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Serotypes of Bacteria Encountered in Childhood Purulent Meningitis in Children in Parakou (Benin) in 2011

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Abstract

Introduction: In the North-Benin, there are three agents causing pediatric purulent meningitis outside the neonatal period. These are: *Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae type b.* The aim of this research work was to investigate bacteria serotypes that caused childhood purulent meningitis in the pediatric unit of the Borgou à Regional University Teaching Hospital (CHUD-Borgou) located in Parakou (North-Benin). Patients and Methods: Through a prospective and descriptive study centered on children aged 0 to 5 years old suspected of meningitis and hospitalized, the cerebrospinal fluid (CSF) samples of those children were analyzed at the WHO reference laboratory in Banjul for serotyping by real time polymerase chain reaction (RT/PCR). Results: Among the 1396 children hospitalized during that period, 366 were suspected of meningitis and had benefitted from lumbar puncture. Among those 366 suspected cases, 51 cases of purulent meningitis were confirmed after CSF cytobacteriological and biochemical test at the CHUD-Borgou laboratory. Among 51 CSF samples in which purulent meningitis was con-

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firmed, 44 were sent to Banjul. In addition, 310 CSF samples from non-confirmed cases of meningitis were also sent to Banjul. In the whole set of samples sent for real time PCR, 151 cases of *Streptococcus pneumoniae* (42.7%) were found, 5 cases of *Neisseria meningitidis* (1.4%) and 1 case of *Haemophilus influenzae* (0.3%) were also encountered. As regards *Streptococcus pneumonia*, the serotypes encountered were: 1, 3, 4, 5, 7F, 8, 9V, 9V/9A, 9N/9L, 14, 18C, 19A, 23F, 33F as well as non typed and non typable serotypes. As for *Neisseria meningitidis*, only serogroup A was found in it. For *Haemophilus influenzae*, only serotype b was identified. Conclusion: Four non vaccine serotypes (8, 9V/9A, 9N/9L and 33F), non typed and non typable serotypes which are not covered by 13-valent pneumococcal conjugate vaccine (PCV 13) were identified. This highlights the need to enhance surveillance of pediatric purulent meningitis and serotyping by RT/PCR of all CSF samples in order to adapt if necessary future new pneumococcal vaccines to circulating non vaccine serotypes.

Keywords

Purulent Meningitis, Children, Bacteria, Serotypes, Benin

1. Introduction

Purulent meningitis cases are common in developing countries and are a leading cause of morbidity and mortality in pediatrics.

Three pathogens are found in most cases: *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* [1]-[14].

Despite the advances that have been achieved in the therapy of childhood purulent meningitis, mortality is still high in developing countries. In fact, similar to other authors, Agossou *et al*. noted that purulent meningitis was the most lethal disease of childhood, specailly pneumococcal meningitis [1] [5]-[15]. To fight purulent meningitis, the country of Benin introduced vaccines against *Haemophilus influenzae type b* (Hib) and *Streptococcus pneumonia* in 2005 and 2011, respectively. Moreover, periodic and regular vaccination campaigns against meningococcal diseases have been conducted in the northern regions of countries located in the African meningitis belt. Thus, it has become vital for Benin to develop an efficient mechanism to ensure the surveillance of pediatric bacterial meningitis (PBM) to determine the distribution of serotypes of the main bacteria that cause meningitis to adapt the best preventive strategies. It was in this context that this research work was initiated; this research was performed within the framework of pediatric bacterial meningitis surveillance in the North Benin. Mid and long term, this study will help assess vaccination impact on the incidence of invasive infections due to these main bacteria.

2. Settings, Materials and Methods

2.1. Settings

This study was conducted at the following locations: the CHUD-Borgou pediatric unit and laboratory in Parakou ,the national laboratory in Cotonou and the WHO reference laboratory in Banjul (Gambia). In the CHUD-Borgou pediatric unit, cerebrospinal fluid (CSF) samples were collected, transported to the CHUD-Borgou laboratory and stored in cryotubes according to the guidelines stated in the regulations manual of the network for the surveillance of *Haemophilus influenza*e type b meningitis and other types of bacterial meningitis in pediatric environments (Hib-MBP) [16]. These CSF samples were then transported to the national laboratory in Cotonou before being sent by DHL to the WHO regional reference laboratory in Banjul. In this laboratory, the bacteria that were found in the CSF samples were serotyped by real-time PCR (RT/PCR) according to WHO regulations [17].

