

## Article

# Invasive Bacterial Meningitis in Mali: Molecular Detection and Serotype Distribution of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*

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## Abstract

Invasive bacterial infections caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* remain a major public health concern. This study aimed to perform the molecular characterization of bacterial strains responsible for meningitis in patients of all ages who met the World Health Organization case definition for meningitis and had cerebrospinal fluid samples collected between January 2021 and December 2022 at the Clinical Bacteriology Laboratory of the National Public Health Institute of Mali. We conducted a surveillance-based observational study using national surveillance data collected between January 2021 and December 2022. Data were collected continuously and in real time throughout the study. The analysis was cross-sectional and descriptive. Data were obtained from samples received at the laboratory, accompanied by individual clinical notification forms. For each sample, demographic data and additional clinical information, including vaccination status, were collected. Infection was diagnosed by isolating invasive strains through culture, confirmed by real-time triplex PCR, and positive cases were further characterized by real-time triplex PCR for serotyping. Overall, 103 infections were confirmed among the 1000 samples received, corresponding to a positivity rate of 10.3%. *S. pneumoniae* predominated with 62%, followed by *H. influenzae* type b with 36% and *N. meningitidis* serogroup X with 2%. The identified serotypes of pneumococcus were predominantly not covered by existing vaccines, particularly serotype 23A (38.30%), while



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others, including serotypes 1 (17.02%) and 3 (10.63%), are included in the PCV13 vaccine. The distribution of cases by age and gender shows a predominance of males, accounting for 60.2% of cases (62/103). The 0–5 age group is by far the largest, accounting for 76.7% of cases (79/103), with males representing 58.22% (46 cases). These findings highlight the importance and the need for continuous monitoring surveillance of circulating strains and strengthening vaccination efforts to improve prevention.

**Keywords:** bacterial meningitis; *Streptococcus pneumoniae*; *Haemophilus influenzae*; *Neisseria meningitidis*; molecular typing; vaccine surveillance; Mali

## 1. Introduction

Invasive bacterial infections caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* remain major public health concerns worldwide [1]. Bacterial meningitis is responsible for millions of cases of morbidity and mortality each year globally [2]. However, the burden is considerably higher in Sub-Saharan Africa, especially in the countries within the “meningitis belt,” which are exposed to a high risk of recurrent meningococcal meningitis epidemics [2,3]. In 2019, an estimated 2.51 million cases of acute bacterial meningitis were reported worldwide, resulting in approximately 236,000 deaths. The burden was particularly high among children under five years of age, accounting for 112,000 deaths and 1.28 million incident cases [1]. In 2024, meningitis cases increased sharply in Africa, with approximately 26,000 cases and nearly 1400 deaths reported across 24 countries. In the first quarter of 2025, more than 5500 suspected cases and nearly 300 deaths were reported in 22 countries [4].

*N. meningitidis*, *H. influenzae*, and *S. pneumoniae* are the leading causes of bacterial meningitis worldwide [5,6]. *N. meningitidis*, also known as meningococcus, is one of the most feared bacterial pathogens, responsible for acute, severe, and fulminant meningitis requiring urgent medical management due to its rapid progression and high lethality [7]. *H. influenzae* is a bacterium capable of causing a wide range of infections, including respiratory tract infections in addition to meningitis [8,9]. *S. pneumoniae*, commonly known as pneumococcus, is an opportunistic pathogen and a frequent cause of bacterial meningitis and other respiratory infection [10–12]. Infections caused by these etiological agents may spread from an initial infectious focus to the central nervous system, leading to severe and potentially life-threatening neurological complications. Although these bacteria are often harmless colonizers, they may occasionally invade the host, resulting in meningitis and septicemia [13].

