Diagnosis of Acute Bacterial Meningitis by PCR and the Problem of Isolating the Causative Bacterial Agents by Culture in Burkina Faso

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Authors' contributions

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ABSTRACT

In Burkina Faso, meningitis is epidemic, pneumococcus, meningococcus and *Haemophilus influenzae* are the most implicated. The aim of this study was to assess the prevalence of ABM characterized by PCR and culture in order to highlight the low yield of the culture. This is a prospective study on 26 health districts of surveillance between 2016 and 2019. The CSF sent were cultured, treated by direct PCR, i.e. from CSF without DNA extraction at the National Meningitis Reference Laboratory of Burkina Faso and at the CDC Atlanta. Several series of direct PCR were carried out for species diagnosis (*lytA*, *sodC* and *hpd*), serotyping of meningococcus and serotyping of pneumococcus (capsular genes) and *Haemophilus influenzae* (capsular genes). Two thousand eight hundred and forty-five (2,845) CSF were treated with 701 positives and a positivity rate of 24.75%. *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae* represent respectively 39.2%, 51.9% and 8.9%. The strains of *Neisseria meningitidis* and *Streptococcus pneumoniae* were sensitive to antibiotics except 3 strains of *Neisseria meningitidis*, resistant to oxacillin. There is a drop in the positivity rate compared to previous years. Pneumococcus remains in the lead with serotype 1 (more than 50%) despite the introduction of PCV13.

1. INTRODUCTION

Bacterial meningitis is a public health problem in the world, and Africa remains the most affected continent, particularly the countries that make up the meningitis belt [1]. This African meningitis belt extends from Senegal in the West to Ethiopia in the East and covers 26 countries, including Burkina Faso, where meningitis is an annual epidemic [2]. Major epidemics occur every two to five years, with an attack rate of up to 1000 cases per 100,000 people [3]. The pathogen most involved in these bacterial meningitis epidemics is *Neisseria meningitidis* (*N. meningitidis*) serogroup A, which has been responsible for most of the epidemics between the years 2000 and 2010 [1]. This situation led to the introduction of the "MenAfriVac®" conjugate vaccine against *N. meningitidis* A in countries affected by these epidemics. Burkina Faso was the first country to introduce this vaccine in December 2010, the same year that it switched from enhanced surveillance to case-by-case surveillance [4]. This surveillance consists of notifying all suspected cases of meningitis, which should be sampled for analysis from the first level health centers to the central level where the National Meningitis Reference Laboratory (NML) is located [5]. The mission of the NRLm is to diagnose the three major etiological agents of acute bacterial meningitis (ABM) which are *Streptococcus pneumoniae* (*S. pneumoniae*), *N. meningitidis* and *Haemophilus influenzae* (*H. influenzae*) and monitor their resistance to antibiotics. This resistance monitoring is compromised by the absence of strains linked to cultures, which are usually negative. The aim of this study was to assess the prevalence of ABM characterized by PCR and culture in order to highlight the low yield of the culture [6, 7].

2. MATERIALS AND METHODS

This was a prospective descriptive study that involved samples of suspected meningitis cases from 25 health districts involved in meningitis surveillance in Burkina Faso between January 2016 and December 2019. The samples consisting of LCS were sent to the mNRL in Ouagadougou. These samples were first processed at the district laboratory and then at the mNRL and CDC in Atlanta. In the health districts, the CSF has been collected at the health facilities and sent to their respective laboratories where it was aliquoted into cryotubes. For each CSF, one portion was packed in a cryotube and another portion was inoculated into trans-isolate (TI) after cytology, Gram stain, and agglutination reaction with sensitized latex particles have been performed. The inoculated TI was kept at room temperature and protected from dust. The aliquoted cryotube was stored at -20°C for no longer than one week. The aliquoted cryotube and inoculated TI have been forwarded to the NRLm no later than 7 days after collection in accordance with the national meningitis surveillance guideline. At the NRLm, the TIs have been incubated at 37°C and then cultured and the strains obtained have been identified on the basis of their morphological characteristics after Gram staining, cultural, biochemical and antigenic by agglutination

