Epidemiological study of bacterial meningitis in Tunisian children, beyond neonatal age, using molecular methods: 2014-2017

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Abstract

Background: Since the 1990s, the epidemiology of bacterial meningitis worldwide has changed thanks to vaccination. In Tunisia, the main causative pathogens were *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* serotype b (Hib). Only Hib vaccination was available during our study period.

Objectives: We performed a laboratory case report based-study of suspected bacterial meningitis in Northern Tunisia from January 2014 to June 2017.

Methods: CSF samples obtained from children beyond neonatal age with suspicion of meningitis were tested by two real time PCRs, targeting pneumococcus, meningococcus and Hib, and conventional methods.

Results: Using real-time PCR, 63 were positive including ten supplementary cases compared to conventional methods. A general decrease of bacterial meningitis cases was demonstrated comparing to previous data. *Pneumococcus* was predominant (69.84%) followed by *meningococcus* (28.57%) and Hib (1.59%). The main serotypes were 14, 19F, 6B and 23F for *pneumococcus* and serogroup B for *meningococcus*. Most cases occurred during cold season and children under one year were the most affected by bacterial meningitis.

Conclusion: Our study suggests the predominance of pneumococcal cases. It may provide valuable data on meningitis epidemiology before the introduction of pneumococcal vaccine, which may be useful for future evaluation.

Keywords: Bacterial meningitis; children; Tunisia; PCR.

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Introduction

Bacterial meningitis is a devastating disease associated with a high mortality rate and significant disability in survivors over the world[1-3]. Clinical diagnosis is difficult to assess, among infants. Moreover, bacteriological conventional methods present a limited sensitivity for low-quality cerebrospinal fluid (CSF) samples and antibiotic pre-treated cases[3,4]. Thus, bacterial meningitis cases remain underestimated, highlighting the need to use methods with high-performance detection such as real time PCR[4].

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Children beyond neonatal age are among the most exposed to bacterial meningitis. Historically, the major causative pathogens for this population worldwide were *Neisseria meningitidis* (*N. meningitidis*), *Streptococcus pneumoniae* (*S. pneumoniae*) and *Haemophilus influenzae* serotype b (Hib)[1]. However, in the last two decades, bacterial meningitis epidemiology changed thanks to the introduction of conjugate vaccines against the three bacteria. Indeed, the vaccination against Hib reduced the number of Hib meningitis cases by 90% in developed countries[5]. Additionally, the successive introduction of pneumococcal conjugate vaccines (PCV7, PCV10 and PCV13) reduced the number of meningitis cases and limited the possible serotypes replacement[5,6]. Furthermore, the conjugate vaccine against *N. meningitidis* serogroup C lowered the number of invasive diseases caused by this bacterium in different region of the world. For instance, in the United Kingdom, where *meningococcus* serogroup C conjugate vaccine was introduced in 1999, the number meningococcal invasive diseases decreased by 81%, from 1998 to 2001, with no evidence of serogroup replacement during the two years following the vaccine introduction[5].

In Tunisia, a middle-income country, these three pathogens were the major agents of bacterial meningitis in children[7,8]. Most frequent serotypes were 14 and 23F for *S. pneumoniae*, serotype b for *H. influenzae* and serogroup B for *N. meningitidis*[8,9]. Hib vaccination was introduced during the 2002-2005 period, in the national vaccination schedule, leading to the decrease of Hib meningitis incidence[8,10]. However, the use of Hib vaccine was interrupted from 2005 to 2011 causing a rise of Hib meningitis cases[8,11]. Since 2011, it has been reintroduced a second time as a routine vaccination. Furthermore, since April 2019, pneumococcal vaccine has been introduced in the national schedule, using PCV10[12]. However, little data about bacterial meningitis epidemiology have been available since 2011 [11]. Most studies focused on characterisation of one pathogen in a very restricted area[13-15] and did not consider the impact of the Hib vaccination reintroduction[11] nor the added value of molecular diagnosis in assessing bacterial meningitis epidemiology.
With the aim of estimating the pathogen distribution of meningitis cases in infants and children in Northern Tunisia, we assessed a laboratory case report based-study over a three and half-year period using molecular and conventional methods.

