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## Epidemiology and antibiotic resistance of bacterial meningitis in Dapaong, northern Togo

Simplice D Karou<sup>1,2\*</sup>, Abago Balaka<sup>3</sup>, Mitiname Bamoké<sup>1</sup>, Daméhan Tchelougou<sup>1</sup>, Maléki Assih<sup>1</sup>, Kokou Anani<sup>1</sup>, Kodjo Agbonoko<sup>4</sup>, Jacques Simporé<sup>2</sup>, Comlan de Souza<sup>1</sup>

<sup>1</sup>Ecole Supérieure des Techniques Biologiques et Alimentaires (ESTBA-UL), Université de Lomé, Togo

<sup>2</sup>Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA/LABIOGENE), Ouagadougou, Burkina Faso

<sup>3</sup>Faculté Mixte de Médecine et de Pharmacie (FMMP-UL), Université de Lomé, Togo

<sup>4</sup>Centre Hospitalier Régional (CHR) de Dapaong, Togo

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### ABSTRACT

**Objective:** To assess the seasonality of the bacterial meningitis and the antibiotic resistance of incriminated bacteria over the last three years in the northern Togo. **Methods:** From January 2007 to January 2010, 533 cerebrospinal fluids (CSF) samples were collected from patients suspected of meningitis in the Regional Hospital of Dapaong (northern Togo). After microscopic examination, samples were cultured for bacterial identification and antibiotic susceptibility. **Results:** The study included 533 patients (306 male and 227 female) aged from 1 day to 55 years [average age (13.00±2.07) years]. Bacterial isolation and identification were attempted for 254/533 (47.65%) samples. The bacterial species identified were: *Neisseria meningitidis* A (*N. meningitidis* A) (58.27%), *Neisseria meningitidis* W135 (*N. meningitidis* W135) (7.09%), *Streptococcus pneumoniae* (*S. pneumoniae*) (26.77%), *Haemophilus influenzae* B (*H. influenzae* B) (6.30%) and Enterobacteriaceae (1.57%). The results indicated that bacterial meningitis occur from November to May with a peak in February for *H. influenzae* and *S. pneumoniae* and March for Neisseriaceae. The distribution of positive CSF with regards to the age showed that subjects between 6 and 12 years followed by subjects of 0 to 5 years were most affected with respective frequencies of 67.82% and 56.52% ( $P<0.001$ ). Susceptibility tests revealed that bacteria have developed resistance to several antibiotics including aminosides (resistance rate >20% for both bacterial strains), macrolides (resistance rate > 30% for *H. influenzae* quinolones (resistance rate >15% for *H. influenzae* and *N. meningitidis* W135). Over three years, the prevalence of *S. pneumoniae* significantly increased from 8.48% to 73.33% ( $P<0.001$ ), while the changes in the prevalence of *H. influenzae* B were not statistically significant: 4.24%, vs. 8.89%, ( $P= 0.233$ ). **Conclusions:** Our results indicate that data in African countries differ depending on geographical location in relation to the African meningitis belt. This underlines the importance of epidemiological surveillance of bacterial meningitis.

## 1. Introduction

Bacterial meningitis results from the invasion of the cerebrospinal fluid by bacterial[1]. It can extend to an infection of the lining surrounding the brain. *Neisseria meningitidis* (*N. meningitidis*) is a major cause of acute meningitis, followed by *Streptococcus pneumoniae* (*S. pneumoniae*) and *Haemophilus influenzae* (*H. influenzae*)[2–4]. By their high infectivity, they can cause epidemics of cerebrospinal

meningitis and septicemia[5]. According to the world health organization, meningococcal infections are endemic in the world, with 50 000 cases per year[6]. The annual incidence of the meningococcal disease is estimated to 1 to 5 cases per 100 000 people in industrialized countries, with a seasonal increase between November and May[7]. Other species commonly found in these infections are staphylococci and some enterobacteria, with *Escherichia coli* (*E. coli*) as the leading agent.

