Figure 2).

3.4. Impact of Vaccination on the Disease. Of the 219 patients confirmed positive for *N. meningitidis*, 33 revealed that they did not know their vaccination status vis-à-vis MenAfricVac in 2012 (Men A conjugate vaccine), 21 said they were not vaccinated, and 9 claimed to have been vaccinated. The patients confirmed positive for *N. meningitidis* serogroups W (53.5%) and C (46.7%) were those whose vaccination status was unknown (Figure 3). Deaths were reported only in the latter with case fatality rates ranging from 9.3 (4/43 for *N. meningitidis* W) to 40% (6/15 for *N. meningitidis* C).

4. Discussion

The introduction in 2012 of MenAfriVac had a beneficial effect on the incidence of confirmed meningitis cases with *N. meningitidis* A. An almost total disappearance was observed in confirmed cases of *N. meningitidis* A, with four cases occurring in 2015 [12]. The results of our surveillance showed a prevalence of 2.15% of meningococcal meningitis from 2016 to 2018. This percentage is lower than that found at the same period in Niger (84.8%) and in Chad (33.2%) [17, 18], both belonging to the African meningitis belt unlike Benin where the northern and central regions are the part found there.

The majority of *N. meningitidis*-positive cases (22.7%) in this study was aged 0 to 4 years and lived in the northern region of the country. Thus, this high percentage of meningococcal meningitis cases is explained by the immature immune system of children and, hence, the health vulnerability of the latter in the face of *N. meningitidis*. This vulnerability of children to meningococcal meningitis has also been noted in several countries (Burkina-Faso, Chad, Mali, Niger, and Togo) of the African meningitis belt [19]. The northern of Benin is characterized by a rainy season (May to October) and a dry season with harmattan (November to April). During the dry season, heat, dry, and dusty wind during the day increases the risk of communicable respiratory diseases [20, 21]. This region is the part of Benin that is found in the African meningitis belt. This is what justifies the fact that the majority of patients living in this region were confirmed positive for *N. meningitidis*. Sidikou et al. showed that, from 2010 to 2018, meningococcal meningitis had a very high incidence in the regions (Niamey, Tillabery, Dosses, Tahoua, and Maradi) which also belong to the African meningitis belt [17].

Serogrouping of isolated *N. meningitidis* strains had shown a high proportion of *N. meningitidis* W (19.6%), the majority of which had been identified during the 2017 epidemiological surveillance (44.4%). These results are in the same direction as those obtained by Sidikou et al. [17] and Mounkoro et al. [22]. From 2016 to 2018, the work of Sidikou et al. [17] revealed a high proportion of *N. meningitidis* W (71%) in Burkina-Faso. Similarly, the work of Mounkoro et al. [22] showed a high proportion of *N. meningitidis* W (32.2%) responsible for the epidemic in Togo in 2016. Apart from the predominance of *N. meningitidis* W, in 2017, it there has been an occurrence of type C meningococcal meningitis. These results are consistent with those obtained by Sidikou et al. [17]. From 2016 to 2018, the work of Sidikou et al. [17] revealed a high proportion of the species of *N. meningitidis* C with a predominance in 2017. Our results show that six years after the introduction of the MenAfricVac conjugate vaccine (2011-2012), the epidemiology of meningococcal meningitis in

Table 1: Frequencies of *N. meningitidis* serogroups stratified by the geographic region, age, appearance of CSF samples, and sex in the Benin republic, 2016–2018.