Methods

Ethics statement and informed consent

The Ethical Committee of Bechir Hamza Children's Hospital in Tunis, Tunisia, approved this study (number 002/2017). The analysis was performed on the leftover of CSF samples obtained for diagnostic purposes. All information related to patient's identity from the sampling of this study (excluding age and sex) was treated confidentially in accordance with ethical guidelines and the Tunisian legal requirements.

Samples and Study design

From January 2014 to June 2017, we performed a prospective laboratory case report based-study. All CSF samples received in the laboratory of Microbiology of Bechir Hamza Children's Hospital, for bacterial meningitis diagnosis, were investigated. This hospital is the main paediatric hospital (a bed capacity of 347 and at least 30.625 admissions in 2017) gathering patients from all Northern regions of Tunisia (11/24 governorates), the most populated area, and also referred patients from other regional hospitals. The investigated samples (n=253) were obtained from children aged from 1 month to 15 years old, presenting various meningitis signs such as high fever, neck stiffness, photophobia, hypotonia and seizures[3,11].
CSF samples were evaluated by bacteriological conventional methods, i.e. bacterial culture, direct examination for microscopic detection of bacteria and Gram staining, and latex agglutination test [16]. Samples, characterised by at least 5 leukocytes per mm^3, were selected and considered as suspected bacterial meningitis cases. These CSF samples were considered for PCR when they had a sufficient volume, i.e. at least 200µL. They were stored at -20°C until molecular investigations..
The reference strain of *Streptococcus pneumoniae* (ATCC 49619) and field strains of *Neisseria meningitidis* and Hib were used as positive controls for real time PCR tests. Field strains were obtained from CSF for *H. influenzae* serotype b meningitis case and blood culture for meningococcaemia case and isolated in the laboratory of Microbiology of Bechir Hamza Children's Hospital in Tunis (Tunisia).

Molecular diagnosis of bacterial meningitis

Bacterial DNA was extracted by heat-shock protocol for positive controls and by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) for CSF samples according to the manufacturer's instructions.
For detection of bacterial meningitis, we performed a multiplex real time PCR for *S. pneumoniae* and *N. meningitidis* detection and a singleplex real time PCR for Hib detection by using TaqMan chemistry[17].

Typing methods

Meningococcal serogroups were identified by latex agglutination test (Pastorex meningitis, BIO-RAD, Marne-la-Coquette, France) and confirmed by PCR [18].
Pneumococcal serotyping was performed by agglutination test (Pneumotest Latex, Statens Serum Institut Diagnostica, Hillerod, Denmark) for strains collected from 2014 to November 2015. Due to the lack of latex agglutination test, several conventional PCRs were performed for strains collected from December 2015 to October 2016 according to the Centers of Disease Control and Prevention (CDC) and World Health Or-

ganization (WHO) recommendations[16] as well as Pai et al.[19], using the Applied Biosystems kit in GeneAmp PCR System 9700 instrument (Applied Biosystems, Foster City, USA) followed by agarose gel electrophoresis.

Statistical analysis

Statistical significance was determined using EpiInfo software 7.2. The Fisher exact test was performed to compare meningococcal detection proportions by molecular and conventional methods (95% confidence intervals).

Results

Detection rates of bacterial meningitis cases

Overall, 4892 CSF samples were obtained from children admitted in the Bechir Hamza Children's Hospital. Based on inclusion criteria, only 253 cases were considered as suspected bacterial meningitis cases, while the others corresponded to viral meningitis cases (4639 cases). All CSF samples (n=253) were investigated by conventional methods and 183 fulfilled the inclusion criteria for PCR testing (sufficient volume of at least 200µL) and were investigated by real time PCR (Table 1). Among the 183 cases, 31 CSF samples were collected after antibiotic treatment.