In all cases, the mortality rate is around 10%[6]. In the absence of any treatment, 5% to 10% of patients die within 24 hours of onset of symptoms and from 10% to 20% of those who survive present severe neurological sequels such as hearing loss and learning disabilities[8,9].

\*Corresponding author: Karou D. Simplicie, Ecole Supérieure des Techniques Biologiques et Alimentaires (ESTBA-UL), Université de Lomé, BP 1515, Lomé, Togo.  
E-mail: simplicekarou@hotmail.com

For decades, Africa is the continent most affected by epidemics of meningococcal meningitis, with an area called “African meningitis belt” that spans from the entire width of the west coast to Sudan and some parts of Ethiopia. The mortality is generally high and is around 13%. The epidemic that occurred in 1996–1997 was particularly devastating. In 10 months, there were 149 166 cases of meningitis and 15 750 deaths in the 22 countries in the African meningitis belt. Benin, Burkina Faso, Gambia, Ghana, Mali, Niger and Togo were particularly affected<sup>[10,11]</sup>.

In Togo, the Savannah region in the north is the area most affected. During the epidemic of the year 1996–1997, it recorded 90% of cases of meningitis and 89% of deaths in the country. However, no current data on the prevalence of bacteria implicated in meningitis infections is available. The present study was conducted to assess the prevalence of bacteria implicated in meningitis and their susceptibility to antibiotics in Dapaong, northern Togo.

## 2. Materials and methods

### 2.1. Patients and pathological products

This study is a retrospective study that covered the period from January 2007 to January 2010 at the Regional Hospital of Dapaong (RHD) in Savannah Region, northern Togo. The Savannah Region is one of the five health regions of the country. It is located in the far north and borders with the three neighboring countries of Togo: Burkina Faso to the north, Ghana to the west and Benin to the east. To the south, it is bordered with the region of Kara. The capital of the region is the city of Dapaong that has the regional hospital. This is the referral health centre in the region.

This study involved 533 patients including 306 male and 227 female with sex ratio of 1.35. Patients ranged in age from 1 day to 55 years with a mean age of (13.00±2.07) years. Patients were divided into five age groups: 0 to 5 years, 6–12 years, 13–19 years, 20–39 years and from 40 to 55 years with as respective effectives 199, 138, 87, 80 and 29. The study population was admitted to the RHD with suspected cerebrospinal infection. Cerebrospinal fluid (CSF) was collected from each patient by lumbar puncture.

### 2.2. Bacteriological techniques

All culture media and antibiotics disks used in this study were provided by BioRad (France). Each CSF was analyzed for the appearance, the quantitative cytology, the gram staining and the bacterial identification and the isolation followed by the antimicrobial susceptibility testing.

The macroscopic study of CSF was made by direct observation. The quantitative cytology consisted of an enumeration of white blood cells of the CSF using Nageotte cell. The bacterial growth and the isolation were assayed by inoculating chocolate agar+PolyViteX or blood agar. Cultures were incubated at 37 °C under CO<sub>2</sub> atmosphere. Negative samples were those for which no colonies were observed after 72 h of incubation. The identification of bacteria was made by the conventional methods used in the center. The susceptibility testing was performed on Muller Hinton agar following the recommendations of the French Society for

Microbiology<sup>[12]</sup>.

### 2.3. Data analysis

Data were recorded with SPSS–12 software for calculation of frequencies. The Fisher test by calculation of  $\chi^2$  values was used to compare frequencies with the software Epi-Info version 6.04. The significance level was set at  $P < 0.05$ .