reactions. An antibiogram was performed for each isolated strain. Cryotubes were used to perform direct PCR, i.e. from CSF without DNA extraction [8]. A first RT-PCR was performed to identify the species, *S. pneumoniae*, *N. meningitidis* and *H. influenzae* respectively by amplification of the *lytA*, *sodC* and *hpd genes* with specific primers (*Pneumococcus Streptococcus Lab Resources and Protocols | CDC)* [9]. This RT-PCR was performed in a final volume of 23 µL containing 12.5 µL of mastermix (Universal TaqMan® PCR Master Mix), 1 µL of each primer, 2 µL of DNA (4 ng). FAM was the single fluorochrome used for all three direct RT-PCR assays for the three pathogens. After species identification, a second direct RT-PCR was performed for *N. meningitidis* and *H. influenzae* respectively serogrouping and serotyping according to the same protocol but this time by searching for the capsular genes. The serotyping of *S. pneumoniae* was performed by 7 quadriplex direct RT-PCR reactions and 8 conventional direct PCR reactions which allow the determination of 21 and 44 serotypes respectively by searching for the capsular genes (*Pneumococcus Streptococcus Lab Resources and Protocols | CDC)* [10,11]. We used FAM, HEX, ROX and CY5 fluorchromes for direct quadriplex PCR in a reaction mixture of 25µl containing 12,5µl of matermix (PerfeCta® MultiPlex qPCR ToughMix, QuantaBio), a volume of primer depending on the type and 2,5µl of sample. Amplifications were performed using Stratagene Mx3005P and AriaMx® Real Time PCR Sytem (Agilent Technologies®, Santa Clara, CA) according to the following program: one cycle at 50°C for 2 minutes, one at 95°C for 10 minutes, 40 cycles at 95°C and 60°C respectively for 15 seconds and one minute [10]. Conventional PCR was followed by electrophoretic migration on a 2% agarose gel prepared with TAE buffer (1.6g per 80mL of Tris Acetate EDTA buffer) at 100V for 180 minutes. This was followed by UV revelation with the Gel Doc® EZ Imager, using BET (Ethidium Bromide) as an intercalating agent. Results were interpreted as follows : Ct < or = 35: positive results for the bacterial species in question; 35 < Ct < or = 40: equivocal results and for Ct > 40 : negative results. Equivocal results are repeated after dilution and declared negative if still equivocal. All steps of the experimental process at the NRLm were performed twice to confirm the results. Then, the non-typable (NT) samples at the NRLm were forwarded to the CDC in Atlanta for testing in the pneumococcal and meningococcal laboratories. At the CDC streptococcal laboratory, pneumococci were serotyped by quadruplex direct RT-PCR on 12 successive reactions.

The different results obtained were recorded on a Microsoft Excel file with an anonymous number to preserve patient anonymity. These data were then exported and analyzed on Epi-info 3.5.1. The figures were made from Microsoft Excel.

3. RESULTS

3.1 Overall Results

A total of 2845 LCS from 25 districts were received and processed at the mNRL between 2016 and 2019 of which, 701 positives for either *N. meningitidis*, *S. pneumoniae* or *H. influenzae* representing a positivity rate of 24.75% (Table 1).

3.2 Distribution of Confirmed Meningitis Cases by District and Month of the Year

The number of confirmed cases and their distribution according to health districts shows that the Diapaga district in the east of the country has the highest number of cases (107/701), especially in 2019 when it alone recorded 60 cases of meningococcal meningitis (Table 2).

The distribution of cases according to the months of the year and the species shows that for all species, the peak of cases is between January and May during our study period (Fig. 1).

3.3 Distribution of Confirmed Meningitis Cases by Species and by Age, Sex and Year

Of 275 positive cases of *N. meningitidis* representing 39.2%, 117 cases were from female patients, 158 cases from male patients, i.e. a sex ratio of 1.35 (Table 1). By age, children aged 6 to 14 years were in the majority (Table 1).

Of 364 pneumococcal positive specimens representing 51.9%, 157 were from female patients and 207 from male patients for a sex ratio of 1.31. Children aged 6 to 14 (older children) were the most affected (Table 1).

Of 62 *H. influenzae* positive samples representing 8.9%, female patients represented 30 cases compared to 32 cases for male patients, i.e. a sex ratio of 1.06. Infants were the most represented (Table 1).

Table 1. Total number of LCS received and LCS positive per year

	Number of case (n)				
LCS received	2016	2017	2018	2019	Total
Positive	257	191	100	153	701
Total	763	671	741	670	2845
Culture	2016	2017	2018	2019	Total
Nm	6	5	1	15	27
Spn	6	1	0	0	7
contaminated	25	22	12	9	68
negative	60	57	23	44	184
Total	97	85	36	68	286
Pathogens diagnosed by PCR	2016	2017	2018	2019	Total
Nm	82	93	16	84	275
Spn	154	93	68	49	701
Hi	21	11	14	16	62
Patients age					
	Hi	*Nm*	*Spn*		
[0d - 28d]	0	3	1		
[29d - 2.5y]	36	90	55		
[2.6y - 5y]	14	123	31		
[6y – 14y]	9	373	110		
[15y – 59y]	2	105	69		
≥ 60y	1	0	7		
Unknown	0	8	1		