A common characteristic of these three invasive bacterial pathogens is the presence of polysaccharide capsules on their cell surface, which serve as serotyping/serogrouping antigens [14]. These capsules also act as protective device, enabling bacteria to evade host immune defenses. Capsule-based vaccines have been developed to target the most prevalent serotypes of *H. influenzae*, *N. meningitidis*, and *S. pneumoniae*. Although vaccination has significantly reduced disease incidence, these pathogens remain major causes of infection among unvaccinated populations. In Africa, the prevalence of invasive bacterial infections remains particularly high due to several factors, including limited access to healthcare services and recurrent seasonal epidemics. Meningitis is highly prevalent in the African meningitis belt, which extends from West Africa to East Africa [15]. Throughout this region, *N. meningitidis* serogroups A, C, W-135, X, and Y are prevalent [16]. Available data indicate that *N. meningitidis* has been responsible for numerous meningitis epidemics. Prior to the introduction of the serogroup A meningococcal conjugate vaccine (MenAfriVac) in

2010, most meningitis cases in the African meningitis belt were caused by *N. meningitidis* serogroup A (NmA). Mass vaccination led to a substantial reduction in NmA cases and the elimination of NmA epidemics in the region. Despite this success, other serogroups, particularly NmW, NmX, and NmC, remain a public health threat and continue to cause outbreaks and periodic epidemics [16,17].

In Mali, bacterial meningitis remains a major public health problem. Data from the World Health Organization (WHO) and the Malian Ministry of Health indicate that meningitis cases are frequently associated with epidemic outbreaks [18]. Mali, which lies within the meningitis belt, has experienced 17 major meningococcal meningitis epidemics since 1940. The most recent outbreak was reported in 2016 in Ouélessébougou, a health district located approximately 80 km from Bamako, and was a localized meningococcal meningitis outbreak mainly caused by NmC and *S. pneumoniae* [16]. Between 2005 and 2007, a study conducted in Mali on 224 positive cerebrospinal fluid samples showed that approximately 114 confirmed meningitis cases were due to *N. meningitidis*, 73 to *S. pneumoniae*, and 44 to *H. influenzae* of type b [19]. More recently, following vaccine introduction, the pathogens most frequently identified in cerebrospinal fluid analyses have been *S. pneumoniae* (56%) and *H. influenzae* [20]. To address the burden of these infections and reduce associated morbidity and mortality, Mali introduced vaccines against these three bacteria into the Expanded Program on Immunization including the *H. influenzae* type b vaccine in 2005 and the pneumococcal conjugate vaccine (PCV13) in 2011 [21]. The MenAfriVac conjugate vaccine was also introduced progressively in 2010 and 2011. As in other countries of the meningitis belt, Malian health authorities strengthened prevention strategies through vaccination as well as microbiological and epidemiological surveillance systems, with support from partners such as the WHO and the Centers for Disease Control and Prevention (CDC, Atlanta). Following the introduction of the serogroup A meningococcal conjugate vaccine [22,23], Mali adopted a case-by-case surveillance strategy. A lumbar puncture systematically performs to collect cerebrospinal fluid prior to any antibiotic therapy in suspected cases, and transporting the samples to the National Reference Laboratory located in Bamako [17,24]. These data raise several important questions such as which serotypes and serogroups are responsible for meningitis? Is there an emergence and/or persistence of non-vaccine serotypes or serogroups? Does vaccination provide adequate protection against these infections?

This study is part of a larger epidemiological surveillance program focusing on the molecular, genomic, and phylogenetic characterization of strains responsible for infections isolated from cerebrospinal fluid (CSF). Our objective was to describe the epidemiology of the three main infectious agents and to assess the impact of vaccination on invasive strains isolated from CSF in Mali.

## 2. Methods

### 2.1. Study Setting and Design

We conducted a surveillance-based observational study using national surveillance data collected between January 2021 and December 2022. Data were collected continuously and in real time throughout the study. The analysis was cross-sectional and descriptive. Data were obtained from samples received at the laboratory, accompanied by individual clinical notification from January 2021 to December 2022. Patients of all ages presenting with suspected meningitis according to World Health Organization (WHO) case definitions and for whom a CSF sample was available were included in the study. The study was conducted at the Clinical Bacteriology Unit of the National Reference Laboratory of the National Public Health Institute (NPHI) of Mali in Bamako. The NPHI receives specimens from all 65 functional districts health facilities across the country.

