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Original Research Article

Characteristics tests of cerebrospinal fluid cytology, chemistry and bacteriology in invasive paediatric bacterial meningitis in Madagascar

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ABSTRACT

Background: Paediatric bacterial meningitis is a major public health problem. CSF laboratory analysis is the key element to confirm the disease but remains difficult to access by clinicians or patients in low-resource settings. We described CSF biological tests results in invasive paediatric bacterial meningitis at the University Hospital Mother and Child of Tsaralalàna (CHUMET) in Madagascar.
Methods: In this retrospective and descriptive study from January 2013 to December 2018, all CSF samples that were confirmed for bacterial meningitis by triplex PCR *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* were enrolled. CSF collected from eligible children were tested by microscopy, culture, soluble antigen at CHUMET laboratory. Residual CSF was referred to the regional reference laboratory (RRL) for real-time polymerase chain reaction (RT-PCR) confirmatory testing and serotyping.
Results: Over the 6-year study period, 2286 CSF were tested by PCR, 141 (6.1%) were positive. The age group of (1-12 months) was the most affected (68.0%). The majority of CSF were cloudy with pleiocytosis >100/mm^3. Hyperproteinorrhea >1 g/l was noted in 48.2% of cases. The sensitivity of gram stain was respectively 56.6% and 75% for *Pneumococcus* and *Meningococcus* detection while for culture it was 28.3% and 66.6%, respectively. The average white cell count was notably higher in meningococcal meningitis and changed significantly according to the pathogens identified (p=0.007).
Conclusions: Paediatric bacterial meningitis diagnosis are based on CSF laboratory testing. Accessibility to multiplex PCR point-of-care tests targeting meningitis pathogens should be made easier for laboratories in low-income countries to improve patient care, monitor pathogen trends and vaccine impact program.

Keywords: CSF, Laboratory, *H. influenzae*, *N. meningitidis*, Paediatric, *S. pneumoniae*

INTRODUCTION

Bacterial meningitis is an invasive infection which affects with predilection children under 5 years of age. It is a medical emergency because of the risk of major neurological sequelae and even mortality if adequate management is delayed.[1] It remains a public health problem in developing countries, especially in Africa, where it is associated with major sequelae and high mortality.[1] Some bacteria can be responsible but mainly three are reported as the most frequently incriminated in paediatrics, which are *Pneumococcus* or *S. pneumoniae*, *Meningococcus* or *N. meningitidis* and *H. influenzae* type b (Hib).[2] The WHO has therefore recommended the introduction of vaccines targeting those pathogens in developing countries and the establishment of an

epidemiologic surveillance system to follow the trends of those bacteria.[3,4] If the hypothesis of meningitis was initially made on a clinical basis, the key to confirm the infection was the biological examination of the CSF. But the accessibility of these exams for patients and doctors in low-income countries is still difficult, due either to the high cost of these tests or to the lack of laboratory capacity, the majority of which don't perform all the tests or partially.[5] In Madagascar, the available data on bacterial meningitis in children are mainly focused on clinical, epidemiological and therapeutical aspects.[6] The aim of this study was to report the findings of cytobacteriological, chemical and molecular CSF tests in patients <5 years old with PCR-confirmed invasive bacterial meningitis.

METHODS

Study site

This retrospective and descriptive study was performed at the laboratory of the Mother and Child Teaching Hospital Tsaralalàna (CHUMET), a public 82-bed paediatric referral hospital, in the capital of Madagascar that offered primarily care to local patients, although there were some patients from elsewhere in the country. This was the only WHO vaccine preventable invasive bacterial disease (VPIBD) surveillance sentinel site in Madagascar since 2012.

Study population

Hospitalized children at CHUMET who fulfilled the WHO bacterial meningitis surveillance eligibility criteria, from January 2013 to December 2018, with a CSF specimen were the study population: a child with sudden onset fever (>38.5°C rectal or 38.0°C axillary) associated with one of the following clinical signs: neck/head stiffness, altered consciousness with no other alternative diagnosis; or with another meningeal sign.[4]

Surveillance circuit

CSF tests done at the sentinel site laboratory (SSL) included chemical (glucose and protein concentrations) and microbiological analyses (microscopy for cytology and gram stain, culture and soluble antigen test for the causative organisms: antigen detection for *S. pneumoniae* using Alere BinaxNOW®, antigen cards and/or the Pastorex™Meningitis Bio-Rad latex agglutination test detecting Hib, *S pneumoniae*, *N. meningitidis* groups (A, B, C, W and Y antigens), *E. Coli* K1 and group B *Streptococci*.