Nm : Neisseria meningitdis ; Spn : Streptococcus pneumoniae ; Hi : Haemophilus influenzae.
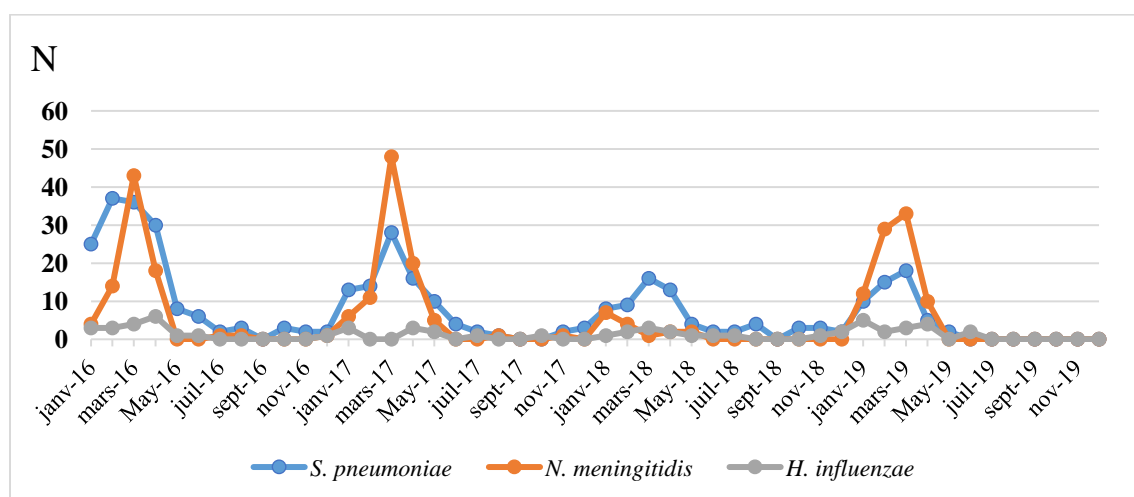*d : days ; y : years*

Fig. 1. Distribution of the number of meningitis cases according to the months during the years 2016 to 2019 according to the species

According to the distribution of species by years the *S. pneumoniae* is the dominant species except in 2019 when it comes in second position after *N. meningitidis*. Indeed we notified for pneumococcus, 59.9% of cases in 2016, 47.4% of cases in 2017, 70.8% of cases in 2018 while in 2019 it is meningococcus that comes in first position with 57.2% of cases (Table 1).

3.4 Frequency of Serotypes and Species Serogroups

The serogrouping of meningococci has made it possible to identify 03 serogroups, namely *N. meningitidis W* (53.4%), *N. meningitidis C* (24.4%), *N. meningitidis X* (22.2%) (Fig. 2).

Table 2. Breakdown of pathogenic species diagnosed by PCR by year and by health district

Sanitary Districts	2016				2017				2018				2019				Cumulative 4 years			
	Hi	Nm	Spn	Total	Hi	Nm	Spn	Total	Hi	Nm	Spn	Total	Hi	Nm	Spn	Total	Hi	Nm	Spn	Total
Bitou	0	5	3	8	1	7	6	14	0	1	0	1	0	0	0	0	1	13	9	23
Boulmigou	2	9	11	22	0	5	5	10	2	1	8	11	5	4	7	16	9	19	31	59
Boulsa	2	1	8	11	0	0	2	2	1	1	6	8	1	2	1	4	4	4	17	25
Boussé	0	3	18	21	0	8	3	11	0	0	3	3	0	0	1	1	0	11	25	36
Dano	0	0	0	0	0	0	0	0	0	0	1	1	0	0	4	4	0	0	5	5
Diapaga	2	4	12	18	0	2	11	13	1	0	10	11	3	60	2	65	6	66	35	107
Diebougou	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1
Garango	0	0	2	2	0	0	0	0	2	0	2	4	0	0	0	0	2	0	4	6
Kombissiri	2	5	4	11	2	12	4	18	0	0	0	0	1	0	4	5	5	17	12	34
Koupela	1	6	5	12	0	2	6	8	0	0	5	5	1	1	1	3	2	9	17	28
Léo	0	0	2	2	0	0	0	0	0	0	1	1	0	7	4	11	0	7	7	14
Manga	2	4	7	13	0	4	1	5	0	1	3	4	0	0	4	4	2	9	15	26
Ouargaye	6	19	15	40	1	15	17	33	0	1	2	3	1	0	3	4	8	35	37	80
Pama	0	0	2	2	1	5	1	7	0	1	3	4	0	4	4	8	1	10	10	21
Pissy	0	0	0	0	0	2	0	2	0	0	4	4	0	0	0	0	0	2	4	6
Pô	0	3	24	27	0	9	2	11	1	5	2	8	1	0	2	3	2	17	30	49
Pouytenga	0	1	7	8	1	4	3	8	2	0	4	6	0	0	3	3	3	5	17	25
Saponé	0	3	4	7	1	5	0	6	0	0	0	0	0	1	2	3	1	9	6	16
Sapouy	1	2	3	6	1	0	5	6	1	0	2	3	2	5	0	7	5	7	10	22
Tenado	0	0	0	0	2	2	3	7	0	0	0	0	0	0	0	0	2	2	3	7
Tenkodogo	1	8	4	13	0	7	3	10	1	2	2	5	1	0	2	3	3	17	11	31
Tougouri	0	2	1	3	0	1	0	1	0	0	2	2	0	0	2	2	0	3	5	8
Zabré	0	1	1	2	0	0	2	2	0	0	1	1	0	3	0	3	0	4	4	8
Ziniaré	0	1	3	4	1	1	3	5	2	0	3	5	0	0	1	1	3	2	10	15
Zorgho	2	5	18	25	0	1	16	17	1	1	3	5	0	0	2	2	3	7	39	49
Total	21	82	154	257	11	92	93	196	14	14	68	96	16	87	49	152	62	275	364	701