## 3. Results

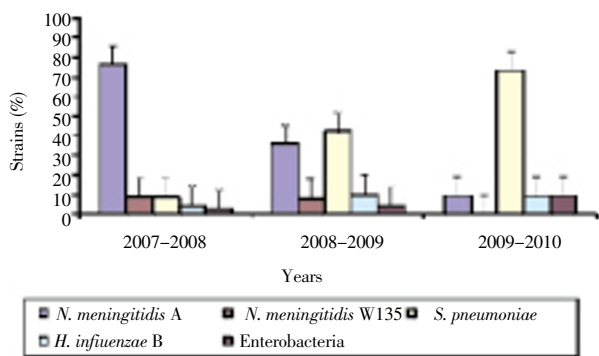
### 3.1. Macroscopic appearance and quantitative cytology of CSF

Of the 533 CSF samples analyzed, the bacterial growth and the identification were attempted for 254, showing a positivity rate of 47.65%. The search for bacterial pathogens was negative for the other samples. Of the 254 positive samples, 150 were from males while 104 were from female subjects. Statistical analysis by the  $\chi^2$  test revealed that the positivity of the CSF was not related to the sex of the patient ( $P = 0.460$ ).

Table 1 shows the macroscopic appearance of the positive samples. Data in the table reveal that the majority of CSF 246 (96.86%) had a cloudy appearance. Two samples (0.78%) were bloody, while one sample (0.39%) presented a normal appearance (crystal clear). Other five samples (1.97%) were purulent. Similarly quantitative cytology of CSF showed that 215 (84.65%) samples had more than 1 000 leucocytes per mm<sup>3</sup>, 35 (13.78%) had between 10 and 1 000 leucocytes per mm<sup>3</sup> and 4 (1.57%) samples had less than 10 leucocytes per mm<sup>3</sup>. Indeed 98.43% had abnormal cytology, constituted of polynuclear cells essentially.

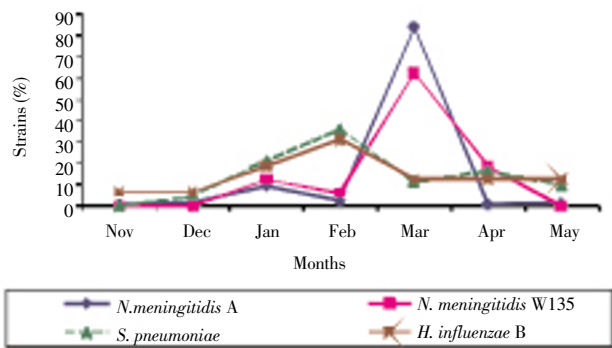
### 3.2. Distribution of bacteria isolated from CSF

The gram staining revealed the presence of bacterial pathogens in 260 samples. This was confirmed by the cultivation of the samples. Thus 260 bacterial strains were isolated from the CSF and distributed as follows: 148 (56.93%) *N. meningitidis* A, 18 (6.93%) *N. meningitidis* W135, 68 (21.15%) *Streptococcus pneumoniae* (*S. pneumoniae*), 16 (6.15%) *Haemophilus influenzae* B (*H. influenzae* B) and 10 (3.84%) enterobacteria. The number of isolates per year was 165, 50 and 45 respectively for 2007–2008, 2008–2009 and 2009–2010. Figure 1 shows the proportion of each species per year. *N. meningitidis* A was the most isolated bacterial species during the first year of the study, and its prevalence significantly decreased from 76.36% to 8.86% through 36% ( $P < 0.001$ ). *N. meningitidis* W135 was not isolated during the final year of the study, but its prevalence did not significantly change during the first two years of the study (8.48% and 8.00%, respectively with  $P = 0.858$ ). The prevalence of *S. pneumoniae* significantly increased in three years from 8.48% to 73.33% ( $P < 0.001$ ). The prevalence of *H. influenzae* B was 4.24%, 10.00% and 8.89% for the three years but the changes were not statistically significant ( $P = 0.233$ ).