Any residual CSF at the SSL was stored at -20°C and shipped to the National institute for communicable diseases (NICD) RRL in South Africa, where RT-PCR molecular testing was performed on all CSF samples received. Total nucleic acid (DNA) were extracted from each CSF on the MagNA pure 96 instrument (Roche) and the extracts were run on the Applied Bio-systems 7500 Fast real-time PCR instrument (Applied Biosystems, Foster City, California, USA) for the detection of ctrA, lytA, and hpd genes for confirmation of *N. meningitidis*, *S. pneumoniae* and *H. influenzae*, respectively.[7]

Cases were considered confirmed bacterial meningitis if any of the pathogens were detected in CSF by any of the laboratory methods.

Patient information was collected on a clinical investigation form including identity (name, date of birth, gender and address), clinical information (diagnosis and date of admission, date of onset, previous antibiotic use and clinical signs), vaccination status, outcome and discharge (date and diagnosis). Laboratory test results were recorded in the laboratory logbook. These sentinel site clinical and laboratory data and PCR results were captured in a database.

Inclusion criteria

All children with PCR positive CSF to one of the major pathogens *S. pneumoniae*, Hib and *N. meningitidis* and analyzed by both gram stain microscopy and/or cytology and/or biochemical examination and/or culture and/or soluble Ag tests with available results were included.

Exclusion criteria

The children with incomplete records were excluded.

The studied variables were patient demographic profile and laboratory test results (macroscopy, microscopy, culture, latex soluble antigens and Binax Now *S. pneumoniae*).

The Chi square test was used for statistical analysis and a $p<0.05$ was considered to be significant. The performance of identification results by the routine examinations compared with the PCR results was expressed in terms of sensitivity and specificity (%).

RESULTS

A total of 4203 CSF samples from eligible children in the surveillance program were received at the CHUMET laboratory during the study period. Of these, 2286 (54.4%) were analyzed by triplex PCR for *N. meningitidis*, Hib and *S. pneumoniae* with a positive rate of 6.1% (n=141).

Children in the age group of 1-12 months most frequently had CSF positive for one of the three invasive bacteria, with mean age of 6 months and ranges from 0 to 59 months. The sex ratio was 1.35.

The macroscopic study showed that 43.2% (n=61) of the patients who had PCR positive CSF had turbid CSF while

it was clear for 6.8% of cases (n=52). Twenty-six CSF (n=26) or 18.4% were haematic.

White cell counts were performed in 136 patients, 38.2% (n=52) of which had a hyperleukocytosis >100 /mm^3. For 36% of the patients (n=35), the white cell count was <10 /mm^3.Of the positive CSF by PCR, gram stains were positive in 51% of cases (n=72) (Figure1).

S. pneumoniae was the common pathogen identified by triplex PCR positive CSF (79%). The age group of 1-12 months had the highest frequency of positive CSF, followed by the 12-24 month age group (Table 1). There were two cases of co-infection with Hib and *Pneumococcus* (Figure 2). The overall sensitivity of the gram stain in detecting *S. pneumoniae* was 56.6% while it was 75% for *Meningococcus* with a specificity more than 99%. The mean white cell counts differed significantly according to the invasive pathogen involved.

Table 1: Pathogens identified by PCR triplex by age group.

PCR results	Age group (month)			
	0-1	**1-12**	**12-24**	**24-59**
S. pneumoniae	6	81	15	10
H. influenzae	3	8	4	0
N. meningitidis	2	7	2	1
S. pneumonia* and *H. influenzae	0	0	2	0
Total	11	96	23	11

Table 2: Mean white cell count, proteinorrachia, and glycorachia values by PCR invasive pathogens identified.

PCR results	Mean		
	White cell count (/mm^3) [min-max]	**Proteinorrachia (g/l) [min-max]**	**Glycorachia (mmol/l) [min-max]**
S. pneumoniae	442 [0-1500]	1,68 [0.01-6.17]	2,47 [0.01-12.63]
H. influenzae	226 [0-9000]	1,48 [0.18-6.12]	3,20 [1.01-5.79]
N. meningitidis	874 [0-4500]	2,82 [1.34-4.65]	1,65 [0.01-7.49]
P value	0.007	0.108	0.132

Min: minimum; max: maximum.