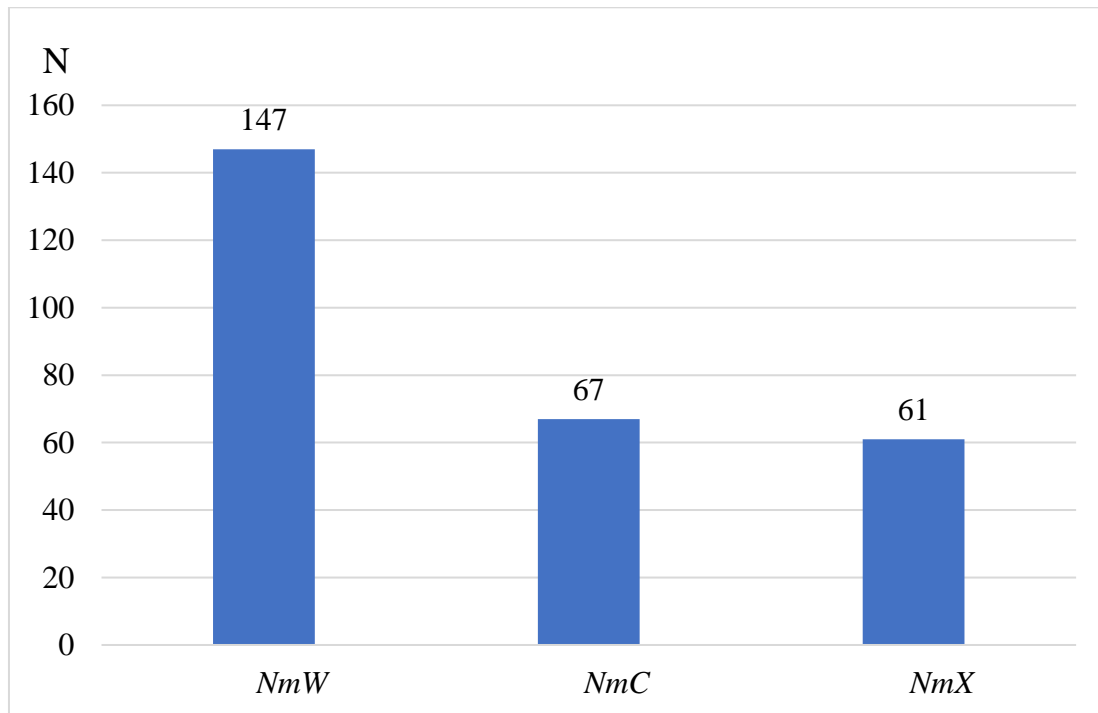*Nm : Neisseria meningitidis ; Spn : Streptococcus pneumoniae; Hi : Haemophilus influenzae*

Fig. 2. Results of pneumococcal species serotyping with rt-PCR and conventional PCR
Nm: *Neisseria meningitides*

A total of 29 different pneumococcal serotypes have been identified, of which 11 serotypes are part of the PCV13 vaccine. Serotype 1 remained dominant with 52.23% followed by serotype 12F/12A/12b/44/46, which represented 9.92%, and non-typeable strains represented 12.12% (Fig. 3). Of the 62 species of *H. influenzae*, 33 were of serotype b and the rest of non-b serotypes representing 53.2% and 46.8% respectively.

3.5 Species Diagnosed by Culture and Antibiotic Susceptibility Profile

Of the 701 PCR-positive samples, 286 were forwarded with TI medium and cultured, representing a transmission rate of 40.8%. This culture allowed the isolation and identification of 34 species, i.e. a culture positivity rate of 11.88%. These were 27 strains of meningococci and 7 strains of pneumococci. We are also reported 68 of contamination cases represented 23,77% rate (Table 2). No *Haemophilus inflenzae* species were isolated (Table 3).