**Figure 1.** Distribution of bacterial strains during three years in Dapaong.

Figure 2 shows the seasonal distribution of bacterial strains. It appears in the figure that no species was isolated between April and October. The period of isolation has two major peaks according to the categories of bacteria. Thus, March is the period of high prevalence of Neisseriaceae *i.e.* *N. meningitidis* A and *N. meningitidis* W135; 80% and 60% of these species were isolated during this month respectively. For *S. pneumoniae* and *H. influenzae* B, February is the peak of isolation, 36% and 31% of strains of *S. pneumoniae* and *H. influenzae* B, respectively, were isolated during this month.



**Figure 2.** Seasonal distribution of bacterial strains in Dapaong.

The distribution of positive CSF samples according to age groups is presented in Table 1. The overall distribution shows a very significant difference between age groups ( $P < 0.001$ ). The persons between 13 and 19 years were the most affected with a frequency of 67.82%, followed by the persons in the range of 6–12 years, 0–5 years and 20–39 years with frequencies of 56.52%, 40.20% and 40.00%, respectively. However statistical analysis revealed that there

**Table 1**

Distribution of positive CSF and bacterial strains in relation with the age of the patients.

Strain	0–5 years (n=199)	6–12 years(n=138)	13–19 years(n=87)	20–39 years(n=80)	40–55 years (n=29)	P
Positive CSF	40.20	56.52	67.82	40.00	31.03	<0.001 <sup>a</sup>
<i>N. meningitidis</i> A	21.60	34.06	41.38	26.25	0.00	0.003 <sup>b</sup>
<i>N. meningitidis</i> W135	2.01	3.62	4.60	3.75	0.00	0.653 <sup>b</sup>
<i>S. pneumoniae</i>	9.05	15.94	12.64	10.00	31.03	0.011 <sup>a</sup>
<i>H. influenzae</i> B	7.04	1.45	0.00	0.00	0.00	0.017 <sup>c</sup>

a: P value by  $\chi^2$  test for the 5 age groups, b: P value by  $\chi^2$  test for the 4 first age groups, c: P value by  $\chi^2$  test for the two first age groups.

was no significant difference between the age of 0–5 years and 20–39 years ( $P = 0.975$ ). With regard to the distribution of bacterial species according to the age of patients, it appears that Neisseriaceae and *H. influenzae* B did not infect patients over 40 years. Only *S. pneumoniae* was found in all age groups with statistically different prevalence ( $P = 0.011$ ). This bacterium mostly affected patients over 40 years with a prevalence of 31.03% in this age group. *H. influenzae* B was found only in people under the 5 years and subjects aged from 6 to 12 years, with a higher prevalence in patients under 5 years (7.04% versus 1.45%,  $P = 0.017$ ).

**3.3. Antibiotic susceptibility of the bacterial strains**

Seven families of antibiotics were tested on the isolated strains as shown in Table 2. The results revealed that *N. meningitidis* W135 presented the most resistant strains to antibiotics. Thus, over 75% of strains resisted to trimethoprim and amikacin. For this species sensitivity was uniform (12.50%) to beta lactam antibiotics. *N. meningitidis* A presented a resistance profile similar to *N. meningitidis* W135. The highest resistance rates were recorded with trimethoprim, amikacin and tobramycin. For this bacterial species quinolones *i.e.* ciprofloxacin, ofloxacin and norfloxacin seemed to be the most active, with resistance rates around 10%. In contrast, macrolides *i.e.* erythromycin, lincomycin and pristinamycin with resistance rates below 10% were the most active on *N. meningitidis* W135. With *S. pneumoniae* resistance rates were relatively low. The highest resistance rates were slightly above 25% for aminoglycosides. No resistance was recorded with certain antibiotics such as lincomycin, pristinamycin and doxycycline. With *H. influenzae* B, no resistance has been recorded with cephalosporins, but noticeable resistance was recorded against antibiotics such as amikacin and pristinamycin. Resistance rates of 46.16% and 35.72% were recorded for these antibiotics respectively.