***Type and period of study*:** This was a cross-sectional and descriptive study with prospective data collection from August 1 to December 31, 2011.

***Study population*:** The study population consisted of children aged 0 to 5 years old who were hospitalized in the CHUD-Borgou pediatric unit and with clinical signs suspicious for meningitis.

***Inclusion criteria*:** Any child aged zero to five years who was suspected of having meningitis and who benefitted from lumbar puncture was included.

***Study variables*:** The study variables were socio-demographic (age, sex, date of admission, and immunization status of children) and bacteriological, particularly involved pathogens and their serotypes.

2.2. Case Definition

A case was considered to be probable bacterial meningitis in a child under 5 years of age if the child showed the following signs or group of signs: fever, stiffness or sluggishness in the neck nape, bulging anterior fontanel, agitation, irritability, refusal to breastfeed, convulsion or altered state of consciousness with CSF cells greater than or equal to 10 leukocytes/mm^3 (>20/mm^3 in a newborn) with prominent neutrophils, CSF protein > 0.40 g/L and hypoglycorrhachia < 0.4 g/L.

A case was considered confirmed if, in addition to the criteria for probable bacterial meningitis mentioned above, bacteria were identified in the CSF during Gram staining or culture or after the detection of bacterial antigen with latex.

2.3. Procedures

In the pediatric unit, two samples of 40 drops of CSF were collected from each child who was suspected of having meningitis and were then immediately transported to the bacteriology laboratory of CHUD-Borgou. Then, approximately 1 ml of CSF was collected in a cryotube using a sterile serological pipette and stored at −20˚C for PCR. The following tests were performed on the remaining fluid: macroscopic and microscopic examination to determine CSF composition, leukocyte count, and determination of bacteria via Gram staining or identification or isolation of soluble antigen after the seeding of a CSF pellet in a specific location or culture. Regarding biochemical parameters, CSF protein and glycorrhachia (CSF glucose concentration) were measured. After these analyses, the CSF samples that were stored in cryotubes at −20˚C for PCR were transported to the national laboratory and stored in an adequate cold chain. At this level, the samples were stored in a freezer at −30˚C. Because the number of samples was significant, the samples were sent by DHL express mail in triple packaging to the WHO sub-regional reference laboratory in Gambia. Serotyping by real-time PCR 1 (RT/PCR) was performed on all CSF samples. Real-time PCR consisted of the selective amplification of a target specific DNA sequence in a heterogeneous group of DNA sequences. The DNA sequence was exponentially amplified through three major repeated steps. In the first step, double-stranded DNA was denatured into a single-strand. In the second step, the sequence of the target complementary single strand was shaped into a ring. In the third step, under the effect of thermostable DNA polymerase, the DNA sequences were extended unidirectionally from 5' to 3' to produce double-stranded DNA molecules. The DNA molecule was replicated and amplified at each step of extension, thus generating millions of copies of the original DNA molecule. Each copy was identified through fluorescent dyes. Real-time PCR combines amplification and detection into one step using fluorescent dyes. For the identification of *Neisseria meningitidis*, two genes were targeted: ctr A and C sod. The first gene encodes a surface adhesin, and the second encodes superoxide dismutase for copper and zinc. For *Haemophilus influenzae*, the gene encoding a lipoprotein called surface protein D was used. For *Streptococcus pneumoniae*, the genes that were used were those encoding pneumolysin (ply), autolysin (lytA) and pneumococcal surface adhesin (psaA). From these genes, the serotypes and serogroups were identified through specific real-time PCR (RT/PCR) [17]. The data were collected, codified and captured using Epi Data software version 3.1 and analyzed using SPSS software version 16.0. The calculation of simple frequencies was used for sample description.