Characteristics	Number of CSF samples (%)	Number of pathogens identified (%)	Number of *N. meningitidis* strain identified (%)	Number of ungrouped *N. meningitidis* strain	Number of *N. meningitidis* serogroups (%)			
					C	A	W	X
Total	2928 (100)	899 (30.7)	219 (24.4)	156 (71.2)	15 (6.8)	1 (0.5)	43 (19.6)	4 (1.8)
Appearance of CSF samples								
Clear	1594 (54.4)	129 (14.3)	24 (11.0)	14 (9.0)	3 (20.0)	0 (0.0)	6 (14.0)	1 (25.0)
Turbid	875 (29.9)	674 (75.0)	182 (83.1)	131 (84.0)	11 (73.3)	1 (100.0)	36 (83.7)	3 (75.0)
Hemorragic	199 (6.8)	38 (4.2)	1 (0.4)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Xanthochromic	112 (3.8)	52 (5.8)	10 (4.6)	8 (5.1)	1 (6.7)	0 (0.0)	1 (2.3)	0 (0.0)
Unknown	148 (5.1)	6 (0.7)	2 (0.9)	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sex								
Male	1639 (56.0)	531 (59.1)	131 (59.8)	92 (59.0)	11 (73.3)	1 (100.0)	24 (55.8)	3 (75.0)
Female	1285 (43.9)	366 (40.7)	87 (39.7)	63 (40.4)	4 (26.7)	—	19 (44.2)	1 (25.0)
Unknown	4 (0.1)	2 (0.2)	1 (0.5)	1 (0.6)	—	—	—	—
Region								
Northern	2907 (99.3)	889 (98.9)	203 (92.7)	145 (92.9)	12 (80.0)	1 (100.0)	42 (97.7)	3 (75.0)
Center	6 (0.2)	3 (0.3)	9 (4.1)	6 (3.8)	2 (13.3)	—	—	1 (25.0)
Southern	15 (0.5)	7 (0.8)	7 (3.2)	5 (3.2)	1 (6.7)	—	1 (2.3)	—
Age (years)	$n = 1965$	$n = 591$	$n = 150$	$n = 87$	$n = 15$	$n = 1$	$n = 43$	$n = 4$
Median (IQR)	4 (1–90)	4 (1–90)	4 (1–13)	6 (4–43)	15 (2–25)	—	9 (2–36)	6 (4.5–7.75)
Age category (Years)								
0–4	892 (45.4)	143 (24.2)	34 (22.7)	14 (16.1)	05 (33.3)	—	14 (32.6)	1 (25.0)
5–9	355 (18.1)	112 (19.0)	28 (18.7)	14 (16.1)	03 (20.0)	—	09 (20.9)	02 (50.0)
10–14	184 (9.4)	77 (13.0)	26 (17.3)	17 (19.5)	04 (26.7)	01 (100.0)	03 (7.0)	01 (25.0)
15–19	106 (5.4)	58 (9.8)	16 (10.7)	14 (16.1)	—	—	02 (4.7)	—
20–24	56 (2.8)	23 (3.9)	03 (2.0)	—	—	—	03 (7.0)	—
25–29	65 (3.3)	35 (6.0)	09 (6.0)	5 (5.7)	—	—	04 (9.3)	—
30–34	57 (2.9)	28 (4.7)	05 (3.3)	—	—	—	05 (11.6)	—
35–39	64 (3.3)	34 (5.8)	05 (3.3)	02 (2.3)	—	—	03 (7.0)	—
40–44	30 (1.5)	13 (2.2)	03 (2.0)	03 (3.4)	—	—	—	—
45–49	47 (2.4)	19 (3.2)	05 (3.3)	05 (5.7)	—	—	—	—
50–54	20 (1.0)	10 (1.7)	05 (3.3)	02 (2.3)	03 (20.0)	—	—	—
55–59	39 (2.0)	21 (3.6)	06 (4.0)	06 (6.9)	—	—	—	—
60–64	13 (0.6)	02 (0.3)	01 (0.7)	01 (1.2)	—	—	—	—
65–69	23 (1.2)	07 (1.2)	03 (2.0)	03 (3.4)	—	—	—	—
≥70	14 (0.7)	09 (1.5)	01 (0.7)	01 (1.2)	—	—	—	—

Benin was marked by the disappearance of cases of *N. meningitidis* A meningitis, an emergence of cases of *N. meningitidis* C and *N. meningitidis* X, and a consistency of the cases of *N. meningitidis* W. As part of this epidemiological surveillance, no case of *N. meningitidis* A was reported in 2018, justifying the continued effectiveness of

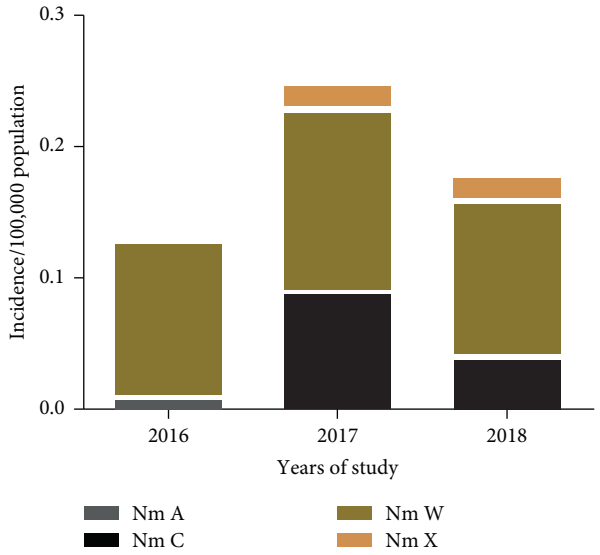

Figure 1: Incidence (per 100,000 inhabitants) of the different *N. meningitidis* serogroups in the Republic of Benin from 2016 to 2018.