Table 1 Yearly distribution of suspected bacterial meningitis tested among children

Year	Number of cases
2014	87
2015	69
2016	81
2017[a]	16
Total	253

[a]Period between January and June

Using real time PCR, 63 cases were detected positive among the 183 investigated cases while using conventional methods, only 53 cases out of 253 had positive results. All positive cases were confirmed by real-time PCR. Furthermore, ten additional positive cases were detected. The 63 positive cases included 44 cases (69.84%) of pneumococcal meningitis followed by 18 cases (28.57%) of meningococcal meningitis and one case (1.59%) of Hib meningitis. The supplementary detected cases corresponded to six meningococcal meningitis and four pneumococcal meningitis cases. Real-time PCR had a significantly higher rate of detection of meningococcal meningitis than conventional methods given that it revealed one-third more positive cases (P=0.005). Among the 31 CSF samples collected after antibiotic treatment, eleven (35.5%) were positive: eight were positive by real time PCR and three were detected by both methods.

Detection rates according to sex and age

For bacterial meningitis cases, approximately two-thirds corresponded to boys (68.25%).

Age information was available for 156 suspected meningitis cases: 49 of them were positive cases whereas 107 of them were negative. Among the positive cases, the average age was 2 years and 4 months (SD= 2 years and 2 months, IQR= 3 years and 6.5 months, range= 2 months-11 years) and the median age was 10 months. Most positive cases corresponded to children aged one year and younger (57.14%). For children aged between 1 and 2 years, the rate was lower (8.16%). For children aged between 3 and 4 years, the rate was 18.37% (Table 2). For the 107 negative cases, most children were aged one year and younger (63.55%) (Table 2).

Table 2 Distribution of negative and positive cases regarding the age

Age (yrs.)	**Negative cases**	**Positive cases**	***S. pneumoniae***	***N. meningitidis***	***H. influenzae b***
1 mo-1	68	28	22	6	0
>1-2	7	4	4	0	0
>2-3	2	0	0	0	0
>3-4	5	9	7	1	1
>4-5	5	0	0	0	0
>5-11	20	8	5	3	0
Total cases	107	49	38	10	1

Distribution of meningitis cases according to years and seasons

Pneumococcal meningitis cases were the most observed during our study period; six cases in 2014 and eighteen cases each in 2015 and 2016. For meningococcal meningitis, the number varied from three cases (2015) to six and seven cases (2016 and 2014 respectively). Only one case of Hib was detected in 2015 (Fig. 1).

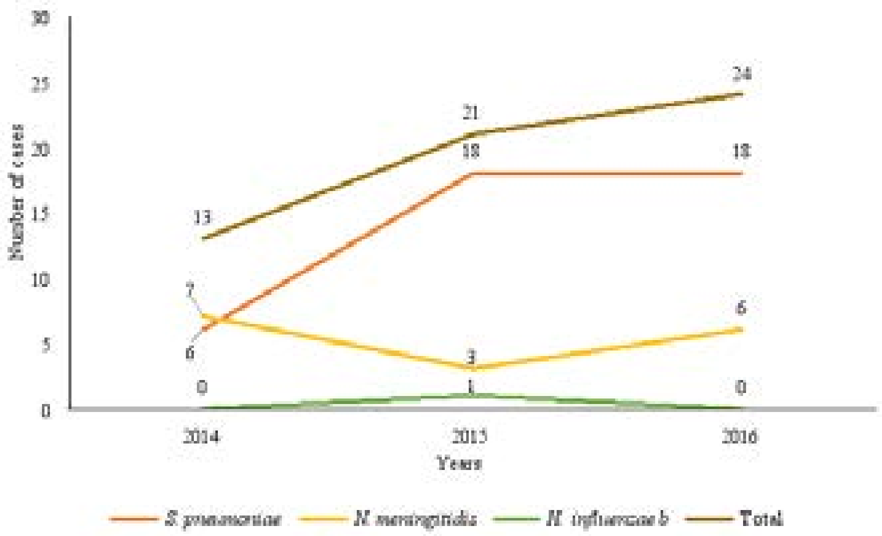

Fig. 1 Number of positive cases of *S. pneumoniae*, *N. meningitidis* and *H. influenzae b* according to years.