**4. Discussion**

This study is a retrospective study that aimed to assess the epidemiological and evolutionary aspects of bacterial meningitis in the savannah region in northern Togo. The study has confirmed 254 cases of bacterial meningitis in 533 suspected subjects, including 148 cases of meningitis caused by *N. meningitidis* A, 18 cases caused by *N. meningitidis* W135, 68 cases by *S. pneumoniae*, 16 cases by *H. influenzae* B and 10 cases due the enterobacteria. These results show that overall, Neisseriaceae were the leading cause of bacterial meningitis in the savannah region in Togo in the three past years. Several studies have reported the prevalence of *N. meningitidis* in cerebrospinal infections[11,13]. Levy *et al*

**Table 2**

Percentage of resistance strains.

Antibiotics	<i>N. meningitidis</i> W135	<i>N. meningitidis</i> A	<i>S. pneumoniae</i>	<i>H. influenzae</i>
Penicillin G	12.50	12.50	7.36	8.75
Ampicillin	12.50	12.50	7.36	8.75
Amoxicillin	12.50	12.50	7.36	8.75
Cefalotine	16.67	12.50	1.47	0.00
Ceftriaxone	16.67	12.50	1.47	0.00
Cefaloxime	15.28	12.50	1.47	0.00
Gentamicin	23.19	22.22	26.47	30.00
Kanamycin	17.46	22.22	26.47	22.22
Tobramycin	50.00	22.22	26.47	22.22
Amikacin	52.38	77.78	26.47	46.16
Ciprofloxacin	10.25	18.75	11.76	12.50
Ofloxacin	8.70	18.75	8.83	25.00
Norfloxacin	8.70	18.75	–	25.00
Erythromycin	–	7.69	5.66	16.66
Lincomycin	–	7.69	0.00	33.33
Prystinamin	–	7.69	0.00	35.72
Tetracycline	–	–	6.25	33.33
Doxycycline	–	–	0.00	33.33
Chlorempenicol	14.86	12.50	2.94	12.50
Trimethoprim	76.10	81.82	11.11	33.33

– not tested.

also found that Neisseriaceae were responsible for over half of cases of bacterial meningitis in children in France with *N. meningitidis* as the leading species. Serogroups B and C were the most encountered; however, these serogroups were not found in this study<sup>[14]</sup>. Our study revealed a rather high prevalence of serogroup A (56.93%). Serogroup W 135 was found in 6.93% cases during the first two years of the study. This strain has often been described as responsible for outbreak among pilgrims to Mecca and sub Saharan Africa<sup>[11]</sup>. Our results showed that the incidence of Neisseriaceae rise especially in March, which corresponds to a hot dry period in the region. The distribution of isolates by year of study revealed that the incidence of Neisseriaceae declined significantly between 2007 and 2010. Indeed, during 2007, a meningitis outbreak was reported in Togo and Burkina Faso; this could explain the high prevalence of these organisms during the year<sup>[10]</sup>. However, our results showed that, unexpectedly, young people from 13 to 19 were more affected than children under 12 years by Neisseriaceae. The vaccination campaigns have often been conducted in the country but the priority is given to children of preschool and school age of 12. This may be a reason but we do not have enough data on this coverage.

Many studies on the causes of bacterial meningitis have often shown a predominance of *S. pneumoniae*<sup>[15]</sup>. In a study in Cameroon, Fonkoua *et al* found the prevalence of 56.2%, 18.5% and 13.4% for *S. pneumoniae*, *H. influenzae* and *N. meningitidis*, respectively<sup>[13]</sup>. *S. pneumoniae* was ranked second after *N. meningitidis* in our study, this is an unexpected, since the first survey in 2000 in the city of Lomé located at 600 km in the south of Dapaong showed that *S. pneumoniae* followed by *H. influenzae* and *N. meningitidis* were the main causative agents of bacterial meningitis in the following proportions 59.7%, 11.2% and 9.3%, respectively<sup>[16]</sup>. A second study conducted in 2007 at the national level has confirmed the predominance of *S. pneumoniae* (39%), followed by *N. meningitidis* (35%) and *H. influenzae* (26%)<sup>[17]</sup>. This inconsistency with our results could be explained by the fact that, Dapaong located in the savannah region in the far north of Togo at the border with Burkina Faso is part of

the African meningitis belt. Our results also showed that *S. pneumoniae* rages on endemospadic mode with a peak in February and affects more adult subjects aged over 40 years. Current data do not explain this situation; this study has not determined the serogroups of *S. pneumoniae* to determine whether they are covered by the vaccines in the country.