3. Results

3.1. Sociodemographic Characteristics and Immunization Status of the Children who Were Involved in the Study

We registered 366 suspected cases of meningitis among the 1396 children aged 0 to 5 years who were hospitalized in the unit during the period of the study. The sex ratio was 1.27. **Table 1** shows the distribution according to the age groups of the 366 children suspected of having meningitis. Regarding immunization status, among the 366 children who were involved in the study, 247 received three doses of Hib out of the 297 who had reached the required age (≥14 weeks of life) to be vaccinated according to the Expanded Program on Immunization

(EPI) schedule that is implemented in Benin. The Hib coverage rate of these children was 83.2%. Regarding the meningococcal vaccine, which is not an EPI vaccine in Benin and is administered only to subjects older than two years, one child out of 122 who could be vaccinated was actually vaccinated. No child from the cohort received the pneumococcal conjugate or polysaccharide vaccine.

3.2. Epidemiological Aspects of Pediatric Purulent Meningitis and Serotyping of Bacteria that Were Identified after Real-Time PCR

Among the 1396 children who were hospitalized during the study period, 51 cases met the criteria for confirmed purulent meningitis, *i.e.*, a hospital incidence of 3.7% and a frequency of 13.9% of children with suspected meningitis.

Figure 1 indicates the distribution of confirmed cases of purulent meningitis according to age group and sex. The sex ratio was 1.55.

Figure 2 shows the graph that specifies the native population and distribution of serotypes of the different bacteria that were identified via RT/PCR in the different CSF samples that were collected from subjects who contracted purulent meningitis and from subjects with suspected meningitis that was not confirmed by cyto-bacteriological testing.

Table 2 shows the bacteriological profile of the CSF samples identified via real-time PCR.

Table 1. Distribution of meningitis suspected cases according to age group in CHUD-Borgou pediatric unit from August to December 2011.

Age (months)	Number	%
[0 - 1[	69	18.9
[1 - 12[	107	29.2
[12 - 24[	68	18.6
[24 - 60]	122	33.3
Total	**366**	**100.0**

Table 2. Bacteriological profile of CSF samples after real time PCR.

	Confirmed cases **N = 44**	%	Non-confirmed cases **N = 310**	%	Suspected cases **N = 354**	%
Streptococcus pneumoniae	23	52.3	128	41.3	151	42.6
Neisseria meningitidis	1	2.3	4	1.3	5	1.4
Haemophilus influenzae	1	2.3	0	0.0	1	0.3
Absence of bacteria	19	43.2	178	57.4	197	55.6
Total	**44**	**100**	**310**	**100**	**354**	100

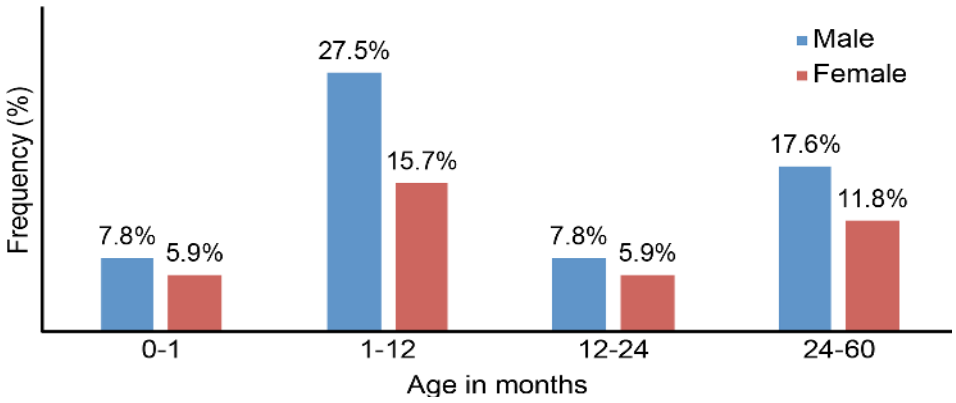

Figure 1. Distribution of confirmed cases of purulent meningitis according to age group and sex in theCHUD-Borgou pediatric unit from August to December 2011.