The distribution of bacterial meningitis cases revealed that most cases had occurred in winter and spring, with 28 cases (44.44%) and 18 cases (28.57%) out of 63 respectively. (Fig. 2).

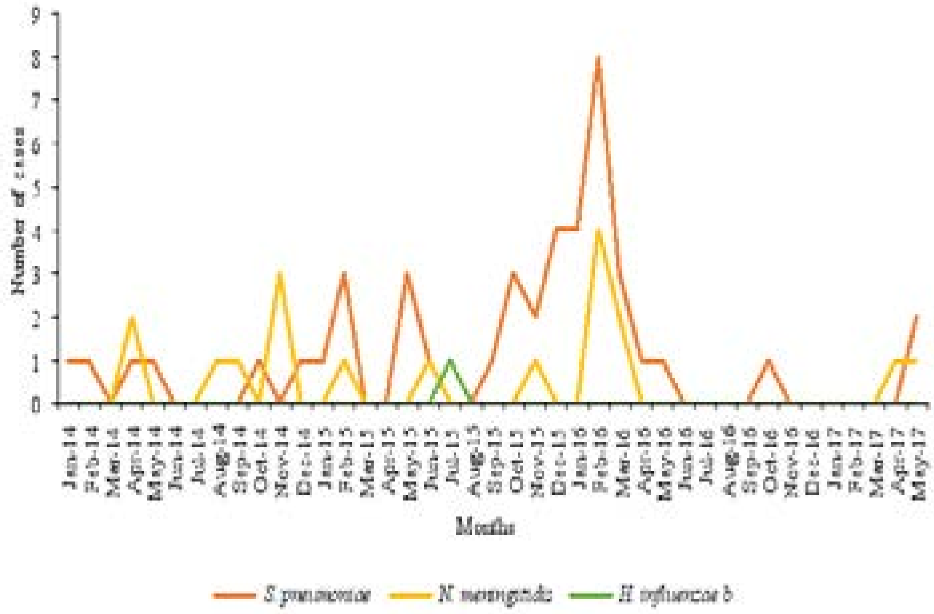

Fig. 2 Monthly distribution of positive bacterial meningitis cases (2014-2017) according to bacterial species.

For pneumococcal meningitis, 23 cases (52.27%) occurred in winter, twelve cases (27.27%) in the spring, eight cases (18.18%) during autumn seasons and only one case (2.27%) occurred in summer 2015. Moreover, we have noted a peak of pneumococcal meningitis (27 cases out of 44 cases) from September 2015 to May 2016.

For meningococcal meningitis, six cases (33.33%) occurred in winter, six cases (33.33%) in spring, two cases (11.11%) in summer and four cases (22.22%) occurred in autumn 2014.

For *H. influenzae* b meningitis, only one case occurred in July 2015.

Typing

Among the 18 meningococcal meningitis cases, 12 were positive by conventional methods: ten by culture and latex agglutination and two by direct examination. All the ten culture/latex agglutination positive samples were *N. meningitidis* serogroup B.

Moreover, 34 pneumococcal strains among the 44 positive cases were obtained and typed. Most *pneumococcal* cases were serotype 14 (9 cases) followed by serotypes 19F (8 cases), 6B (4 cases), 23F (4 cases), 6A/B (2 cases), 18 (2 cases), 19A (2 case), 6A (1 case), 12F (1 case) and 9V (1 case). Fluctuations of different serotypes isolates were observed. The peak of pneumococcal meningitis recorded from September 2015 to May 2016 was the consequence of a slight rise of different serotypes, mainly serotype 14 (7 cases) followed by serotype 19F (5 cases) and serotype 23F (4 cases).