Limitations of our study include the use of surveillance data from 2021 to 2022, which may not fully reflect current epidemiological trends. Additionally, there is a potential underestimation of disease burden due to variations in healthcare access and diagnostic capacity. Furthermore, incomplete vaccination records limit the assessment of associations between vaccination status and protection against identified strains.

## 2.2. Data Collection and Storage

All CSF samples obtained by lumbar puncture and collected in either sterile dry tubes or Trans-Isolate (T-I) medium were included. Samples were collected from patients with suspected meningitis referred from hospitals and health facilities throughout the country. Data were obtained from CSF samples received at the laboratory, accompanied by individual clinical notification forms. For each CSF sample, demographic data and additional clinical information, including vaccination status, were recorded.

## 2.3. Identification and Typing

All CSF samples were analyzed using both conventional bacteriological and molecular techniques. Infection was diagnosed by isolating invasive strains through culture and confirmed by real-time multiplex PCR and positive cases were further characterized by real-time triplex PCR [25].

All isolates were identified according to standard bacteriological procedures. Real-time PCR (RT-PCR) was performed directly on CSF samples without prior nucleic acid extraction. A multiplex PCR targeting three specific genes *sodC*, *hpd*, and *lytA* was used for the simultaneous identification of *N. meningitidis*, *H. influenzae*, and *S. pneumoniae*, respectively [25]. A second PCR assay was used for typing. Direct serotyping of pneumococcus-positive CSF samples was performed as previously described [25]. Serotyping was carried out using a real-time triplex PCR based on a panel of seven triplex reactions targeting pneumococcal serotypes frequently isolated in Africa. The assay consisted of adding 2 µL of CSF directly to 23 µL of reaction mixture containing TaqMan® Universal PCR Master Mix, primers, probes, and PCR-grade water. Amplification and detection were performed using the AriaMx real-time PCR system (Agilent Technologies, Santa Clara, CA, USA).

The triplex primer/probe sets used were as follows: Triplex 1 (1, 5, 23F); Triplex 2 (4, 6A/6B/6C/6D, 9V/9A); Triplex 3 (14, 18C/18B/18A/18F, 19F); Triplex 4 (3, 7F/7A, 19A); Triplex 5 (6C/6D, 12F/12A/12B/44/46, 22F/22A); Triplex 6 (15A/15F, 23A, 33F/33A/37); and Triplex 7 (2, 11A/11D, 16F) [25].

This molecular typing protocol uses multiplex real-time PCR to directly detect and characterize three bacterial species from a clinical sample *N. meningitidis* (*ctrA/sodC* gene), *H. influenzae* (*hpd* gene), and *S. pneumoniae* (*lytA* gene). Based on CDC recommendations, it not only confirms the species but also determines the specific capsular types, targeting serogroups A, B, C, Y, W135, and X for *N. meningitidis* and serotype b for *H. influenzae*. The procedure involves preparing a multiplex reaction mix with specific primers, TaqMan probes, and master mix in a 96-well plate before adding the clinical sample alongside positive, negative, and internal inhibition controls. The assay consisted of adding 2 µL of CSF directly to 23 µL of reaction mixture containing TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), primers (Biosearch Technologies, Petaluma, CA, USA), probes (Biosearch Technologies, Petaluma, CA, USA), and PCR-grade water. Amplification and detection were performed using the AriaMx real-time PCR system (Agilent Technologies, Santa Clara, CA, USA). Following amplification (initial denaturation at 95 °C, then 45 cycles of 95 °C/15 s and 60 °C/60 s), Ct values ≤ 35 were considered positive; Ct values in the range of 36–40 equivocal; and Ct values > 40 negative.

## 2.4. Statistical Analysis

The variables studied included patient age and sex, year and month of CSF collection, vaccination status, and the detected serotypes and serogroups. Data were analyzed using Epi Info™ software (version 7.2.6.0; Centers for Disease Control and Prevention [CDC], Atlanta, GA, USA). Descriptive analyses were performed to determine the frequencies of serotypes and serogroups and to assess their coverage by the PCV13 and MenA conjugate vaccines. Results were expressed as counts and percentages ( $n$ , %), with percentages calculated relative to the total number of samples analyzed for each method.