3.5.1 N. meningitides

Of the 27 meningococcal strains isolated in culture, 23 were susceptibility tested. The antibiotics tested were penicillin G, oxacillin,

ceftriaxone and chloramphenicol. All antibiotics tested on the isolated meningococcal strains were active except for 3/7 oxacillin discs which were inactive on meningococcal serogroup W (Table 3).

3.5.2 S. Pneumonia

All 07 pneumococcal strains isolated were susceptible to the antibiotics tested except for 1/4 of the strains that were resistant to penicillin G, 1/5 resistant to oxacillin and 1/1 resistant to cotrimoxazole (Table 3).

4. DISCUSSION

4.1 Prevalence of confirmed meningitis cases

This study showed a decrease in the overall positivity rate which is estimated at 24.75%. Looking at the positivity rate by year, there was a progressive decrease from 2016 to 2018 with a slight increase in 2019 compared to 2018 (Table 1). However, previous studies in Burkina Faso have shown a higher positivity rate, 80.42%, 27.6%, 41.14%, 32.52%, 43.3% and 29.05% respectively in 2010, 2011, 2012, 2013, 2014 and 2015 [12]. Of this overall positivity rate, the Diapaga health district stands out as the district

that reported the most cases, especially in 2019. These results can be explained by the fact that this district bordering Niger was in an epidemic of *N. meningitidis C*, which justifies the predominance of meningococcus over pneumococcus in 2019 [13]. However, in general, pneumococcus is the predominant species and this trend is evident in other studies conducted previously in Burkina Faso [12,14].

Indeed, between 2011 and 2015, we note an emergence of pneumococcal meningitis compared to meningococcal meningitis, whereas in 2010, the year of the introduction of "MenAfriVac", meningococcal meningitis was predominant [4,12,15,16]. The same observation was made by an other author who found between 2011 and 2013 a predominance of pneumococcus followed by meningococcus [17].
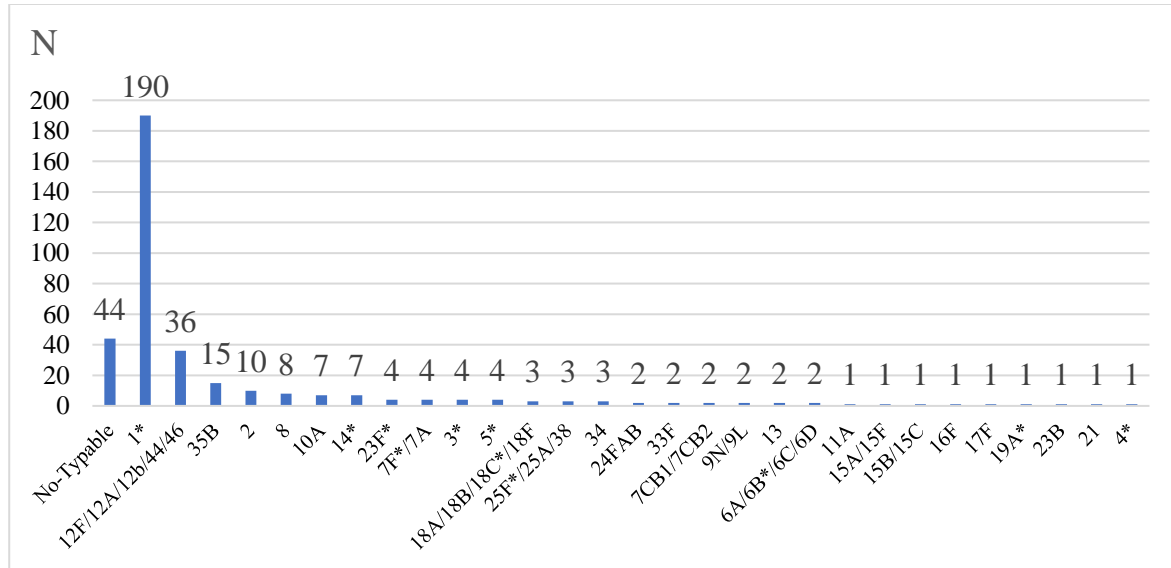

Fig. 3. Results of pneumococcal species serotyping with rt-PCR and conventional PCR
* : Included in PCV13

Table 3. Antibiotic susceptibility profile of *N. meningitidis* and S*. pneumoniae*

Antibiotics	*N. meningitidis C*		*N. meningitidis W*		*N. meningitidis X*		*S. pneumoniae*	
	S	R	S	R	S	R	S	R
Chloramphenicol	10	0	11	0	2	0	4	0
Ceftriaxone	10	0	11	0	2	0	-	-
Oxacilline	10	0	7	3	2	0	5	1
Penicilline G	10	0	11	0	2	0	4	1
Cotrimoxazole	-	-	-	-	-	-	1	1