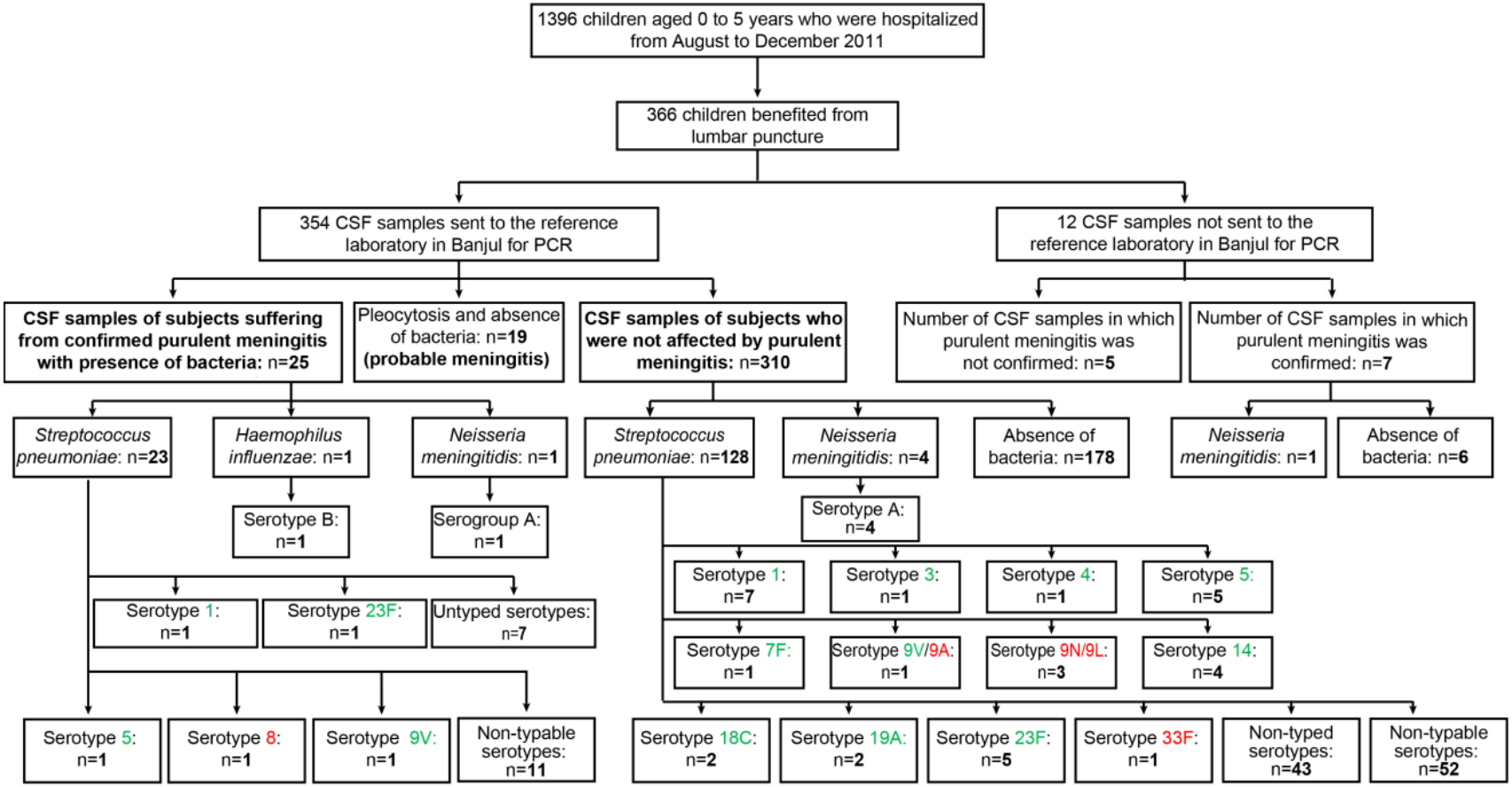

Figure 2. Flow chart indicating the distribution of serotypes according to bacteria found by PCR.

Table 3 indicates the distribution of the *Streptococcus pneumoniae* serotypes that were found in the 151 CSF samples of subjects with confirmed or non-confirmed cases of purulent meningitis, from which *Streptococcus pneumoniae* was isolated via RT/PCR.