Comparisons between culture and PCR detection were performed using Fisher's exact test. Associations between age groups and vaccination status were assessed using the chi-square test or Fisher's exact test, as appropriate. The association between age group, sex, and PCR positivity was further evaluated using multivariable logistic regression, with results reported as odds ratios (ORs) and 95% confidence intervals (CIs), and a  $p$ -value  $< 0.05$  considered statistically significant.

The association between vaccination status and serotype group (vaccine vs. non-vaccine) was assessed using Fisher's exact test. A logistic regression model was then fitted to estimate the odds of infection with non-vaccine serotypes according to vaccination status. Odds ratios (ORs) and 95% confidence intervals (CIs) were reported.

Among PCR-positive cases, pathogen-specific analyses were conducted. A binary outcome comparing *S. pneumoniae* with other pathogens was explored; however, due to sparse data and quasi-complete separation, regression estimates were unstable. Therefore, these associations were primarily assessed using contingency tables and chi-square or Fisher's exact tests.

No statistical testing was performed for MenAfriVac due to insufficient sample size. Statistical significance was defined as  $p < 0.05$ .

## 3. Results

During the study period, a total of 1000 CSF samples were collected from patients clinically suspected of meningitis. The overall prevalence was 10.3%, including six cases confirmed by bacterial culture (0.6%) and 103 cases confirmed by PCR (10.3%) (Table 1). The distribution of cases by age and sex showed a male predominance, with males accounting for 60.2% of cases (62/103) compared with 39.8% among females (41/103). Children aged 0–5 years represented the majority of cases, accounting for 76.7% (79/103), with a higher proportion among male (46 cases) than female (33 cases). The 5–14 years age group accounted for 19.4% of cases (20/103), also with a male predominance (14 versus 6). In contrast, older age groups, >14–18 years and >18 years, were poorly represented, each accounting for only 1.9% of cases (2/103), with an equal distribution between the sexes.

**Table 1.** Distribution of the etiological agents of bacterial meningitis according to the diagnostic method (culture and PCR).

|                                     | Culture (N = 6) | PCR (N = 103) | $p$ -Value |
|-------------------------------------|-----------------|---------------|------------|
| <i>S. pneumoniae</i> $n$ (%)        | 6 (100)         | 64 (62.00)    | $<0.0001$  |
| <i>H. influenzae</i> type b $n$ (%) | 0 (0)           | 37 (36.00)    | $<0.0001$  |
| <i>N. meningitidis</i> $n$ (%)      | 0 (0)           | 2 (2.00)      | 0.47       |

Values are expressed as counts and percentages  $n$  (%). Percentages were calculated relative to the total number of samples analyzed for each method (culture:  $N = 6$ ; PCR:  $N = 103$ ). PCR refers to a real-time PCR assay for the molecular detection of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*. Comparisons between culture and PCR results were performed using Fisher's exact test. A  $p$ -value  $< 0.05$  was considered statistically significant.

In bivariate analysis, no statistically significant association was observed between age group and infection status ( $p = 0.122$ ), although the chi-square approximation was

considered unreliable due to small expected cell counts in several categories. No association was observed between sex and infection ( $p = 0.86$ ).

In multivariate logistic regression analysis, children aged 0–5 years were significantly more likely to have bacterial meningitis compared with older age groups (OR = 4.74, 95% CI: 1.45–29.16,  $p = 0.032$ ). The 5–14 years age group showed a non-significant trend (OR = 4.04, 95% CI: 1.13–25.72,  $p = 0.064$ ), while no association was observed for older age groups. Sex was not significantly associated with infection risk (OR = 1.04, 95% CI not significant,  $p = 0.83$ ).