S: sensitive ; R: resistance

Even though pneumococcus is the pathogen most incriminated in cases of meningitis in this study, the highest epidemic peaks are attributable to meningococcus during the 4 years of the study depending on the months of the year. The peaks are all observed between January and May for each species. There is therefore a correlation between the results obtained and the seasonal cycle in Burkina Faso. This correlation has been demonstrated in other previous studies in Burkina Faso and in other countries such as Niger where the peak of cases is almost at the same period [18–20]. Indeed, the dry season, which extends from November to

May, is characterized by the harmattan wind, which is favorable to the dissemination of pathogens, leading to an outbreak of cases from January to May [2]. On the other hand, the rainy season, which extends from June to October, is characterized by high rainfall, during which a drop in the number of meningitis cases is observed as soon as the first rains appear. In addition to the season, socio-demographic parameters such as age had an impact on the occurence of cases, depending on the pathogène.

4.2 Distribution of Cases by Socio-Demographic Characteristics

The distribution of cases according to socio-demographic characteristics shows that meningitis affects both men and women, although in our study we noted a slight predominance of men (ratio of about 1.3). The distribution according to species shows that pneumococcus and meningococcus affect almost all age groups. But with *H. influenzae*, it is mainly children who are most affected, especially infants from 29 days to 36 months. This observation was made in countries others than Burkina Faso by an others authors [17,21].

4.3 Frequency of Serogroups and Serotypes Identified by PCR by Year

The serogroups and serotypes identified in this study are diverse but we did not report *N. meningitidis A and N. meningitidis Y*, which confirms the disappearance of the serogroup A observed in previous studies in Burkina Faso. Indeed, since the introduction of the *N. meningitidis A* vaccine in 2010, it is only in 2014 that surveillance has reported cases of *N. meningitidis A* meningitis [22]. NmW remains the predominant serogroup, with an increase in cases of *N. meningitidis* C meningitis. This rise in serogroup C could be explained by the outbreak of *N. meningitidis* C meningitis in Niger, a country bordering Burkina Faso [18]. For *S. pneumoniae* serotype 1 remains in the lead with about 52% followed by serotype F12 with about 9.9%. Serotype 1 since 2007 remains the majority serotype of pneumococcal meningitis cases until now despite the introduction of the PCV13 vaccine in October 2013 which takes into account serotype 1 and not F12 [7,23,24]. This predominance of serotype 1 is also observed in other countries of the sub-region such as Niger where the prevalence between 2003 and 2011 was 54.3% [25]. For *H. influenzae* we reported 33 b serotypes against 29 non-b serotypes. This result differs from those of others studies which obtained a prevalence of 119 b serotypes against 7 non-b serotypes between 2010 and 2015 in Burkina Faso [12,18]. This could be explained by the decrease in the incidence of meningitis in general according to data from the epidemiological surveillance service of the Population Health Protection Department, a central department of the Ministry of Health. Almost all cases, as well as serotypes and serogroupes were diagnosed by PCR due to the difficulties observed in diagnosing by culture.

4.4 Culture Results and Susceptibility Profile of Isolated Strains

The culture showed a low positivity rate (11.88%) among PCR positive samples, a low TI transmission rate for culture (40.8%) and a high contamination rate (23.77%). This low rate of positivity can be linked to several factors, including antibiotic therapy before sampling, either in hospitals or in the community, and self-medication, which is a recurrent phenomenon. It is also necessary to take into account the delayed incolution of LCS in TI the conditions of conservation and transport of samples from peripheral health facilities to the national loboratory. Isolation difficulties are more marked with pneumococcus, as out of 363 pneumococci identified by PCR, only 7 strains were isolated by culture. This is probably related to the more demanding nature of this germ than meningococcus. This low positivity rate is shown in other studies carried out in Burkina Faso, such as the one in Kambiré, which noted between 2010 and 2015, 2185 contaminations, 1204 positive cultures out of approximately 7474 CSFs cultured, i.e. a positivity rate of approximately 16%. On the other hand, PCR gave 5625 positive results on about 16000 samples, i.e. a positivity rate of about 38% [12]. A sick bed inoculation experiments could improve pathogens' chances of survival, and raising stakeholders' awareness of the TI inoculation process could increase the rate of TI transmission and reduce the contamination rate. These difficulties reported have an impact on antibiogram data.

Of the limited data collected, no meningococcal resistance was observed with chloramphenicol, cefriaxone and penicillin G. However, three serogroup W strains were resistant to oxacillin. This situation differs from that observed in Cameroon where found 4/20 penicillin-resistant strains, 2/18 ampicillin-resistant strains and 100% susceptibility to cefotaxime were found [26]. As for pneumococcal strains, the small number of strains does not allow an objective interpretation of the sensitivity level.

