EPIDEMIOLOGY OF BACTERIAL MENINGITIS IN TUNISIAN CHILDREN(2000-2008)

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INTRODUCTION

Bacterial meningitis is a medical emergency associated with high mortality and neurological sequelae world wide. It is most commonly caused by *N. meningitidis* and *S. pneumoniae*. Before 1990s, *H. influenzae* b was the leading cause of bacterial meningitis among children in the range of age 3 months to 3 years, but Hib introduction vaccine as routine immunizations in all children in many countries have reduced the occurrence of invasive diseases due to this species. Analysis of cerebrospinal fluid (CSF) through diagnostic lumbar puncture (LP) is the cornerstone of effective diagnosis and management. CSF examination provide confirmation of the diagnosis and may yield vital data regarding aetiology, antibiotic sensitivities and important prognostic information. Establishing an aetiological diagnosis is essential in the public health management of meningitis.

We reported epidemiological characteristics of laboratory confirmed cases of bacterial meningitis in children during nine years (2000-2008).

MATERIAL AND METHODS

❖ We analyzed all laboratory confirmed bacterial meningitis between January 2000 and December 2008 in Children's Hospital of Tunis.

❖ Data on demographics and vaccination status were noted.

❖ All cerebrospinal fluid (CSF) specimens were studied by conventional methods :

➢ Macroscopic appearance : tubidity, blood staining or xanthchromia.

➢ Culture on specific media: Mueller Hinton, blood, chocolate agar supplemented with polyvitex (bio Mérieux) and brain hearth infusion (BIO-RAD). All the media were incubated at 37°C, under 5% CO_2 for blood and chocolate agar

➢ Microscopic procedure :

- Count of the total number of cells using a counting chamber (Nageotte)
- Differential count and Gram stain : prepare 2 smears using a cytocentrifuge, one stained with Methylene blue to make the differential count and the other stained with Gram stain to detect bacteria.

➢ Detection of bacterial antigen in CSF : using passive agglutination of sensitized latex particles (Pastorex meningitis, BIO-RAD).

➢ Since 2006, we introduced PCR to amplify the *bex A, crgA* and *ply* genes specific of *H influenzae N. meningitidis* and *S. pneumoniae* respectively directly from CSF. In the cases where PCR was positive, a second PCR was conducted to determin the serotype for *H. influenzae* and serogroup for *N. meningitis*.

❖ For all strains isolated :

➢ Strains identification was conducted by conventional methods

➢ Serotypes and serogroups were determined by slide agglutination procedure using *H influenzae* typing anti-sera (Difco) and meningococcal typing antisera (BIO-RAD).

➢ Antibiotic susceptibility using disk diffusion tests was performed according to CA-SFM guidelines.

- β-lactamase production was revealed by using a nitrocefin-impregnated disk (bio Mérieux)
- Penicillin non susceptible pneumococci (PNSP) and meningococci (PNSM) strains were defined by an inhibitory diameter of 5µg oxacillin disk less then 26 an 18 mm respectively.
- PNSP and PNSM were confirmed by the MIC determination of beta-lactams using E-test method (AB BIODISK).

RESULTS

❖ During the study period we have collected 328 laboratory confirmed cases of bacterial meningitis (fig 1) : 106 cases of *S. pneumoniae* (32.3%), 85 cases of *N. meningitidis* (26%), and 67 cases of *H. influenzae* (20.4%) (fig 2). Most cases (67.1%) occurred in children under 3 years of age (fig 3).

❖ Before the introduction of Hib conjugate vaccine (2000-2002), *H. influenzae* were the first species responsible for a great number of cases (40%) especially in children under three years of ages. During the period of vaccine generalisation (2003-2005) we noted a diminution in the number of cases of *H. influenzae* meningitis and this species become third place after *N. meningitidis* and *S. pneumoniae*. Now that Hib vaccine was been stopped, the number of Hib meningitis cases has increased (fig 4).

❖ *S. pneumoniae* : The most frequent serotype among *S. pneumoniae* isolates is the 14 (17.3%) followed by the serotype 23F (15.5%). PNSP accounted for 40.4%, the majority of them was of low level resistance (38.3%) (fig 5).

❖ *N. meningitidis* : the majority of strains belong to serogroup B (76.3%) followed by C (13.1%), and A (7.9%). PNSM accounted for 66.7%, the majority of them was of low level resistance (63.5%) (fig 6).

❖ *H. influenzae* : 93.3% of *H. influenzae* strains belong to serotype b, so this serotype is the most virulent one among Hi isolates. Conjugate vaccine has dramatically reduced the incidence of Hib meningitis in countries where good coverage has been achieved (USA and several European countries). Resistance to ampicillin was almost 46%, all with beta-lactamase production (fig 7).

