Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

Microbiological Profile of Meningitis: Analyzes of Cerebrospinal Fluid at the Laboratory of the National Institute of Public Health in 2017

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Received: 14 January 2020; **Accepted:** 28 February 2020

Citation: MS Sow, ST Diallo, A Camara, et al. Microbiological Profile of Meningitis: Analyzes of Cerebrospinal Fluid at the Laboratory of the National Institute of Public Health in 2017. Microbiol Infect Dis. 2020; 4(1): 1-5.

ABSTRACT

Objective: *Identify the etiogies of meningitis in Guinea.*

Methods: *This retrospective and descriptive study was carried out at the Microbiology Unit of the laboratory of the National Institute of Public Health (Guinea), using data from samples of Cerebro-Spinal Liquid (LCS) recorded in the database during the year 2017 of the said unit. The samples received were analyzed by various bacteriological and mycotic examinations (Gram stain, staining with Indian ink for Cryptococcus research, culture,) immunological (agglutination tests allowing the detection of antigens of the germs responsible for meningitis) and molecular (the polymerase chain reaction). The analyzes were performed with version 20 SPSS software.*

Results: *Data from 347 LCS samples were analyzed. Among which 31 samples were confirmed for meningitis, corresponding to (9%). These positive samples were dominated by men, 58% with a sex ratio of 1.3. The mean age of the patients was 28.95 ± 26.66 years. 29 samples were analyzed by microscopy, among them 13 showed germs, that is (44.83%). The agglutination tests were carried out on 25 samples, 15 of which had germs (60%). Out of 31 samples having benefited from the culture, 11 allowed the isolation and identification of bacteria, or 35.48%. In our study, Cryptococcusneoformans was the most found with 14 cases, or 46.16, followed by Streptococcus pneumoniae and Hæmophilus influenzae type B with respectively, 8 and 6, ie 25.81 and 19.35%.*

Conclusion: *It emerges from this study that the frequency of meningitis diagnosed in the laboratory of the national institute of public health in 2017 was 9%. The most frequent etiologies were Cryptococcusneoformans, Streptococcus pneumoniae and Hæmophilus influenzae type B.*

Keywords

Meningitis, SMIT, Donka.

Introduction

Meningitis is an inflammation of the brain envelopes (the meninges), causing abnormalities in the cerebrospinal fluid (CSF). This process extends throughout the subarachnoid space (ESA) from the brain to the spinal cord. It is most often caused by an infection (bacterial, viral, parasitic or fungal), but can be secondary to chemical irritation, subarachnoid hemorrhage, neoplasm or system disease [1].

It is estimated that more than 1.2 million cases of bacterial meningitis recur worldwide each year [2]. It is often formidable, especially in developing countries where health conditions are sometimes precarious. Analysis of the cerebrospinal fluid (LCS) remains an emergency examination, which is difficult to treat [3].

Despite the progress made in terms of diagnosis and therapy, meningitis remains burdened with heavy mortality, exceeding 35% in severe forms and significant permanent sequelae such as deafness, epilepsy, cerebral palsy, mental retardation [2]. Thus globally the World Health Organization (WHO) reported 407 850 meningitis deaths in 2011, 237 85 2 Africa [4].

The Republic of Guinea is part of the meningitis belt, sixteen out

of thirty-eight health districts are located in the band of this belt. Sporadic cases and outbreaks are reported every year. In recent years, the country has experienced a resurgence of meningitis epidemics [5]. The national directorate for the prevention and fight against the disease notified in 2011, 288 cases including 18 deaths in twenty-two health districts and four communes of Conakry. Neisseria meningitidis A was the responsible germ. In 2012, 186 cases including 13 deaths in 21 health districts and two communes of Conakry were recorded. Since 2002, the emergence of the Neisseria meningitidis serogroup W135 (MnW135) has been observed [6]. In order to optimize the isolation of the agent, several examinations must be used including direct examination, culture, the search for soluble antigens and the molecular detection of certain organisms by Polymerase Chain Reaction (PCR) [7]. PCR techniques have made it possible, wherever they have been performed, to improve the diagnostic performance of meningitis thanks to their high sensitivity and specificity [8].

Objective of this study

Study the etiologies of meningitis in the laboratory of the National Institute of Public Health of the Republic of Guinea.

Methodology

Type, period and framework

This retrospective and descriptive study was carried out at the Microbiology Unit of the laboratory of the National Institute of Public Health of the Republic of Guinea, using data from samples of Cerebro-Spinal Liquid (LCS) recorded in the base meningitis data of 1st January to December 31, 2017 of said unit.

Population

Inclusion: All samples from the LCS for suspected bacterial meningitis, collected in the various prefectures of Guinea and sent to the national laboratory of the INSP were included.