5. CONCLUSION

This study showed a low positivity rate for culture compared with PCR. It also showed a low IT transmission rate for culture and a high contamination rate. There is therefore a need to take measures to improve the performance of culture, which remains the reference technique

for diagnosis and in-depth case management based on antibiogram. The development of inoculation at the patient's bedside and the sensitization of agents on the importance and inoculation of TI could help improve the yield of culture in the surveillance of meningitis in Burkina Faso. The search for resistance genes using PCR with primers specific to each antibiotic could give an idea of the pathogens likely to develop resistance to the antibiotics tested. However, the presence of the gene will not necessarily mean that the pathogen is resistant, as the gene may be present without being expressed, which means that improving crop yield remains essential.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mazamay S, Guégan JF, Diallo N, Bompangue D, Bokabo E, Muyembe JJ, et al. An overview of bacterial meningitis epidemics in Africa from 1928 to 2018 with a focus on epidemics "outside-the-belt". BMC Infect Dis. Déc. 2021;21(1): 1027.
   DOI: 10.1186/s12879-021-06724-1

2. Lapeyssonie L. Cerebral-spinal menigitis in Africa. Bulletin of the World Health Organization [Internet]. 1963;28 Supplement.
   Available:https://apps.who.int/iris/bitstream/handle/10665/72037/bulletin_supp%20_Vol28.pdf

3. Broutin H, Philippon S, Constantin de Magny G, Courel MF, Sultan B, Guégan JF. Comparative study of meningitis dynamics across nine African countries: a global perspective. Int J Health Geogr. 2007;6(1):29.
   DOI: 10.1186/1476-072X-6-29.

4. Novak RT, Kambou JL, Diomandé FV, Tarbangdo TF, Ouédraogo-Traoré R, Sangaré L, et al. Serogroup A meningococcal conjugate vaccination in Burkina Faso: analysis of national surveillance data. The Lancet Infectious Diseases. 2012;12(10):757-64.
   DOI : 10.1016/S1473-3099(12)70168-8.

5. WHO. Standard Operating Procedures for Surveillance, Preparedness and Response to Meningitis Epidemics in Africa [Internet]; 2018.
   Available:https://apps.who.int/iris/bitstream/handle/10665/312142/9789290313113-fre.pdf. Accessed on 07/29/2023.

6. Soeters HM, Kambiré D, Sawadogo G, Ouédraogo-Traoré R, Bicaba B, Medah I, et al. Impact of 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Meningitis, Burkina Faso, 2016–2017. The Journal of Infectious Diseases. 2019;220(Supplement_4):S253-62.
   DOI: 10.1093/infdis/jiz301

7. Kambiré D, Soeters HM, Ouédraogo-Traoré R, Medah I, Sangaré L, Yaméogo I, et al. Early impact of 13-valent pneumococcal conjugate vaccine on pneumococcal meningitis—Burkina Faso, 2014–2015. Journal of Infection. Mars. 2018;76(3):270-9.
   DOI: 10.1016/j.jinf.2017.12.002

8. Vuong J, Collard JM, Whaley MJ, Bassira I, Seidou I, Diarra S, et al. Development of Real-Time PCR Methods for the Detection of Bacterial Meningitis Pathogens without DNA Extraction. Chaturvedi V, éditeur. PLoS ONE. 1 févr. 2016;11(2):e0147765.
   DOI: 10.1371/journal.pone.0147765

9. Carvalho MDGS, Tondella ML, McCaustland K, Weidlich L, McGee L, Mayer LW, et al. Evaluation and Improvement of Real-Time PCR Assays Targeting *lytA* , *ply* , and *psaA* Genes for Detection of Pneumococcal DNA. J Clin Microbiol. Août. 2007;45(8):2460-6.
   DOI: 10.1128/JCM.02498-06

10. Velusamy S, Tran T, Mongkolrattanothai T, Walker H, McGee L, Beall B. Expanded sequential quadriplex real-time polymerase chain reaction (PCR) for identifying pneumococcal serotypes, penicillin susceptibility, and resistance markers. Diagnostic Microbiology and Infectious Disease. Juin. 2020;97(2):115037.
DOI : 10.1016/j.diagmicrobio.2020.115037

11. Ouattara M, Whaley MJ, Jenkins LT, Schwartz SB, Traoré RO, Diarra S, et al. Triplex real-time PCR assay for the detection of Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae directly from clinical specimens without extraction of DNA. Diagnostic Microbiology and Infectious Disease. mars 2019;93(3):188-90.
DOI: 10.1016/j.diagmicrobio.2018.10.008.

12. Kambiré D, Ouédraogo AS, sanou Mahamoudou, Tamboura Mamadou, Ky Ba A, Aké F, et al. Real-time PCR as a diagnostic tool in the epidemiological surveillance of purulent meningitis in Burkina Faso from 2010-2015. African Journal of Medical Biology / Revue Africaine de Biologie Médicale. Volume 2 (No. 4). 2017;303-10.