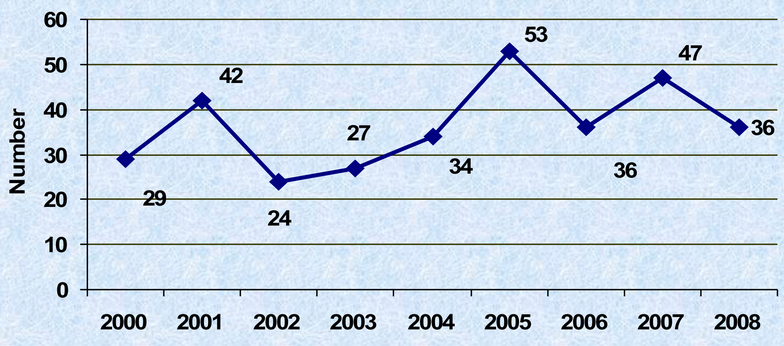

Fig 1. Annual distribution of all bacterial meningitis cases

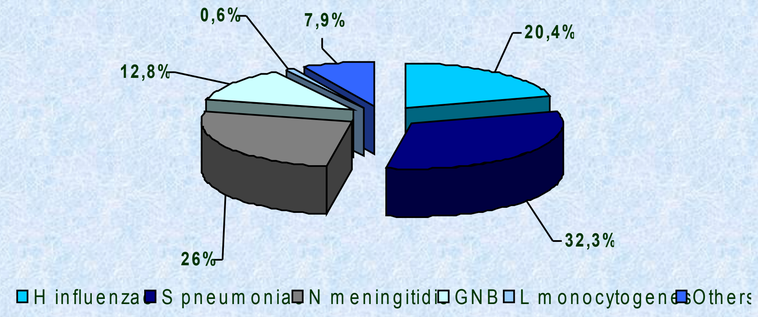

Fig 2. Distribution of all confirmed bacterial meningitis cases regarding to aetiology

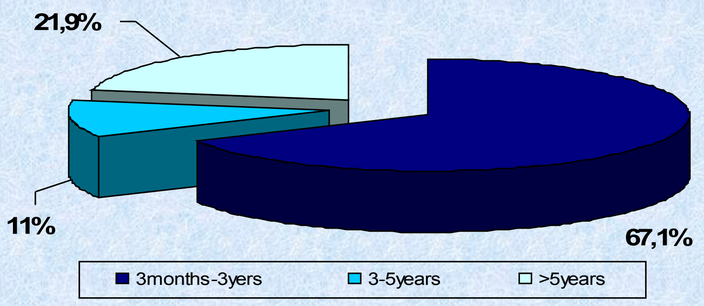

Fig 3. age distribution of all confirmed bacterial meningitis cases

Fig 4. Evolution of meningitis cases caused by *S. pneumoniae*, *N. meningitis* and *H. influenzae*

Table 1. Bacterial meningitis distribution regarding to tests positivity and aetiology

	N.meningitidis	*S.pneumoniae*	*H.influenzae*	others	Total
Positive culture	53	82	53	67	255
Bacterial antigen +/- posive direct Gram staining	22	18	13	3	56
(+)PCR	10	6	1	-	17
TOTAL	85	106	67	70	328

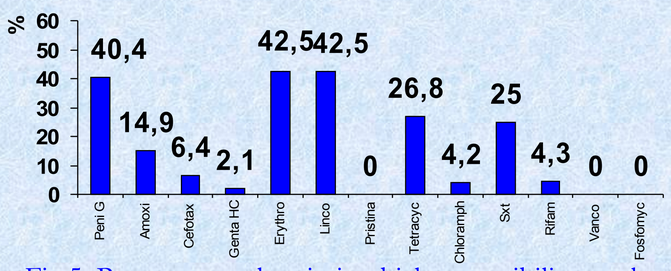

Fig 5. Pneumococcal antimicrobial susceptibility results

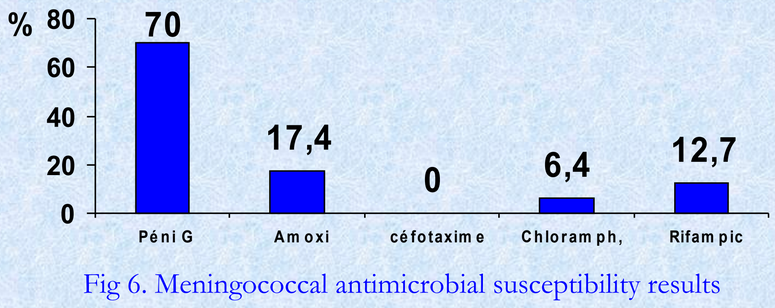

Fig 6. Meningococcal antimicrobial susceptibility results

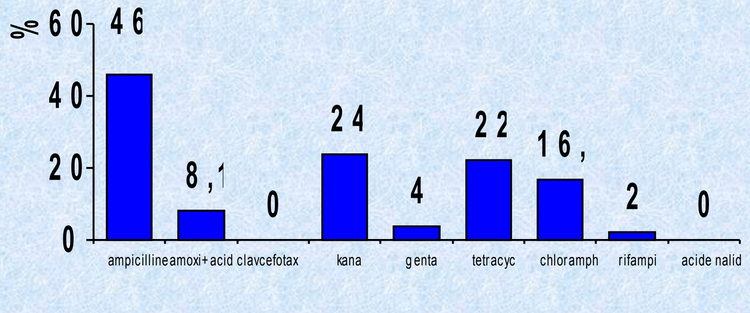

Fig 7 *H. influenzae* antimicrobial susceptibility results

CONCLUSION

The predominant bacterial species causing bacterial meningitis in tunisian childrens' were *S. pneumoniae* and *N. meningitidis*. *H. influenzae* b is very common in unvaccinated population such as demonstrated by the increasing number of Hib meningitis after vaccine stopping (2006-2008). The data presented in this study justify the reintroduction of conjugate Hib vaccine and the introduction of pneumococcal conjugate vaccine in all tunisian children.

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