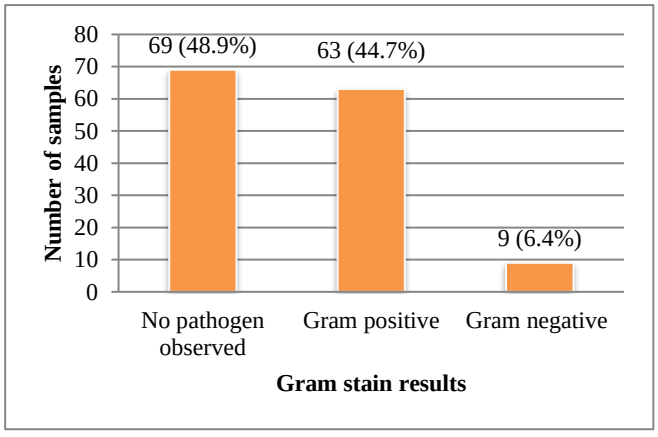

Figure 1: Gram stain results.

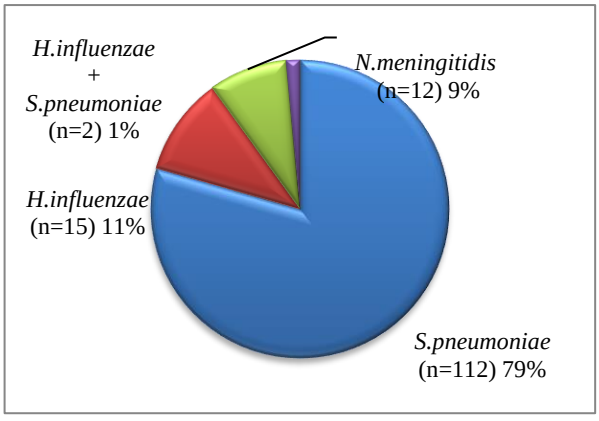

Figure 2: Pathogen identified by PCR triplex *S. pneumoniae, H. influenzae* and *N. meningitidis*.

One hundred and thirty (n=130) CSF were analyzed for chemical tests. Protein concentration was high (>100 mg/dl or >1 g/l) for 52.3% of cases (n=68) while for the glucose concentration, 40.7% (n=53) of patients presented a concentration <2.20 mmol/l. The mean values for white cell count, protein and glucose concentrations by pathogen identified are shown in Table 2.

Culture was done for 139 PCR positive CSF and it was positive in 28.7% of cases. It was sterile in 71.2% (n=99) of the cases. *S. pneumoniae* was the most frequently isolated organism in culture (23%, n=32) followed by *N. meningitidis* (6%, n=8). The overall sensitivity of culture for detection of *Pneumococcus* was 28% and 67% for *Meningococcus*. Of the 15 identified by PCR, no Hib was isolated in culture.

Of the 141 CSF positive by PCR, a total of 125 were tested by latex agglutination of soluble antigen. As for culture, no Hib was identified by this test. *Pneumococcus*

detection sensitivity was 53% and 33% for *Meningococcus*. Among positive CSF by PCR, 40 samples (n=40, 28.3%) were tested for *S. pneumoniae* soluble antigen by the BINAX NOW test (AlereR). The overall sensitivity of this test was 74.3%.

DISCUSSION

The PCR technique was selected to confirm the bacterial presence in the CSF because of its high sensitivity, even for patients with previous antibiotherapy. It detects the organism in spite of the low level of DNA, which usually limit interpretation of the routine bacteriological result (microscopic examination, culture). However, the risk of contamination was the major limitation. The PCR positivity rate was 6.1%. This differed from the findings of Goita et al who reported a rate of 15.2%.[8] The incidence of bacterial meningitis may differ by age group, terrain, country and the availability of immunization programs for vaccine preventable diseases.

The turbid appearance of the CSF was predominant in our study, which was similar to the findings of other authors.[9-11] This aspect was related to the pleiocytosis in the CSF and occurred when there were 200 white blood cells/mm^3, particularly in the presence of neutrophilic granulocytes.[12] It was important for the operator to note this appearance, as it can indicate a bacterial origin of the meningitis and required the initiation of an immediate antibiotic treatment to minimize mortality risks and complications. According to Carbonelle approximately 10% of meningococcal meningitis may occur with normal CSF.[12]

The cytological analysis of the positive CSF by PCR revealed that 38.2% of patients had more than 100 leukocytes/mm^3. The typical characteristic of purulent meningitis was the presence of high CSF cellularity (>500/mm^3) with predominantly altered neutrophilic cells. In some cases, no white cells can be found in CSF, although bacterial inoculum was high.[12] A recent study by Zimmermann et al had reported no association between the absence of pleocytosis and the presence of specific organisms in meningitis.[13] These findings suggested that absence of pleiocytosis was not a reliable exclusion of bacterial meningitis and must be interpreted in context of disease duration.