13. Dinanibè K, Issa T, Gautier OWH, Mahamoudou S, Wilfrid OLSL, Mahamoudou S, et al. Meningitis Outbreak Caused by <i>Neisseria meningitidis</i> Serogroup C ST 10217 in 2019 in Diapaga, Burkina Faso. AID. 2023;13(01):66-76.
DOI: 10.4236/aid.2023.131008.

14. Sanou M, Palenfo D, Bisseye C, Nagalo BM, Simporé J, Sangaré L, et al. Molecular diagnosis of bacterial meningitis in Burkina Faso. Tropical Medicine and Health. 2013;23(1):93-9.
DOI: 10.1684/mst.2013.0148.

15. WHO. WEEKLY EPIDEMIOLOGICAL RECORD : Epidemic meningitis control in countries of the African meningitis belt, 2016. 31 mars.2017;(NO 13):145-54. PMID: 28361528.

16. WHO. WEEKLY EPIDEMIOLOGICAL RECORD; Pneumococcal meningitis outbreaks in sub-Saharan Africa. 10 juin 2016;(NO 23):298-302.
Available:https://apps.who.int/iris/handle/10665/254456

17. Kambiré D, Soeters HM, Ouédraogo-Traoré R, Medah I, Sangare L, Yaméogo I, et al. Nationwide Trends in Bacterial Meningitis before the Introduction of 13-Valent Pneumococcal Conjugate Vaccine—Burkina Faso, 2011–2013. Hozbor DF, éditeur. PLoS ONE. 2016; 11(11):e0166384.
DOI: 10.1371/journal.pone.0166384

18. Soeters HM, Diallo AO, Bicaba BW, Kadadé G, Dembélé AY, Acyl MA, et al. Bacterial Meningitis Epidemiology in Five Countries in the Meningitis Belt of Sub-Saharan Africa, 2015–2017. The Journal of InfectiousDiseases.
2019;220(Supplement_4):S165-74.
DOI: 10.1093/infdis/jiz358.

19. Kourna Hama M, Khan D, Laouali B, Okoi C, Yam A, Haladou M, et al. Pediatric Bacterial Meningitis Surveillance in Niger: Increased Importance of Neisseria meningitidis Serogroup C, and a Decrease in Streptococcus pneumoniae Following 13-Valent Pneumococcal Conjugate Vaccine Introduction. Clinical Infectious Diseases.2019;69(Supplement_2):S133-9.
DOI: 10.1093/cid/ciz598.

20. Traore Y, Tameklo TA, Njanpop-Lafourcade B, Lourd M, Yaro S, Niamba D, et al. Incidence, Seasonality, Age Distribution, and Mortality of Pneumococcal Meningitis in Burkina Faso and Togo. CLIN INFECT DIS. mars 2009;48(s2):S181-9.
DOI : 10.1086/596498.

21. Boni-Cisse C, Jarju S, Bancroft RE, Lepri NA, Kone H, Kofi N, et al. Etiology of Bacterial Meningitis Among Children <5 Years Old in Côte d'Ivoire: Findings of Hospital-based Surveillance Before and After Pneumococcal Conjugate Vaccine Introduction. Clinical Infectious Diseases.
2019;69(Supplement_2):S114-20.
DOI: 10.1093/cid/ciz475.

22. Kristiansen PA, Ba AK, Ouédraogo AS, Sanou I, Ouédraogo R, Sangaré L, et al. Persistent low carriage of serogroup A Neisseria meningitidistwo years after mass vaccination with the meningococcal conjugate vaccine, MenAfriVac. BMC Infect Dis. 2014;14(1):663.
DOI : 10.1186/s12879-014-0663-4

23. Mueller JE, Yaro S, Ouédraogo MS, Levina N, Njanpop-Lafourcade BM, Tall H, et al. Pneumococci in the African Meningitis Belt: Meningitis Incidence and Carriage Prevalence in Children and Adults. Beall B, éditeur. PLoS ONE. 2012;7(12):e52464.
DOI: 10.1371/journal.pone.0052464.

24. Jefferies JM, Macdonald E, Faust SN, Clarke SC. 13-valent pneumococcal conjugate vaccine (PCV13). Human Vaccines. oct 2011;7(10): 1012-8.
DOI: 10.4161/hv.7.10.16794.

25. Collard JM, Alio Sanda A kader, Jusot JF. Determination of Pneumococcal Serotypes in Meningitis Cases in Niger, 2003–2011. Aguilar L, éditeur. PLoS ONE. 2013;8(3):e60432.
DOI : 10.1371/journal.pone.0060432.

26. Mabiala Babela JR, Ollandzobo LC, Nika ER, Moyen G. Neisseria meningitidis susceptibility in North-Cameroon. Médecine et Maladies Infectieuses. 2014;44(9):441-3.
DOI: 10.1371/journal.pone.0060432.

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