Performance of the Eazyplex® CSF direct B in Cerebrospinal Fluid for the Etiological Diagnosis of Community-Acquired Bacterial Meningitis

Giulia Menchinelli[1], Barbara Fiori[1], Tiziana D'Inzeo[1], Flora Marzia Liotti[1], Giulia De Angelis[1], Liliana Giordano[1], Maria Federica Ventriglia[1], Flavio De Maio[1], Massimo Fantoni[2], Rita Murri[2], Maurizio Sanguinetti[1], Teresa Spanu[1]

Institutes of [1]Microbiology, and [2]Infectious Diseases, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario Agostino Gemelli, Rome, Italy

Gemelli
Fondazione Policlinico Universitario A. Gemelli
Università Cattolica del Sacro Cuore

INTRODUCTION

Community-acquired bacterial meningitis (CABM) is a rare but life-threatening disease. Delaying diagnosis and treatment can have disastrous consequences so early recognition and rapid adequate treatment are essential for reducing mortality and morbidity. The gold standard for the diagnosis of CABM is cerebrospinal fluid (CSF) chemical-cytological analyses, microscopic Gram stain examination and culture of which have long turnaround times and poor clinical sensitivity in patients treated with antibiotics before sample collection.

The specificity and sensitivity of the microscopic examination depend on the infectious microorganism, being estimated around 25-35% in *L. monocytogenes* meningitis, 50% in those caused by *H. influenzae*, 70- 90% in meningococcal meningitis and 90% in pneumococcal meningitis. The positive percentage of the culture method varies depending on the etiological agents: 96% for meningitis caused by *H. influenzae*, 87% for the pneumococcal and 82% in the meningococcal meningitis. However, both methods have limited sensitivity in samples of patients previously treated with antibiotics. In contrast, several studies have shown that the use of culture independent molecular assays can rapidly identify cultivable and non-cultivable organisms even in treated patients.

AIM OF THE STUDY

We performed a prospective clinical study to investigate the diagnostic value of the of Eazyplex ®CSF direct B (Amplex Diagnostics GmbH, Germany), a loop-mediated isothermal amplification (LAMP)-based system, designed to identify 4 common agents of CABM in 30 min: *Listeria monocytogenes, Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae* comparing the results obtained by culture and / or molecular method.

MATHERIAL AND MATHODS

Setting, and study design: The study was performed at the Microbiology laboratory of the A. Gemelli Hospital Foundation, Catholic University of the Sacred Heart in Rome from July 2016 to April 2018, with approval of the local Ethics Committee. Children and adults patients with clinically suspected CABM were enrolled.

Diagnostic protocol. The CFS samples were collected by lumbar puncture of patients. Chemical-physical analysis and microbiological analysis were performed. Upon arrival in the Microbiology laboratory, the sample was splitted into two aliquots: an aliquot was used for seeding on 5% TSA and chocolate PVX agar plates and thioglycollate broth which were incubated at 35-37 ° C in 5% CO2 for 2 to 5 days; the remaining part was subjected to cytocentrifugation and used for microscopic examination after Gram staining, the second aliquot was subjected to molecular investigation. using the Eazyplex ®CSF direct B testing system. The identification of microorganisms grown on culture media was obtained by mass spectrometry. (Fig.1)

The eazyplex® SuperBug CRE system (Amplex Biosystems GmbH, Giessen, Germany) is a qualitative genotypic diagnostic test, consisting of a freeze-dried, ready-to-use mixture for an isothermal amplification reaction able to detect *Listeria monocytogenes, Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumonia.* Amplification products that are generated by loop-mediated isothermal amplification (LAMP) are visualized by real-time fluorescence measurement. The results were automatically provided by the system after about 20 min.

Data analysis. The diagnostic accuracy of the Eazyplex CSF assay was evaluated comparing the results with that of the culture method and PCR and 16S rRNA sequencing. Results were classified as true positive (TP), true negatives (TN), falsenegatives (FN) or false positives (FP) .