Figure 3 shows the distribution of the *Streptococcus pneumoniae* serotypes that were found in CSF samples from which *Streptococcus pneumoniae* was identified via RT/PCR and typed at the WHO Reference Laboratory in Banjul, depending on whether the samples were PCV_{13} (vaccine serotypes) or not(non-vaccine serotypes).

4. Discussion

This study was initiated within the framework of surveillance of pediatric bacterial meningitis (PBM) and aimed to determine the hospital attendance of pediatric purulent meningitis, the different bacteria that were implicated

Table 3. Distribution of *Streptococcus pneumoniae* serotypes isolated by RT/PCR at the WHO regional reference laboratory in Banjul on CSF samples collected from confirmed or non-confirmed purulent meningitis cases.

Serotypes	Cconfirmed cases	%	Non-confirmed cases	%	**Ssuspected cases**	**%**
1	1	4.3	7	5.5	**8**	**5.3**
3	0	0.0	1	0.8	**1**	**0.7**
4	0	0.0	1	0.8	**1**	**0.7**
5	1	4.3	5	3.9	**6**	**3.9**
7F	0	0.0	1	0.8	**1**	**0.7**
8	1	4.3	0	0.0	**1**	**0.7**
9V	1	4.3	0	0.0	**1**	**0.7**
9V/9A	0	0.0	1	0.8	**1**	**0.7**
9N/9L	0	0/0	3	2.3	**3**	**1.9**
14	0	0.0	4	3.1	**4**	**2.6**
18C	0	0.0	2	1.6	**2**	**1.3**
19A	0	0.0	2	1.6	**2**	**1.3**
23F	1	4.3	5	3.9	**6**	**3.9**
33F	0	0.0	1	0.8	**1**	**0.7**
Non-typed*	7	30.4	43	33.6	**50**	**33.1**
Non-typable**	11	47.8	52	40.6	**63**	**41.7**
Total	**23**	**100**	**128**	**100**	**151**	**100**

**Non-typable = Sufficient DNA, but isolated *S. pneumoniae* could have not been typed with probes available (40 CDC Serotype primers).
*Non-typed = DNA insufficient for serotyping.

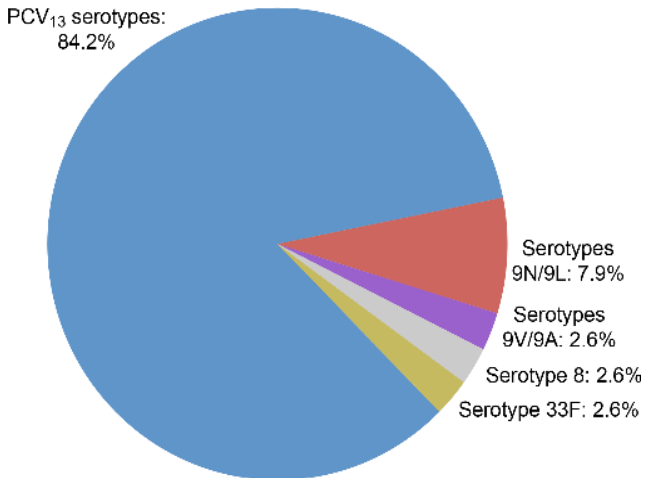

Figure 3. Distribution of *Streptococcus pneumoniae* serotypes that were identified via RT/PCR in CSF samples.

and their serotypes. The hospital attendance of bacterial meningitis was estimated at 3.7% in 2011 at CHUD-Borgou. Moreover, this study identified the bacteria that caused childhood purulent meningitis. By order of frequency, the incriminated bacteria were as follows: *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae*. In addition, this research work identified the serotypes of the different bacteria that were encountered. Regarding *Streptococcus pneumoniae*, this work helped to identify the serotypes covered by PCV $_{13}$ (vaccine serotypes: VS) and those that are not covered PCV_{13} (non-vaccine serotypes: NVS).

Given the results, we argue that this research work is relevant because it helped to identify other *Streptococcus pneumonia* serotypes that are not covered by PCV_{13}.