Discussion

Bacterial meningitis is a major concern for public health worldwide and especially in developing countries[5,16,20,21]. This study describes the distribution of the three main causative bacteria of meningitis cases in Northern Tunisian children beyond neonates, after the reintroduction of Hib vaccination, and before the introduction of pneumococcal vaccination, from January 2014 to June 2017. It presents valuable results, which can be enlightening on bacterial meningitis in Tunisia given that it concerns the most populated governorates in Tunisia (5.347.531 inhabitants among 11.007.326 recorded in the Tunisian 2014 census by the National Institute of Statistics)[22].

This study suggested an important decrease or absence of Hib cases in Northern Tunisia (only one case detected in our study) and the predominance of pneumococcal cases. Such epidemiological study should be followed by others studies from Central and Southern Tunisia targeting the majority of health centres to confirm this conclusion. Our results are informative at the national stage and may contribute to reshaping the general view on national vaccination strategy by adapting the selection of pneumococcal vaccine to the circulating serotypes. At the regional level, it may be also of great interest to other low- and middle-income countries experiencing the same situation such as Egypt, Malaysia or Iran[23,24].

On the other hand, the added value of this study was the use of real time PCR, which allowed the detection of 10/62 additional cases to those obtained by conventional methods, especially for meningococcal cases (P=0.005). Indeed, it was previously demonstrated that molecular methods, targeting microbial genome, have the advantage to detect unviable bacterium. Therefore, they could overcome *N. meningitidis* alteration during transportation at ambient temperature and detect more positive cases[17,25]. Our significant results highlighted the need for an optimum framework to preserve meningococcus viability especially rapid transportation at 37°C[25].

Our findings demonstrated that bacterial meningitis is still causing damage in children beyond neonates in Northern Tunisia. However, our results revealed a general decrease in the number of bacterial meningitis cases during the study period in comparison with the number of cases obtained in the same region between 2000 and 2011 [8]. Indeed, Smaoui et al. study (2012) reported 24 to 53 meningitis cases[8] per year, whereas in our study, we found an average of 16 bacterial meningitis cases per year (13 to 24 cases per year between 2014 and 2016) despite using sensitive detection methods.
This result may be explained, first, by the depletion of Hib meningitis cases thanks to the reintroduction of Hib vaccination in 2011: ninety-nine cases were recorded between 2000 and 2011 [8] and one case was recorded from 2014 to mid-2017. Hib vaccination was introduced in 2002 and interrupted from 2005 to 2011 leading to the increase of Hib meningitis cases[8,11]. Our findings might suggest the efficiency of the reintroduction of Hib conjugate vaccine since 2011, as previously demonstrated in other regions of the world. Indeed, in high income countries, Hib meningitis cases decreased by 90% after introduction of Hib vaccination and in the middle- and low-income countries such as Morocco, they decreased by 75%[5,26]. Nowadays, this pathogen is considered to be eradicated in certain regions such as Europe[3]. Nevertheless, the recorded case corresponded to a vaccinated patient. This patient was given all Hib vaccine doses and thus immunological investigation was recommended. Other reports described Hib meningitis cases after vaccination and explained that it might be due to immunodeficiency or non-respect of vaccination schedule such as lack of booster shots[27].