*H. influenzae* B is a major causative agent of bacterial meningitis in children in countries where vaccination against this bacterium has not been widespread. The current impact of meningitis due to *H. influenzae* in most African countries are poorly understood because the statements of meningitis are not exhaustive, due to lack of diagnostic and inadequate information gathering system<sup>[18]</sup>. In Togo, data on the epidemiology of this agent are scanty. In this study, our results showed that the bacterium was ranked third behind Neisseriaceae and *S. pneumoniae* with prevalence around 10%, raging in endemospadic mode with a peak in February and affecting preferably children under 5 years. However, some authors found no seasonal effect on the prevalence of the organism<sup>[19]</sup>. In this study, the prevalence of the organism has not varied significantly over the last three years and remains lower than that found in Madagascar<sup>[18]</sup>. Indeed in that study, the authors have shown that *H. influenzae* with an incidence around 32% was the second leading cause of bacterial meningitis in children before *S. pneumoniae* and after *N. meningitidis*.

We were also interested in the susceptibility of these bacteria to antibiotics. The advantage of this aspect of the study lies in the fact that without waiting for the identification of the agent, an empiric antibiotic therapy is often introduced as the presumption diagnosis, taking into account the epidemiology of bacteria involved and their sensitivity to antibiotics<sup>[20–22]</sup>. Our results showed that Neisseriaceae have developed resistance to all antibiotics tested, the lowest rate of resistance is around 8%. For *N. meningitidis* A, the lowest rate of resistance was recorded with quinolones. Overall, more than 80% of strains were sensitive to beta-lactams. These results are similar to those of Dagnra *et al* who reported rates of sensitivity around 83% for penicillin G and 100% for cefotaxime<sup>[16]</sup>. In contrast this

study showed that *N. meningitidis* was more sensitive to chlormphenicol than aminopenicillins.

The aminoglycoside antibiotics have been the least efficient on *S. pneumoniae*, and over 25% of strains were resistant to these antibiotics. Béré *et al* in Burkina Faso found that over 95% of strains were resistant to amikacin and more than 25% were resistant to aminopenicillins<sup>[15]</sup>. Resistance rates recorded with aminopenicillins in this study are below the values recorded in Burkina Faso. However these rates are similar to those found by Fonkoua *et al* in Cameroon<sup>[13]</sup>. The study of Dagnran *et al* showed that erythromycin was more active on *S. pneumoniae* as chlormphenicol and aminopenicillins. Our results showed an opposite situation since chlormphenicol was more active than erythromycin although the other macrolides showed 100% sensitivity.

Concerning *H. influenzae*, beta-lactam antibiotics appear to be of choice in the treatment of infections. The resistance rate was approximately 8% for penicillins and 0% for cephalosporins. These results are not consistent with those of Dagnran *et al* who found that chlormphenicol and macrolides were more active than beta-lactams on *H. influenzae*. However, these results agree well with results of studies in Madagascar and Senegal that demonstrated that *H. influenzae* was sensitive to third generation cephalosporins but has a high resistance to chlormphenicol<sup>[18,23–26]</sup>. Other studies in Central Africa have shown that aminoglycosides were not very effective on *H. influenzae* but that the activity of aminopenicillins was reduced<sup>[13,27]</sup>.

It appears from this study that, the data in African countries differ depending on geographical location in relation to the African meningitis belt. This underlines the importance of epidemiological surveillance of bacterial meningitis.

### Conflict of interest statement

We declare no conflict of interest.

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