Exclusion: have been excluded of this study the samples to insufficient amount did not allow the realization of examinations.

Tools and technical data gathering: punctures the spinal fluid are performed by nursing teams in care centers before all suspicion s meningitis. Direct examinations with G ram staining were carried out in laboratory units. Other samples were transported to the laboratory of the national institute of public health in Trans-isolate environments or in dry tubes within 72 hours maximum. Samples received on t dry ube were first observed s in assessing the macroscopic appearance, and cytology was carried out. For the demonstration of the germ, the bacterial culture followed by the identification and or the polymerase chain reaction (PCR) were carried out.

Other analyzes have been also carried out: Gram stain, latex agglutination for research streptococcus, meningococcal and the Hae mophilus influenzae B. Mark of Cryptococcus neoformans by staining the china ink was made for ec negative hantillons and those for which there was a clinical orientation.

Data were collected on a sheet of Inquiry pre ed tablie to this effect then entered on a basis of Excel.

Characteristics e teristics of the test and description techniques

Staining of G ram: examination allowing the orientation of the etiological diagnosis towards the type of bacteria possible to be responsible for the disease. It distinguishes two (02) types according to the peptidoglycan content of the bacterial membrane. We used the Gram color kit of 4 series of 250ml which includes Gentiel violet which colors all bacteria blue for one minute, lugol which bites in one minute too, 95° alcohol which discolours bacteria less rich in peptidoglycan in 10-30 seconds and safranin which recolours bacteria in one minute. The bacterium is said to be gram positive when after the staining procedure it remains colored blue or purple and it is gram negative when it turns pink.
A microscopic examination the art form s bacteria were observed and coccobacilli were distinguished of Cocci.

Culture: is the gold standard for the etiological diagnosis of meningitis. It consists in seeding the samples on a medium favorable to the multiplication of bacteria. The result is conclusive in 18 to 24 hours. In our context, the most used medium was chocolate agar medium, which is cooked blood mixed with agar associated with a base, thus giving the preparation a chocolatey appearance, where all the bacteria responsible for meningitis can grow. anaerobic condition.

Coloring with Indian ink: examination allowing the search for cryptococcus in the LCS, after coloring, under the microscope the presence of a rounded, clear and shiny background indicates the presence of Cryptococcus ne oformans.

Immuno -detection: examination to detect antigen in the LCS (Ag) responsible for meningitis germs. LCS samples were centrifuged é s, then heated ed for five (5) minutes before the 'used ation of s antibody (Ab) for agglutination tests on the reading table. The test is positive if one of the samples agglutinates when there is a meeting between the Ag and its specific Ab. In this study used the kit included: Ac anti NmA, ac anti NMW / Y ac anti- Hib, ac anti S. pneumoniae and ac anti-Strep B.

The polymerase chain reaction (PCR): it studies the structure, the functional properties, the degradation and synthesis of molecules constituent microorganisms. It is the polymerase chain reaction which makes it possible to detect and amplify the DNA and / or RNA of the microorganism causing the disease. In this study real-time PCR was the method used. This PCR uses a fluorescent probe which allows the quantification and characterization of the amplicon formed in real time.

CSF samples of a volume of 200 microliter s were centrifuged at 12000 rpm for 20 min, DNA was extracted from pellets obtained by centrifugation with a preparation kit of bacterial DNA.

Detection of N. meningitidis, H. influenzae and S. pneumoniae by real-time PCR

Real-time PCR by Taqman type probes is based on the labeling of the latter at their 5′ end with a fluorochemist (reporter), for example FAM, and at their 3′ end by a fluorescent suppressor (quencher) or no, for example BHQ1, which inhibits the emission of the reporter when they are nearby. During the PCR, if the probe is hybridized to its target, it is hydrolyzed by DNA polymerase. The reporter thus separated from the quencher emits a signal proportional to the number of hydrolyzed probes, measurable at the time of elongation. The oligonucleotide primers and the probes marked respectively by the fluorochromes: FAM, HEX and Cy5 originate respectively from the sequences of the genes ctrA (meningococcus), Hpd3 (Hæmophilus) and LytA (streptococcus).

The results of the real-time PCR by the type of probes Taqman are based on the reading of the fluorescence detected by the thermal cycler during the a mplification. The thermal cycler calculates the background noise of each reaction, as well as the cycle threshold value (Ct) which is the number of PCR cycles for which the fluorescence of the measured signal exceeds the background noise threshold value indicating the amplification of the target sequence. If no increase in fluorescence is observed after 45 cycles, the sample is considered to be negative.

Study variables

The epidemiological variables were frequency, sex (man, woman), age in years dichotomized in 4 sections (less than a year; 1 year to 10 years; 11 years to 17 years; 18 years and over) and provenance (Conakry and h or Conakry). Biological variables were Gram stain, latex agglutination tests, bacterial culture, polymerase chain reaction (PCR) and India ink staining of cerebrospinal fluid.