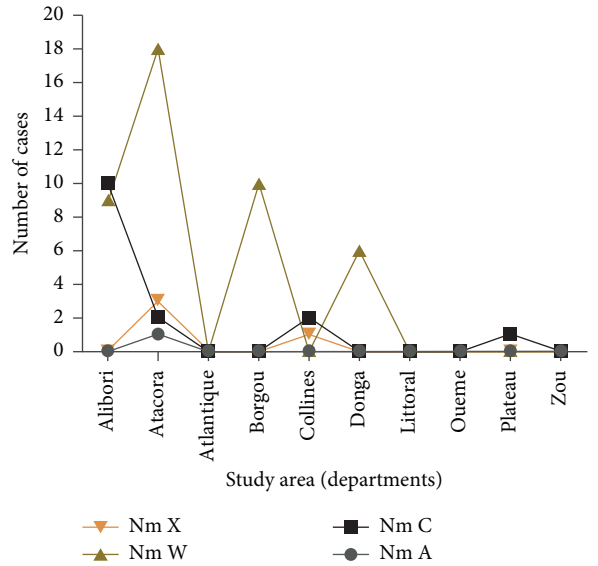

Figure 2: Distribution of the frequency of *N. meningitidis* serogroups according to the departments in the Republic of Benin from 2016 to 2018.

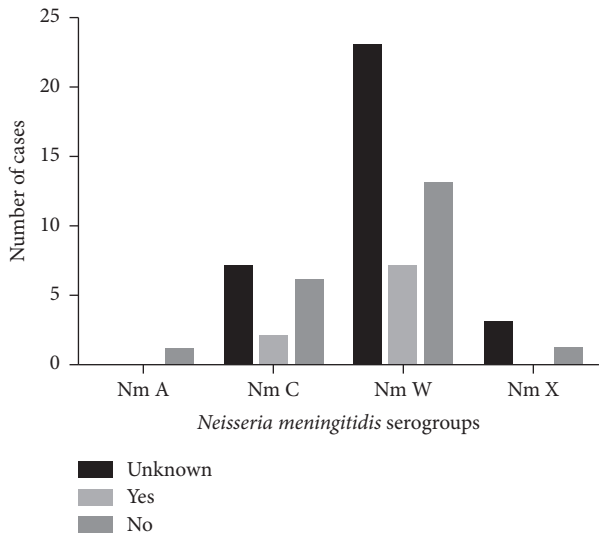

Figure 3: Frequency of the different serogroups of *N. meningitidis* according to the immunization status in the Republic of Benin from 2016 to 2018.

MenAfricVac. These results support other analyses that show the remarkable and continuous impact of MenAfricVac on carriage and meningococcal meningitis type A throughout the meningitis belt [23]. Combined with the high acceptance by the community [24], the low cost of the vaccine, and the long duration of protective immunity [25] (shorter in children aged <5 years [26]), these results provide convincing evidence of the continuous deployment of MenAfricVac in Benin and in high-risk countries within or at the periphery of the meningitis belt, via mass immunization and the introduction into the systematic vaccination system of children aged 9 to 18 months [27, 28]. This remarkable disappearance of *N. meningitidis* A disease has been observed in other countries which have introduced MenAfricVac [29]. The circulation of *N. meningitidis* serogroups (C, W, and X) has also been observed in other countries in the meningitis belt which also introduced MenAfricVac: Niger [17], Burkina- Faso, Chad, Mali [19, 23], and Côte d'Ivoire [30].