According to vaccination status among PCR positive cases, most patients were vaccinated (47/103), while 33 were unvaccinated and 23 had unknown vaccination status. Vaccinated patients had received at least one of the following vaccines: PCV13 (*S. pneumoniae*), Hib (*H. influenzae* type b), or the MenA conjugated vaccine is used widely on immunization program (*N. meningitidis*) (Table 2). ACYW is used currently only for traveler. The distribution of pathogens according to vaccination status showed no statistically significant association (Fisher's exact test,  $p = 0.26$ ). Pneumococcus was the predominant pathogen across all vaccination groups. Although a higher proportion of *H. influenzae* type b cases was observed among unvaccinated individuals, this difference did not reach statistical significance.

**Table 2.** Distribution of bacterial meningitis according to vaccination status.

|                        |        | PCV-13 Vaccination<br>( $p$ -Value † > 0.05) |                          |                    | Hib Vaccination<br>( $p$ -Value ‡ > 0.05) |                          |                    | MenAfriVac (Men A)<br>( $p$ -Value > 0.05) |                          |                    |
|------------------------|--------|----------------------------------------------|--------------------------|--------------------|-------------------------------------------|--------------------------|--------------------|--------------------------------------------|--------------------------|--------------------|
|                        |        | Vaccinated<br>$n$ (%)                        | Un-Vaccinated<br>$n$ (%) | Unknown<br>$n$ (%) | Vaccinated<br>$n$ (%)                     | Un-Vaccinated<br>$n$ (%) | Unknown<br>$n$ (%) | Vaccinated<br>$n$ (%)                      | Un-Vaccinated<br>$n$ (%) | Unknown<br>$n$ (%) |
| Age<br>group<br>(year) | 0–5    | 21 (32.8)                                    | 11 (17.2)                | 12 (18.8)          | 13 (35.1)                                 | 16 (43.2)                | 6 (16.2)           | 0 (0)                                      | 0 (0)                    | 0 (0)              |
|                        | >5–14  | 10 (15.6)                                    | 4 (6.3)                  | 2 (3.1)            | 0 (0)                                     | 0 (0)                    | 2 (5.4)            | 1 (50.0)                                   | 1 (50.0)                 | 0 (0)              |
|                        | >14–18 | 1 (1.6)                                      | 1 (1.6)                  | 0 (0)              | 0 (0)                                     | 0 (0)                    | 0 (0)              | 0 (0)                                      | 0 (0)                    | 0 (0)              |
|                        | >18    | 1 (1.6)                                      | 0 (0)                    | 1 (1.6)            | 0 (0)                                     | 0 (0)                    | 0 (0)              | 0 (0)                                      | 0 (0)                    | 0 (0)              |
|                        | Total  | 33 (51.6)                                    | 16 (25.0)                | 15 (23.4)          | 13 (35.1)                                 | 16 (43.2)                | 8 (21.6)           | 1 (50.0)                                   | 1 (50.0)                 | 0 (0)              |

Data are presented as counts and percentages ( $n$ , %). †  $p$ -value was calculated using the  $\chi^2$  test for the PCV. ‡  $p$ -value was calculated using Fisher's exact test for the Hib vaccine due to small sample size. No statistical analysis was performed for MenAfriVac due to insufficient sample size ( $N = 2$ ). The threshold for statistical significance was set at  $p < 0.05$ . Unknown corresponds to cases for which vaccination information was unavailable.

Among the 1000 patients included, 566 were vaccinated, 171 were unvaccinated, and 263 had unknown vaccination status. The proportion of positive cases was higher among unvaccinated persons (19.3%) compared with vaccinated persons (8.3%) and those with unknown vaccination status (8.7%). Bivariate analysis showed a statistically significant association between vaccination status and bacterial positivity ( $\chi^2 = 18.1$ ;  $p < 0.001$ ). In multivariate logistic regression analysis adjusted for age and sex, vaccination was associated with a significantly reduced risk of invasive bacterial infection (OR = 0.38; 95% CI: 0.23–0.62;  $p < 0.001$ ). In contrast, no significant association was observed between bacterial positivity and age groups or sex after adjustment ( $p > 0.05$ ).