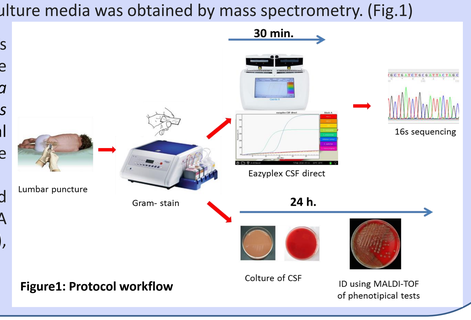


Figure1: Protocol workflow

RESULTS

During the study period, 56 samples of CSF for patients with suspected CABM were analyzed. Thirty-six percent of patients received antibiotics before hospital admission. A bacterial etiology has been demonstrated in 31 patients. The demographic charactherstics and laboratory parameters are presented in **Table 1**

Characteristichs	Samples with CSF bacteriological results	
Patients (n=56)	Positives (n=31)	Negatives (n=25)
Males	14	11
Age grup		
0-6 months	5	7
7 months -5 years	3	3
6-19 years	4	3
20-34 years	1	1
35-64 years	10	7
>=65 years	8	4
Blood chemistry tests		
Leukocyte count (cell/mm3)		
< 10000	6	8
> 10000	25	17
C-reactive Protein (mg/L)[c]		
< 5	0	6
6-100	3	8
> 100	20	4
CSF chemistry tests		
CSF/blood glucose ratio <= 0,36	26	2
Proteins (mg/dl) mean+SD	365±227	121±162
Leukocyte count (cell/mm³)		
0-100	4	21
101-500	5	2
501-1000	1	2
>1000	21	0
Positive blood cultures [d]	11	9

Table1. Demographic charactherstics and laboratory parameters.
CSF, cerebtospinal fluid
[a],PCR was determined in 41 patients.
[b], Blood cultures were drawn from all but two patients.

Bacterial etiology diagnosis was made in 55.3% (31/56) of patients (Figure 2); the main species identified were *S. pneumoniae and N. meningitidis.*

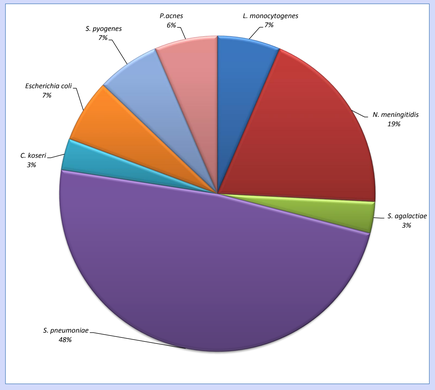
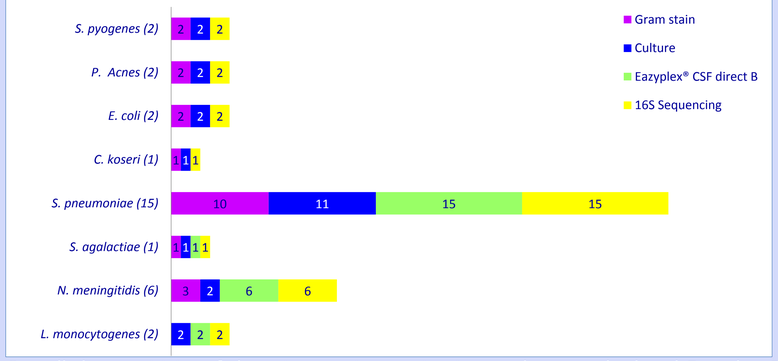


Figure 2: Distribution of etiologic agents in 31 patients with bacterial meningitis

Figure 3. Comparison of microscopic examination, culture , Eazyplex CSF B and PCR 16 S results



As reported in **Figure 2**, microscopic examination revealed the presence of microorganisms in 21 cases. The culture-based method yielded 23 bacterial isolates. The Eazyplex CSF B assay yielded a positive result in 24 cases. The results for the 8 discrepant cases (molecular-positive and culture-negative), all obtained in patients pre-treated with antibiotics, were confirmed by molecular method. The 7 cases with culture-positive and Eazyplex CSF B-negative results were due to the presence of bacteria not included in the panel.

Overall the sensitivity of the microscopic examination, culture method and Eazyplex CSF B assay were 67.7%, 74.2% and 77.4% respectively. Considering only the microorganisms included in the panel, the sensitivity of the microscopic examination, of the culture method and of the Eazyplex CSF B assay were 58.3%, 66.7% and 100%, respectively. The specificity of Eazyplex CSF B was 100%. Of the 25 patients with a negative result on the CSF sample, 1 had a bacteraemia by *Acinetobacter baumannii*, 1 by *E. coli*, 1 by *Klebsiella pneumoniae*, 1 by *S. agalactiae*, 2 by *N. meningitidis*, 2 by *Staphylococcus aureus*, 1 by *S. mitis*. A viral etiology has been documented in 4 patients (2 Epstein-Barr virus, 1 Cytomegalovirus and 1 Herpes simplex 1).

CONCLUSIONS

These results suggest that the Eazyplex ®CSF direct B assay can provide rapid, accurate, and reliable identification of bacterial pathogens responsible for CABM, although there is room for improvement in the identification of certain species, especially *Haemophilus influenzae* and *E.coli* to increase the accuracy of microbiological diagnostics and patient management.

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