Geographically, the northern region is made up of the departments of Alibori, Atacora, Borgou, and Donga, and the department of Collines are the departments most involved in the surveillance of meningitis in Benin. This study showed that there is a distribution of serogroups of *N. meningitidis* which is not proportional within these five departments belonging to the African meningitis belt. This disproportionality could be explained by the lack of health structure and health surveillance in certain departments and, especially, the diversity of sociocultural and economic habits from one department to another. *N. meningitidis* C was in high proportion in the patients of the departments of Alibori (66.7%), while *N. meningitidis* W was found in high proportion (Figure 2) in patients living in the departments of Atacora (41.9%), Borgou (23.3%), Alibori (20.9%), and Collines (14.0%) (Figure 2). As for *N. meningitidis* X, it had been found in 75% patients living in the Department of Collines. These results further confirm the emergence of meningococcal meningococcus from serogroups C and X.

When analyzing the data, small proportions of specific meningococcal serogroups were detected in the departments not belonging to the African meningitis (*N. meningitidis* C (6.7%) and *N. meningitidis* W (1.2%) in the department of Plateau). In Benin, from 2016 to 2018, Godjedo et al. have already shown that, with the case-by-case surveillance system, there had been cases of meningitis and death in the department of the Plateau (12.5% fatality) [16]. This demonstrates that strong surveillance systems are capable of detecting small changes in the distribution of serogroups

that could have important implications for public health strategies in the coming seasons.

It should be noted that more than half (52.4%) of the patients identified positive with one of the four serogroups of *N. meningitidis* identified do not know their vaccination status and 33.3% (21/63) of them had declared not be vaccinated. A high proportion of *N. meningitidis* W-positive patients (53.5%) with an unknown vaccination status and 30.2% (13/43) were noted. However, the lethality was lower in these patients (9.3%) than in those positive for *N. meningitidis* C (40.0%). This shows the importance of vaccination campaigns in the fight against meningococcal meningitis in Benin.

During this study, a very high percentage (71.2%) of cases of *N. meningitidis* were not grouped (Table 1). This high percentage is due to the numerous polyagglutinable and incompletely identified strains. This could be due to the conditions and processing of CSF samples from sampling, through transport and storage of these before diagnosis. Suboptimal sample storage, transport, processing, and handling practices (for example, multiple freeze-thaw cycles) can negatively affect the quality of samples and lead to nonconclusive results when tested by culture, latex, and/or rt-PCR [18]. We must also add that the cold chain breaks sometimes during the storage of latex tests (Pastorex) due to the lack of availability of electrical energy. This can lead to the ineffectiveness of these tests during diagnosis and, hence, the multitude of cases of ungrouped *N. meningitidis*. As a result, the total number of serogroup cases (A, C, W, and X) obtained in this study does not really reflect the incidence of meningococcal meningitis. As a limitation of the epidemiological surveillance system for meningitis in Benin, our study showed the absence on-site of an rt-PCR machine for the diagnosis of the disease. However, several authors showed that rt-PCR is the most appropriate and reliable diagnostic or confirmation method [31, 32].

5. Conclusions

The results of this study showed that six years after the introduction of MenAfricVac, there has been a remarkable decrease or even disappearance of cases of meningitis with *N. meningitidis* A and an emergence of cases of meningitis with *N. meningitidis* C with several deaths in 2017. At the same time, there was an insignificant decrease in *N. meningitidis* W meningitis from 2017 to 2018. This investigation confirmed the strong presence of meningococcal meningitis cases in the northern region of Benin. However, cases of meningococcal C, W, and X meningitis have been noted in the southern region (with deaths). This underlines the importance of setting up an efficient surveillance system at the national level and strengthening the capacities of laboratories to make in-depth diagnoses (PCR and sequencing), to support the development and introduction of the next generation of meningococcal meningitis vaccines in Benin and sub-Saharan Africa.

Data Availability

Data can be obtained from the corresponding author on request.

Ethical Approval

In Benin, ethical approval was not required for routine surveillance of meningitis, as it had been approved as part of the systematic diagnosis algorithm in the Ministry of Health.

Consent

Informed consent was sought from parents or guardians of surveillance participants.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

TPG, MNP, AJA, and HB wrote the protocol. TPG, AJA, and MH were involved in field work, protocol implementation, data analysis, and interpretation and wrote the draft of the manuscript. AJA and TVD made substantial contributions to conception and study design, data analysis, and interpretation. MNP, LB-M, and HB participated in the critical revision of the manuscript.

Acknowledgments

The authors thank all the staff of the epidemiological surveillance service of the Ministry of Health of Benin and all the health personnel who participated in this surveillance. The authors also thank Dr. Lawaly Manzo for his contribution to the writing of this manuscript.

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