The gram stain was positive in 51% of cases, similar to the results of Meghraoui who reported 50% positivity of cases, but differed from Diffo whose results revealed 28% positivity due to antibiotic treatment.[14,15] A number of previous studies had reported the sensitivity of gram stain to range between 60 and 97% and specificity to around 100% without antibiotic treatment. With early treatment, sensitivity was commonly between 40% and 60% or less.[16] In our study, it was respectively 56.6% and 75% in *Pneumococcus* and *Meningococcus* detection.

The efficiency of this method depended on the amount of bacterial load in the sample, which can be reduced substantially if antibiotics were used.[17] Additionally to cytological analysis, the presence of bacteria on gram stain will confirm the bacterial meningitis and will give orientation about the involved species.

Glycorachy was not dependent on any threshold level but should be compared with blood glucose concentration and should be at least half of the latter. Nevertheless, any decrease of glycorachia under 2.20 mmol/l would suggest a pyogenic or tuberculosis meningitis rather than viral etiology. However, low levels of glucose concentration were not specific of bacterial infections since it can be found in other situations (inherited metabolic diseases).[18] And when it was too low, it indicated a bad outcome.

Culture positivity rate in this study was higher than the finding of Malki and Carlyse but lower than Bouskraoui et al results in 2014 where culture was positive in 54.5% of cases.[15,19,20]

These differences can be related to a prior antibiotic administration before lumbar puncture and to the inadequate pre analytical conditions of CSF which can lead to the negativation of culture despite the infection.[14] These invasive organisms were very sensitive to extreme temperature fluctuations. A very low bacterial inoculum could also explain the culture negativity. According to a recent study, a prior antibiotherapy reduced the positivity rate of the CSF cultures from 95 to 68%.[21]

This study found that culture was more sensitive in identifying *Meningococcus* than *Pneumococcus* (28.3% versus 66.6%). Prior antibiotic treatment may strongly impact the culture. In Madagascar, amoxicillin or ampicillin were widely available in ambulatory practice, sometimes as self-medication by the parents. *S. pneumoniae* was more susceptible to these antibiotics while for *N. meningitidis*, reduced susceptibility to penicillin G and aminopenicillins was more common and had been reported. The high sensitivity of PCR enabled the amplification of the DNA, although bacterial growth may be inhibited by the antibiotics, which explained the findings in this study. No Hib was isolated in culture among the fifteen identified by PCR. Nutritional requirement of the species and the use of antibiotic prior to hospitalization could explain this result.

Despite low sensitivity of the culture, this method remained the gold standard for the diagnosis of bacterial meningitis and allowed antibiotic susceptibility testing and antimicrobial resistance monitoring.[14]

The agglutination test for soluble antigen detection had low sensitivity and for meningococcus, performance of agglutination reagents depended on the serogroup involved.[22] Although the BINAX *S. pneumoniae* test was used in only 28.3% of the CSF tested, its sensitivity in detecting the pathogen was better than the other

bacteriologic test (direct examination, culture, latex soluble ag). This rapid diagnostic test, easy to use and requiring no special equipment, was advantageous in the regional healthcare structures where the laboratory's capacity to perform complete microbiological tests was often lacking. This test offered possibility to improve patients care and it should be used as a complementary tool to microscopic and biochemistry tests.

New vaccine introduction into vaccination program of Ministry of Health for many countries had changed the epidemiology of bacterial meningitis. *H. influenzae* type b meningitis had practically disappeared since the introduction of the Hib vaccine in routine immunizations. Several studies in African countries had reported a predominance of *S. pneumoniae*.[8,14,15,23]

Meningococcus represented 8.5% of PCR identified pathogens. It was the only bacterial meningitis that can cause epidemics. For a country, an epidemic situation can be defined by an unacceptable incidence rate requiring action.

This study was limited by being conducted in a single paediatric hospital of Madagascar and might not reflect the trends and the situation in other facilities.

CONCLUSION

This study highlighted the role of the laboratory in the diagnosis of bacterial meningitis. A variety of biological tests are actually available and the most sensitive to detect pathogens commonly involved is the PCR analysis. But in resource-limited countries, this test is not always accessible to all laboratories in the lower levels of the health pyramid. Microscopy tests should then be used in association with biochemical analysis and/or culture and or the rapid diagnostic test for soluble antigen by BinaxNow *S. pneumoniae*, which is user-friendly for lab technicians, to overcome the issue of the above tests sensitivity. However, development of multiplex PCR point-of-care tests targeting meningitis pathogens is encouraged, and access for laboratories in low-income countries should be made easier to improve patient care and monitor pathogen trends.

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