Concerning the validity of the results, the type of study, the method of CSF collection and the transport and analytical procedures allowed us to avoid the difficulties associated with different sources of possible bias. For example, the unavailability of latex for the identification of soluble antigens in CSF did not enable us to make a presumptive etiological diagnosis of the bacteria that were involved in childhood purulent meningitis at CHUD-Borgou, given the high number of CSF samples that were collected from patients who were affected by meningitis with bacteria-free neutrophil pleiocytosis. However, the case definition criteria and possibility of real-time PCR for CSF samples in the event of confirmed meningitis, as well as in the event of non-confirmed meningitis, helped us avoid this difficulty. The absence of bacteria from the CSF during direct examination and culture was almost certainly due to inappropriate antibiotic self-medication, resulting in a substantial decrease in bacterial sensitivity to the usual antibiotics. This decrease represents a source of complications of bacterial infectious diseases and death among children [13].

4.1. Sociodemographic Characteristics and Immunization Status of the Children who Were Involved in the Study

Considering the group age from 1 to 24 months, which accounted for 47.8% of suspected cases, 56.9% of confirmed cases were identified. This age group would therefore be the most exposed. These results are consistent with those of other studies, such as that conducted by Agossou *et al.* in 2009 in the same unit [5] and that of Soltani *et al.*, which was carried out in Tunisia in 2005 [18]. The vulnerability of children from this age group is the outcome of a gradual decline in passive immunity due to maternal antibodies and is the reason why it is necessary to vaccinate these children against the main bacteria that cause invasive infections, such as bacterial meningitis. The male predominance that was found among the subjects who were suspected of having meningitis was also noted in the children with verified infections. Male susceptibility to purulent meningitis has been reported by many African authors [5] [9] [18]. Regarding the vaccine coverage of the children from our series, 83% were vaccinated against *Haemophilus influenzae*; this is an acceptable rate. However, to protect children against preventable infections by vaccination, it is necessary to reach a regional and national coverage rate of approximately 90% to 95%. Regarding the meningococcal meningitis vaccine, only one child was vaccinated. This low coverage may be because although this vaccine is not an EPI vaccine, the Ministry of Health of Benin organizes vaccination campaigns against meningococcal A and C bacteria in the northern part of the country only once every three years.

4.2. Childhood Hospital Attendance for Purulent Meningitis in the CHUD-Borgou Pediatric Unit in 2011 and the Serotyping of Bacteria that Were Identified after RT/PCR

The 3.7% attendance rate that was found in this study is close to that published by Orega *et al.* in Abidjan in 1997 (3.4%) [19]. This result is near the 2% frequency that was reported by Agossou *et al.* in 2009 during a study that was conducted in the same unit but whose investigated age group was different [5].

The proportion of confirmed cases of meningitis among all suspected cases was 13.9% in our study. This proportion is similar to that reported by Papavasileiou *et al.* (15.76%) in Greece in 2011 [20].

Regarding the bacteria that were found in the CSF samples via PCR, among the 44 cases of confirmed purulent meningitis in which the CSF was tested in Banjul, RT/PCR was positive in 25 cases (56.8%) and identified the three main bacteria: *Streptococcus pneumoniae*, *Haemophilus influenza* and *Neisseria meningitidis.* In Egypt in 2007, Afifi *et al.* reported a lower positivity proportion of these three bacteria via PCR (13%) than was reported in this study from a study that was performed between 1998 and 2004 [21].

This study confirms once again what may be characterized as “epidemiologic transition”, which has been occurring over the last thirty years. In fact, from 1970 to 1990, epidemic meningitis was feared in the northern

region of Benin, which is within the Lapeyssonnie meningitis belt or African meningitis belt; in contrast, the southern part is the endemic area of Hib pneumococcal meningitis [1]-[5].