Analysis

The qualitative variables were analyzed in frequency and on average ± confidence interval and the quantitative variables in percentage. Statistical analyzes are done with SPSS version20 software.

Ethical consideration

The protocol was submitted to the Public Health Chair at the training unit and of research (UFR) pharmacy from the University Gamal Abdel Nasser of Conakry and accepted, and anonymity was respected.

Results

347 samples were collected in 2017, of which 31 cases were positive, a frequency of 9% (Table 1). Of the 31 cases of meningitis 18 were of the male sex, that is 58% against 13 of the female sex, t 42% with a sex ratio M / F = 1.3. The average age of our patients was 11.53 +/- 15.26 years with extremes 1 month-80 years. The age group most represented was that of 18 years and over with 12 cases or 38.7%, followed by that of less than a year 09 cases or 29.03% (Table 2). More than half (55%) lived outside Conakry.

Only 6% (n = 2) patients were vaccinated. 312 samples benefited from the direct examination after Gram staining before the other examinations were performed, however the other 35 samples could not be analyzed by direct examination because of their transport in the trans- isolate medium. S ur 31 meningitis observed only two cases have not benefited from the staining Gram.

variables		Numbers	Percentages (%)
Frequency		31	9
Sex	Male	18	58
	Female	13	42
Age range	less than a year	9	29
	1- 10 years	8	26
	11-17ans	2	6
	18 and over	12	39
Origin	Conakry	14	45
	Outside Conakry	17	55
Vaccination status	Vaccinated	2	6
	Not vaccinated	29	94

Table 1: Epidemiological profile of the 31 cases of meningitis diagnosed in the laboratory of the national institute of public health in 2017.

Exams	Total exams	
	Positive number and percentage (%)	Total
Gram coloring	13 (44.83)	29 (93.55)
Agglutination tests	15 (60)	25 (80.65)
Culture	11 (35.48)	31 (100)
PCR	06 (40)	15 (48.39)
Chinese ink	14 (100)	14 (45.16)

Table 2: Distribution of the 31 cases of meningitis diagnosed in the laboratory of the national institute of public health in 2017 according to the frequency of detection of the examinations carried out.

Thus in our study, the microscopic examination after Gram staining carried out on 29 samples made it possible to note the presence of germ in 13 samples, ie 44.83%. The latex agglutination test, carried out on 25 samples, was positive in 15 cases, ie 60%. Out of 31 samples, the bacterial culture allowed the isolation and identification of bacteria in 11 samples, representing a 35.48% confirmation rate.

For this first year of PCR in the etiological diagnosis of meningitis in Guinea out of the 347 samples, there were 41 who benefited from PCR.

Our 15 samples from cases confirmed, the a PCR identified 6 cases, a confirmation rate of 40%. The microscopic examination after coloring with Indian ink carried out on 14 samples was 100% positive (Table 3). D years our study the cryptococcal not oformans was most found BC ec 14 cases or 45.16% followed by S Streptococcus pneumoniae and the He mophilus influenzae B respectively 8 and 6 cases or 25.81% and 19.35%.

Gram staining revealed 8 cases of DGP, 4 cases of BGN and 01 case of DGN with respective frequencies of 27.59%, 13.79% and 3.45%.

In our study, soluble Ag detected 7 cases (28%) of S t reptococcus pneumoniae, 5 cases (20%) of Hib, group B streptococcus and NmW had the lowest numbers respectively 2 and 1 case 8% and 4%.

Exams		Numbers	Percentages (%)
Gram stain (n = 29)	Gram negative bacillus	4	13.79
	Gram positive diplococcus	8	27.59
	Gram negative diplococcus	1	3.45
	Negative	16	55.17
Agglutination test (n = 25)	Neisseriae meningitidis type W / Y	1	4
	Hemophilus influenzae B	5	20
	Streptococcus pneumoniae	7	28
	Streptococcus B	2	8
	Negative	10	40
Culture (n = 31)	Neisseriae meningitidis W	1	33.33
	Streptococcus pneumoniae	6	19.35
	Hemophilus influenzae B	4	12.9
	Negative	20	64.52
PCR (n = 16)	Streptococcus pneumoniae	4	26.67
	Hemophilus influenzae B	2	13.33
	Negative	9	60
Indian ink (n = 14)	Cryptococcus neoformans	14	100
	Negative	0	0

Table 3: Distribution of the 31 cases of meningitis diagnosed in the laboratory of the national institute of public health in 2017 according to the examinations carried out.

In our series, the culture confirmed 6 cases of S treptococcus pneumoniae, 4 cases of Hib and 1 case of NmW with respective frequencies of 19.35%; 12.90% and 3.33%.