Among the confirmed cases, *S. pneumoniae* serotype 23A (38.3%) and serotype 1 (17.0%) were the most prevalent. All *H. influenzae* isolates were type b (Hib), while only serogroup X was detected for *N. meningitidis*. The majority of *S. pneumoniae* and *H. influenzae* infections occurred in children aged 0–5 years. The distribution of bacterial pathogens differed significantly across age groups ( $p = 0.0076$ ). *H. influenzae* type b was predominantly observed in children under 5 years, whereas *S. pneumoniae* affected all age groups. *N. meningitidis* serogroup X was identified only in older children.

Serotyping was performed on 47 of 64 *S. pneumoniae* positive samples. All detected serotypes were included in the real-time triplex PCR analysis (Tables 3 and 4). Serotyping could not be performed for 17 samples because our molecular serotyping by the real-time triplex PCR molecular typing assay targets only 21 serotypes. Among children aged

0–5 years, the proportion of serotypes covered by the PCV13 vaccine was 41.4%, increasing to 50% among children aged 5–14 years. In contrast, among older adolescents and adults, only a single PCV13-included serotype was identified, accounting for all observed cases in these age groups. Among pneumococcal isolates, the most frequently identified serotype was serotype 23A, followed by serotype 1 and serotype 3. Overall, 39% of isolates were classified as vaccine-type serotypes, while 34.4% were non-vaccine types. A substantial proportion (26.6%) of isolates had unknown serotype.

**Table 3.** Distribution of patients by identified serotypes in the real-time triplex PCR assay.

| Bacteria                       | Serotypes   | Number<br>n (%) | Age Group (Years) |       |        |     |
|--------------------------------|-------------|-----------------|-------------------|-------|--------|-----|
|                                |             |                 | 0–5               | >5–14 | >14–18 | >18 |
| <i>S. pneumoniae</i> (N = 47)  | 1           | 8 (17.02)       | 1                 | 5     | 1      | 1   |
|                                | 3           | 5 (10.63)       | 3                 | 2     | 0      | 0   |
|                                | 5           | 1 (2.12)        | 1                 | 0     | 0      | 0   |
|                                | 6A/B/C/D    | 2 (4.25)        | 2                 | 0     | 0      | 0   |
|                                | 11A/11D     | 1 (2.12)        | 0                 | 1     | 0      | 0   |
|                                | 12FAB/44/46 | 2 (4.25)        | 1                 | 1     | 0      | 0   |
|                                | 14          | 3 (6.38)        | 2                 | 1     | 0      | 0   |
|                                | 22F/22A     | 4 (9.51)        | 2                 | 2     | 0      | 0   |
|                                | 23A         | 18 (38.30)      | 14                | 4     | 0      | 0   |
|                                | 23F         | 3 (6.38)        | 3                 | 0     | 0      | 0   |
|                                | Total       | 47 (100)        | 29                | 16    | 1      | 1   |
| <i>H. influenzae</i> (N = 37)  | Hib         | 37 (100)        | 35                | 2     | 0      | 0   |
| <i>N. meningitidis</i> (N = 2) | NmX         | 2 (100)         | 0                 | 2     | 0      | 0   |

The 13-valent pneumococcal conjugate vaccine (PCV13) includes 13 purified capsular polysaccharides of *Streptococcus pneumoniae* (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F).

**Table 4.** Distribution and prevalence of non-vaccine serotypes not covered by PCV13.

| Serotypes   | Number<br>n (%) | Age Group (Years) |       |        |     |
|-------------|-----------------|-------------------|-------|--------|-----|
|             |                 | 0–5               | >5–14 | >14–18 | >18 |
| 11A/11D     | 1 (2.12)        | 0                 | 1     | 0      | 0   |
| 12FAB/44/46 | 2 (4.25)        | 1                 | 1     | 0      | 0   |
| 22F/22A     | 4 (9.51)        | 2                 | 2     | 0      | 0   |
| 23A         | 18 (38.30)      | 14                | 4     | 0      | 0   |
| Total       | 25 (100)        | 17                | 8     | 0      | 0   |

The study reveals a high prevalence of serotype 23A (38.3%), particularly in children under 5 years. These findings highlight the emergence of non-vaccine serotypes in the pediatric population and underscore the need to adapt vaccination strategies to cover these new variants.