Regarding the bacteria that cause childhood purulent meningitis, this study leads us to distinguish two situations that may be described as paradoxes when determining different serotypes by real-time PCR (RT/PCR). We remark in **Table 2** that CSF samples, even when sterile during culture, may contain genetic material of bacteria, causing a false positive during RT/PCR. Among the 44 CSF samples that were collected from subjects who were affected by purulent meningitis, 52.3% of the cases were due to *Streptococcus pneumoniae*, followed by *Neisseria meningitidis* and *Haemophilus influenza* in the same proportions (2.3%). In addition, 41.3% of the CSF samples that were collected from subjects who were suspected of having purulent meningitis that was not confirmed by cytobacteriological testing and culture tested positive via RT/PCR for *Streptococcus pneumoniae*. Four CSF samples in this group (1.3%) tested positive via RT/PCR for *Neisseria meningitidis*, which is why some authors recommend matching classical CSF assays at the laboratory [cytology, Gram staining, Latex Particle Agglutination (LPA) and culture] with PCR for the diagnosis of bacterial meningitis [21] [22]. In fact, in China in 2014, Wang *et al.* compared three methods of the etiological diagnosis of neonatal bacterial meningitis using two PCR-based molecular assays: real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR based-reverse line blot hybridization (mPCR/RLB) compared with bacterial culture results. At the end of this work, it was demonstrated that RT/PCR provided evidence of the etiology of bacterial meningitis in 45% of cases; in contrast, these values was 29% and 9%, respectively, for mPCR/RLB and bacterial culture [22]. Moreover, Khan *et al.* tested 166 CSF samples from children aged 1 to 59 months who were suspected of having meningitis according to WHO criteria and declared negative during culture using PCR and LPA. These authors found that PCR was positive in 39.15% and that LPA was positive in 15.66% of cases, with the identification of the three bacteria: *S. pneumoniae*, *N.meningitidis and H.influenzae type b* [21].

Regarding the identification of bacterial serotypes that caused childhood purulent meningitis during this study, for *Neisseria meningitidis*, only serogroup A was found in the samples of both the subject who was affected by meningococcal meningitis and some subjects who were not affected by meningitis but tested positive during RT/PCR for that bacterium. Regarding *Haemophilus influenzae*, serotype b was involved. These findings enhance the knowledge of those two bacteria demonstrated by the research works of Lalya *et al.* and Agossou *et al.* in Benin [3] [5]. Regarding meningococcal meningitis, serogroup A was incriminated in more than 80% in the studies that have been conducted at the national level [1]-[5] A review of regional and African literature on *Haemophilus* and meningococcal meningitis notes that serotype b for *Haemophilus* and serogroup A for meningococcal meningitis are circulated more in Sub-Saharan Africa, with some cases of serogroups C and W135, whereas serogroup B is responsible for meningococcal meningitis in Europe [3]-[5] [8] [12] [13] [20] [23]-[25]. This variability is why, within the framework of universal immunization policy, since 2005, Benin has introduced the Hib vaccine into the EPI and has organized free-of-charge mass campaigns against meningococcal meningitis A and C for the benefit of children older one year and adults in the northern part of the country once every three years. Moreover, meningococcal vaccines (A, C, W135 and Y) have been mandatory since 2003 for Benin pilgrims traveling to Mecca to prevent outbreaks of epidemics from North Africa and the Far and Middle East, where serogroups A, B, C, W135 and Y circulate [12] [13] [23]-[25].

Regarding *Streptococcus pneumoniae*, this research shows that 15.8% of circulating serotypes were not covered by PCV_{13}, which is the vaccine that was introduced into the EPI in Benin in August 2011.According to this study, none of the children who were suspected of having meningitis received the 13-valent pneumococcal conjugate vaccine (PCV_{13}). Thus, before the introduction of PCV_{13} in Benin, serotypes other than the vaccine serotypes generated invasive diseases in children. These include not only serotype 8, which was found in the CSF of one subject who met the purulent meningitis definition criteria, but also serotypes 9N/9L, 9V/9A and 33F, which were identified in CSF samples of subjects who did not meet the cytobacteriological criteria of purulent meningitis.

In Benin, as in almost all West and Central African countries, antibacterial self-medication within the population has become a normal behavior; this situation may be due to the high rate of subjects who have been declared as not suffering from purulent meningitis. These subjects were identified as carriers of disease-causing bacteria via RT/PCR. With the introduction of PCV_{13} into the EPI in Benin, the idea that non-vaccine pneumococcal serotypes are responsible for childhood invasive infections has emerged. In fact, as noted by several studies in Europe, Asia and Australia, on the American continent and even in Africa, the introduction of 7-, 10- or 13-valent pneumococcal conjugate vaccine into national immunization programs (NIPs) on a large