The PCR carried out found 4 cases of S treptococcus pneumoniae or 26.67% and 2 cases of Hib or 13.33%. The C ryptococcus neoformans was found in 100% of échanti llons tested with ink.

Discussion

The objective of this study was to identify the contribution of biological examinations of cerebrospinal fluid (LCS) to the etiological diagnoses of meningitis in the laboratory of the I NSP. The insufficiency in quality and quantity of certain samples, the systematic failure to carry out all the examinations on all the samples and the rupture of the primers for the PCR during our study constituted the main difficulties.

The absence of a search for viruses, parasites, specific bacteria in the LCS at the LNR / INSP and the lack of culture in certain environments such as Sabouraud were our main limitations in this study.

The data analysis of 347 patients was used to calculate the wearer frequency of e seed s of the meningitis is 9%. This frequency of meningitis in our study was higher than that found by *S. Mezghani* Maalej in Tunisia in 2005 who found in their study 2.41% of meningitis [9]. The male predominance is also found by Dao S et al. in Mali in 2008 where they reported a male predominance of 61.3% against 38.7% of the female sex with a sex ratio of 1.58 [10].

The high rate of meningitis in people over 18 and under 1 year of age could be explained by the fact that people in extreme ages are the most vulnerable because of the weak immune systems. Most of our samples came from the interior of the country, 55% compared to 45% who came from Conakry. Other authors reported in their study in 2013, 123 samples out of 480 suspected cases all from Upper Guinea [11], this could be explained by the fact that most of the districts of Upper Guinea are located in the meningitis belt tape.

The low vaccination rate found by this study is much lower than that found by Ouatara et al. in their study in Burkina Faso where they reported that vaccination coverage was estimated at 93.5% based on the vaccination card plus the history of vaccination [11]. The absence of vaccines against certain germs responsible for meningitis in the Expanded Program on Immunization (EPI) and the lack of vaccine culture in the population explains this result.

The low percentage of confirmation by PCR observed in our study is different from those found by A. Nikiema et al. in Burkina Faso where PCR had the highest confirmation percentage 64% [12]. The low confirmation rate per culture could be explained in part by the constraints imposed by the culture, the reference test, requiring appropriate logistics for the conservation and rapid transport of LCS samples from peripheral centers to reference laboratories or confirmation by culture is carried out. To this is added the low sensitivity of the culture in the event of prior antibiotic therapy and often, the microbial density in the LCS which varies according to the species in question and the phase of the disease.

The predominance of cryptococcus in our series corroborates those found by Traoré FA et al. in Guinea who also reported in their study a predominance of cryptococcus with 58% [13] and the predominance of pneumococcus among bacterial germs also corroborates that found by Nambei WS et al. in Bangui in 2012 where they observed a predominance of S treptococcus pneumoniae among Bacterial germs, ie 82% [14]

The predominance of Cryptococcus could be explained by the fact that part of our study population was immunocompromised.

In our study, the soluble Ag detected 28% of S treptococcus pneumoniae, 20% of Hib, the NmW had the lowest percentage 4%. This result is comparable to that found by Dao et al. in 2008 where latex agglutination tests identified 50% of S treptococcus pneumoniae, 32% H emophilus influenzae and 18% N eisseria meningitidis [10].

In our series, the culture confirmed 6 cases of S treptococcus pneumoniae, 4 cases of Hib and 1 case of NmW with respective

frequencies of 19.35%; 12.90% and 3.33%. Our result is very close to that found by M Sanou et al. in 2013 in Burkina Faso where the culture found 18% of S treptococcus pneumoniae, on the other hand N eisseria meningitidis had a high percentage 79% and H emophilus influenzae B, a low percentage of 4% [8].

The PCR carried out in our study found 4 cases of S treptococcus pneumoniae (26.67%) and 2 cases of Hib (13.33%).

Our result is different from that found by M Sanou et al. in 2013 where the PCR identified 59.3% of N eisseria meningitidis, 34.3% of S treptococcus pneumoniae and 3.7% of H emophilus influenzae B [8].

Conclusion

At the end of this study, it appears that the frequency of meningitis diagnosed at the National Laboratory of the National Institute of Public Health in 2017 was 9%. The most frequent causes were the C ryptococcus neofo summary into the S Streptococcus pneumonea and Haemophilus influenzae B. The frequency of detection varied depending on the exams, this shows that the negativity of one of the exams does not exclude meningitis and all the LCS analysis techniques are important to perform and complement each other. Their systematic implementation in the event of suspected meningitis would allow diagnosis and microbiological monitoring as well as their effective and appropriate treatment. However, the PCR made it possible to detect 3 samples which were negative in culture. This study was limited by the lack of research of the virus and parasites in samples of CSF, it will be another s studys.

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