Among cases with known serotype and vaccination status, no significant association was observed between vaccination status and serotype group ( $p = 0.495$ ). In logistic regression analysis, vaccinated individuals had higher odds of infection with non-vaccine serotypes compared to unvaccinated individuals (OR = 2.04; 95% CI: 0.53–8.46), although this association was not statistically significant ( $p = 0.308$ ).

The monthly distribution of meningitis cases showed marked seasonality, with a peak observed in November (18%), and high proportions in July and December (15% each). In contrast, the lowest incidences were recorded in August (3%) and May (4%).

#### 4. Discussion

In this study, the overall prevalence rate was 10.3%. This prevalence reflects only laboratory-confirmed invasive bacterial meningitis cases identified through the national surveillance system and analyzed at the National Institute of Public Health, rather than the total burden of meningitis occurring in Mali. Although vaccination programs have contributed to improving the epidemiological situation, with national vaccination coverage

among children under five years reaching approximately 94% in 2023, underdiagnosis, underreporting, limited access to healthcare, prior antibiotic use before lumbar puncture, and restricted laboratory diagnostic capacity may contribute to an underestimation of the true burden of disease. Therefore, these findings should not be interpreted with cautions. *S. pneumoniae* was identified as the main pathogen, accounting for 62% of invasive bacterial meningitis cases. This high prevalence is consistent with findings from a previous study conducted in Mali, which also reported a predominance of *S. pneumoniae* among the causative agents of meningitis in children [21], suggesting that this bacterium remains the principal pathogen of invasive meningitis in the region. These results underscore the continued importance of preventive strategies targeting *S. pneumoniae*, particularly vaccination according to the recommended immunization schedule (WHO/CDC/Gavi) [4,26,27].

This predominance is consistent with data from the African region. A study conducted in Burkina Faso reported a proportional morbidity of 7% for pneumococcal meningitis over a 20-year period [28]. Moreover, pneumococcal meningitis outbreaks have been documented in the African meningitis belt between 2000 and 2018 [29]. In our study, the emergence of serotypes not included in current vaccines, such as ST-23A and others, highlights the need for continuous surveillance and adaptation of vaccine formulations to cover these emerging strains. Recent studies have shown an increase in the prevalence of non-vaccine serotypes in several regions, particularly in Africa. A study conducted in Morocco revealed that 43% of *S. pneumoniae* strains isolated from hospitalized children were non-vaccine serotypes (PCV10) [30]. Although serotype 23A was not specifically mentioned in this study, other research has identified its emergence in African countries as South Africa [31]. The phenomenon of serotype replacement, where non-vaccine serotypes become more prevalent following the introduction of vaccination, is well documented. An international study demonstrated that, following the introduction of PCV13, invasive pneumococcal infections caused by non-vaccine serotypes became predominant in several countries, including in Africa [32].

The detection of *H. influenzae* type b in 36% of cases indicates a significant persistence of this pathogen despite the introduction of the Hib vaccine in many African countries. A study conducted in the pediatric department of CHU Gabriel Touré in Mali investigated invasive Hib infection in hospitalized children. It showed that Hib remains an important cause of invasive infections despite vaccine introduction, with high mortality (approximately 21%) and occurrence in unvaccinated children in approximately 21% of cases [33]. A meta-analysis demonstrated that the incidence of Hib meningitis significantly decreased after vaccine introduction, from 4.84 to 0.67 cases per 100,000 child-years [34]. In Morocco, the introduction of the Hib vaccine in 2007 led to a 75% reduction in confirmed Hib meningitis cases among children under 5 years of age [35].