scale has led not only to considerable reductions in the incidences of invasive infections due to vaccine serotypes but also to the emergence of other non-vaccine serotypes that cause childhood invasive infections [13] [15] [23] [26]-[34]. Moreover, according to the findings of a study that was conducted in Denmark between 1977 and 2007 and centered on a cohort of 18,858 patients of all ages who suffered from pneumococcal invasive infection, Zitta B. Harboe *et al.* found that serotypes 3, 10A, 11A, 15B, 16F, 17F, 19F, 31 and 35F were associated with a greater mortality than serotype 1, which is the serotype that was encountered most frequently among the 77 serotypes that were counted during this study, not including non-serotypable isolates. The same study demonstrated that among sick children under five years of age, serotypes 1, 4, 6A, 6B, 7F, 9V, 14, 18C, 19F and 23F were responsible for 80% of invasive pneumococcal infections. In addition serotypes 1, 3, 4, 14, 7F, 8, 9V, 12F and 23F were the most common in patients older than five years, accounting for 67% of cases.

Similar to serotypes 9 V/9A, 9N/9L, 33F and numerous non-typable and non-typed *Streptococcus pneumoniae* isolates that are not included in PCV_{13}, serotype 8, which was identified in our study, requires vigilance, as does the surveillance of the emergence of new serotypes in our area of the country, where resources are limited. Given the capacity for the recombination of *Streptococcus pneumonia* and the excessive use of antibiotics, often at inappropriate doses by the Benin population, there is concern for the emergence of not only non-vaccine serotypes but also vaccine serotypes that are resistant to antibiotics, such as serotype 14 reported by Lee *et al* and Antonio *et al*. [33] [35] [36]. In fact, studies that have been conducted in the West African sub-region, especially in Gambia, Ghana, Niger, Burkina-Faso and Togo, have demonstrated that serotype 14 had a very high clonal expansion associated with hypervirulent sequence type 63 (ST63), which causes meningitis in Burkina-Faso, Ghana and Niger [37]-[39]. Moreover, ST63 may also express serotypes 15A, 19A, 19F and 23F, indicating its capacity to change serotypes. In this study, we identified serotype 14 in 2.6% of children who were suspected of having meningitis and from whose CSF *Streptococcus pneumoniae* was isolated. In addition, serotype 1, which caused lethal pneumococcal meningitis in Burkina Faso, was the most frequently encountered serotype in this study. This result will likely motivate Benin health authorities charged with the monitoring of bacterial meningitis cases among all children and adults to take adequate preventive measures in the event of an epidemic.

During the phase preceding the introduction of the pneumococcal vaccine in Benin, an unpublished preliminary study that was carried out at the two sentinel sites in the Northern part of the country noted that among 318 CSF samples from sick children who were suspected of having meningitis that were analyzed at the reference laboratory in Banjul, serotype 13, which is not included in PCV_{13}, was identified with vaccine serotypes 1, 5, 14 and 19F.

The distinctive feature of this study was the identification of pneumococcal sequences in the CSF of patients who were suspected of having meningitis and had similar cytobacteriological and chemical characteristics. This result is likely related to the assay that was used ,i.e real-time PCR, which has the advantage of detecting proteins found in pneumococcal capsules, even under non-pathogenic conditions, and amplifying them exponentially, thus enabling their classification. The significant number of non-typable and non-typed samples due to a lack of genetic material or appropriate probes did not enable us to classify all of the *Streptococcus pneumoniae* isolates that were identified during this study. Analysis of CSF samples that we continue to send to the WHO regional laboratory in Banjul may enable us to determine the profile of the serotypes that still cause invasive infections in children less than five years of age in North Benin.

5. Conclusion

This study helped us to define the serotypic profile of the bacteria that caused purulent meningitis in children less than five years old in Benin. The results agree with the surveillance of meningitis cases, which has become an essential tool not only for measuring the impact of PCV_{13} introduction into EPI in Benin but also for determining the profile of the non-vaccine serotypes that are still circulating. This study may contribute to advocacy toward pharmaceutical companies for the development of a new pneumococcal conjugate vaccine integrating circulating non-vaccine serotypes that cause invasive infections among children.

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