Second, we also noted a global decrease of meningococcal cases. For instance, between 2000 and 2011, meningococcal cases varied from 4 to 21 per year [8] while we recorded 3 cases to 7 cases per year between 2014 and 2016. The high frequency recorded between 2000 and 2011 was mostly related to an outbreak that occurred in 2004-2005 (about 35 cases)[14,28]. Thus, continuous surveillance of meningococcal meningitis should be carried out. In other regions of the world such as sub-Saharan Africa and India, after the introduction of Hib and pneumococcal conjugate vaccines, *N. meningitidis* became a major cause of bacterial meningitis[5] and caused seasonal outbreaks with significant mortality. Also, in European countries, it became the leading cause of bacterial meningitis[3,29-31]. Several meningococcal conjugate vaccines have been introduced in different regions of the world, such as conjugate meningococcal serogroup C and protein-based serogroup B vaccines in Europe since 1999 and 2015 respectively, and meningococcal serogroup A conjugate vaccine in sub-Saharan Africa since 2010. Many reports proved their effectiveness in the following years, especially meningococcal C and A vaccines, by the significant decrease of meningococcal meningitis cases caused by these serogroups[32,33].
In contrast, the number of pneumococcal meningitis cases remains the same. Indeed, during the 2000-2011 period, an average of 13 cases per year (157 total cases) was reported and in our study period, we found an average of 14 cases (44 total cases). However, the main cases (27/44 cases) were obtained between September 2015 and May 2016. The most detected serotypes were 14, 19F, 23F, 6B and 19A. These fluctuations may describe the natural epidemiological evolution of these serotypes which are endemic within the population, be- cause of a possible weakening of the herd immunity. Nevertheless, we could not rule out the possible occur- rence of outbreaks, especially for serotype 14. More precise characterization methods were needed to confirm this hypothesis. Additionally, the unchanged number of pneumococcal cases highlighted the need to introduce pneumococcal conjugate vaccines in the Tunisian immunization schedule as recommended by WHO in 200734. Indeed, in April 2019, pneumococcal conjugate vaccine was introduced in the Tunisian vaccination program using PCV10 with three doses at two, four and eleven months[12]. This introduction might lead to a change in bacterial meningitis epidemiology in Tunisia.
The same issue was noticed in other countries[35]. For example, in Morocco, the introduction of pneumococcal conjugate vaccine, since 2010, has reduced the incidence of pneumococcal meningitis in the under-five years from 34.6 cases (2007-2010) to 13.5 cases (2011-2014) per 100 000 inhabitants[36]. Same results were shown in Western Europe countries following the introduction of PCV7 then later with PCV10 and or PCV13 [37]. In addition to the depletion of meningitis cases, the in-

troduction of pneumococcal vaccines may improve the childhood health status by preventing severe respiratory infection, especially for preterm infant. We think that it will be important to maintain this vaccination with high coverage and to avoid interruption, as previously experimented with Hib vaccination in Tunisia. For instance, in Kuwait, despite the introduction of PCV13 vaccine in 2010, *S. pneumoniae* remained the major cause of bacterial meningitis in children[38]. Sadeq et al study explained that it might be due to the low vaccine coverage, especially for infants less than six months and to a possible non-match of the common serotypes in Kuwait with coverage of PCV13 vaccine serotypes[38]. Thus, serotyping investigations should provide valuable information that enables to adjust vaccination strategies to the epidemiological context[14]. Otherwise, the use of these conjugate vaccines may give rise to the emergence of other uncovered serotypes, such as 6A, 19A and 3 when using PCV10 and 24F, 22F, 8 and 15A if using PCV13/PCV10 [37].

Even though our study was restricted to the Northern regions of Tunisia, it presented valuable and different results from previous studies in the area[8,10,11]. In this study, we did not include neonatal meningitis cases because the epidemiology is different from children beyond neonatal age since other main pathogens, such as *Escherichia coli*, *Listeria monocytogenes* and *Streptococcus agalactiae*, are implicated. Furthermore, results obtained in our laboratory during the study period showed that *S. pneumoniae*, *N. meningitis* and *H. influenzae* serotype b were not detected among neonatal cases (unpublished data). Also, a depth analysis of 6A/6B pneumococcal strains detected by PCR should be carried for precise serotype identification.

In conclusion, this study suggested a depletion or absence of Hib meningitis cases and the predominance of pneumococcal cases in Northern Tunisia, between 2014 and mid-2017. This finding gave an idea of a possible efficiency of Hib vaccination reintroduction, which should be confirmed by other studies targeting other regions of the country, and might be instructive at the local and regional level. On the other hand, it provided valuable data on meningitis epidemiology before the introduction of pneumococcal vaccine, which will be important to evaluate the effectiveness and the serotype coverage of the recently introduced pneumococcal vaccine PCV10.

Conflict of interest

None declared.

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