Although representing only 2% of cases in our study, *N. meningitidis* serogroup X is concerning due to the absence of a specific vaccine. Serogroup X outbreaks have been reported in several African countries, including Burkina Faso and other Sub-Saharan African nations [36,37]. In response to this threat, the pentavalent NmCV-5 vaccine, covering serogroups A, C, W, Y, and X, was prequalified in 2023, and Nigeria became the first country in the world to deploy the new Men5CV vaccine recommended by the World Health Organization in 2024 [38]. The fact that 86% of patients in our study were unvaccinated highlights gaps in vaccination programs. Factors such as limited access to healthcare, conflicts, and population displacement can hinder the effective implementation of vaccination campaigns. In Togo, despite an administrative coverage of 100% for the third dose of the PCV13 vaccine in the Savanes region, a pneumococcal meningitis outbreak was reported in 2023, primarily affecting adolescents and young adults [39].

The monthly distribution of meningitis cases revealed a marked seasonal pattern, with a peak observed in November and relatively high proportions in July and December. Conversely, the lowest incidences were recorded in August and May. This seasonal variation is consistent with observations reported by the WHO and the Centers for Disease Control and Prevention, which have documented periodic increases in bacterial meningitis during specific times of the year. In Sub-Saharan Africa, particularly within the meningitis belt, the dry season is characterized by low humidity, dusty winds, and pronounced temperature fluctuations. These environmental conditions are known to damage the nasopharyngeal mucosa, thereby facilitating bacterial invasion and increasing susceptibility to invasive meningitis, especially due to *Neisseria meningitidis* and *S. pneumoniae*. The absence of a significant association between vaccination status and serotype group may be explained by several factors. First, the limited number of cases with complete serotype and vaccination data may have reduced the statistical power to detect a difference. Second, the circulation of non-vaccine serotypes, potentially driven by serotype replacement following pneumococcal conjugate vaccine (PCV) introduction, could reduce the observable impact of vaccination on serotype distribution. Finally, incomplete vaccination schedules or waning immunity in some children may also contribute to the persistence of vaccine-type or mixed serotype distribution patterns. The observed seasonal trend underscores the need for strengthened epidemiological surveillance and timely, targeted prevention strategies. These include optimizing vaccination campaigns, improving early case detection, and enhancing health system preparedness during periods associated with increased incidence of invasive bacterial meningitis. The limited microbiological yield of CSF cultures constitutes a major constraint despite the availability of trans-isolate medium in the country. This situation is frequently observed in African contexts for several reasons such as the administration of antibiotics prior to lumbar puncture, which inhibits bacterial growth in culture; inappropriate transport and storage conditions of samples, often due to a lack of cold chain or adequate transport media; and the intrinsically lower sensitivity of culture compared to molecular methods such as real-time PCR. Strengthening the use of PCR in routine diagnostics and implementing protocols to limit empirical antibiotic use before sampling can improve pathogen detection. Uncertainty regarding the vaccination status of many patients reduces the ability to establish a direct correlation between vaccination and protection against the identified strains. This issue is related to the absence of vaccination cards, which is common in low-income or rural areas; lack of electronic vaccination data, limiting traceability; and vaccination coverage often overestimated in administrative data. Systematic verification of vaccination status in studies and the improvement of electronic vaccination registries are essential to ensure higher data quality and a better understanding of vaccine effectiveness.

## 5. Conclusions

The results of this study highlight the critical importance of strengthening vaccination programs, enhancing epidemiological surveillance, and adapting vaccination strategies to include emerging serotypes and serogroups. An integrated approach combining vaccination, surveillance, and research is essential to reduce the morbidity and mortality associated with invasive bacterial meningitis in Africa. Despite these limitations, the study remains valuable for monitoring bacterial strains in Mali. Improved microbiological diagnostic logistics and more rigorous documentation of vaccination status would further increase the robustness of the findings.

Genomic surveillance is essential for monitoring three interconnected phenomena driven by vaccine pressure. First, non-typable *S. pneumoniae* strains are emerging as a significant reservoir of antimicrobial resistance. Second, the burden of non-PCV serotypes is increasing through serotype replacement, as observed with serotype 23A following

vaccine introduction. Finally, the emergence of *N. meningitidis* serogroup X (NmX) in Africa demonstrates how pathogens can fill ecological niches left vacant by serogroup A vaccination, highlighting the urgent need for broader multivalent vaccines.

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