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ORIGINAL ARTICLE

Implementation of the eazyplex® CSF direct panel assay for rapid laboratory diagnosis of bacterial meningitis: 32-month experience at a tertiary care university hospital

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Abstract

We aimed to report a 32-month laboratory experience with the eazyplex® CSF direct panel assay for the rapid diagnosis of meningitis due to six most common bacterial species (*Escherichia coli*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*). We included all cerebrospinal fluid (CSF) samples from patients admitted with a clinical suspicion of meningitis/encephalitis between May 2016 and December 2018 at our hospital. In addition to the eazyplex® assay, both Gram stain microscopy and culture were performed, and results were confirmed with 16S rRNA PCR/sequencing. Patients' demographics and relevant clinical information were collected. Of 135 studied patients, 44 (32.6%) had a microbiologically documented diagnosis of meningitis. Overall, we identified 21 *S. pneumoniae*, 10 *N. meningitidis*, 6 *L. monocytogenes*, 3 *E. coli*, 2 *Streptococcus pyogenes*, 1 *S. agalactiae*, and 1 *Citrobacter koseri* as aetiological agents. The eazyplex® assay allowed identification in 40 (90.9%) cases, with four not identified cases due to microorganisms not included in the panel at the time of testing. Thirty-two (72.7%) cases had positive culture results, whereas 28 (63.6%) cases had positive Gram stain results. Notably, combining Gram stain and eazyplex® assay allowed identification in 100% of cases. After notification of rapid results, physicians modified the empiric antibiotic therapy, which became appropriate in three patients (all with *L. monocytogenes* meningitis). The eazyplex® CSF panel assay worked better than culture in detecting the most common agents of bacterial meningitis and accelerated the diagnosis leading to timely initiation or continuation of appropriate antibiotic therapy.

Keywords eazyplex® CSF · Bacterial meningitis · Rapid diagnosis · Molecular assay · Antibiotic therapy

Brunella Posteraro and Teresa Spanu contributed equally to this work.

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Introduction

Among central nervous system infections, meningitis—a meningeal inflammation typically defined as an abnormal white cell count in the cerebrospinal fluid (CSF) [1, 2]—is mostly due to viruses (e.g. enterovirus [EV]) and bacteria (e.g. *Streptococcus pneumoniae*) [3, 4], whereas fungi (e.g. *Cryptococcus neoformans*) and parasites (e.g. *Toxoplasma gondii*) are less frequent causes. Bacterial organisms such as *Streptococcus agalactiae* and *Escherichia coli* are leading causes of neonatal meningitis [5]. Despite being uncommon compared with viral infections [6, 7], bacterial infections need rapid ruling out because of their substantial mortality and long-term morbidity [8–13].

According to recent data, mortality remains as high as 30% in pneumococcal meningitis and 5–10% in meningococcal (i.e. *Neisseria meningitidis*) meningitis [1, 14]. Thus, CSF sampling via lumbar puncture is central to distinguishing meningitis from other diseases as well as bacterial from non-bacterial aetiologies. For most patients, CSF sampling (and starting empiric antimicrobial treatment) should occur within few hours of admission [15, 16], unless it is strictly necessary to wait for computed tomography of the head before lumbar puncture. In this case, if a patient receives antimicrobial treatment, the diagnostic yield of CSF culture will be inevitably low, thus posing the necessity of a microbiology laboratory diagnosis based on nucleic acid amplification tests (NAATs) [17].

Current meningitis management guidelines recommend CSF molecular testing combined with CSF Gram staining and culture [16, 18], which are routine CSF examinations for aerobic bacteria—regardless of the extent of suspicion for infection—in many laboratories worldwide [17]. Typically, NAATs are singleplex and/or in-house real-time PCR assays targeting specifically a single microbial pathogen and, thus, display a high sensitivity and specificity [19]. Instead, as broader tests, multiplex molecular assays have the advantage of targeting more infectious agents eventually responsible for a given clinical syndrome (e.g. meningitis/encephalitis [ME]), thereby reducing the risk of missed diagnoses [19]. One of these assays, namely the FilmArray® ME (BioMérieux, France, previously BioFire Diagnostics, USA) panel, has been USA Food and Drug Administration approved to allow the simultaneous detection of 14 different viral, bacterial, or yeast targets in CSF samples [20]. However, clinical validation data of similar molecular tests, such as the Allplex Meningitis panel (SeeGene, Seoul, Republic of Korea), the eazyplex® CSF direct panel (Amplex Biosystems GmbH, Giessen, Germany), or other recently developed assays, are still missing or scarce [19].

Since 2016—before the advent of the FilmArray® ME panel for routine clinical use—we have implemented the loop-mediated isothermal amplification (LAMP)–based eazyplex® CSF direct panel assay—which detects six bacteria, i.e. *E. coli*, *Haemophilus influenzae*, *Listeria monocytogenes*, *N. meningitidis*, *S. agalactiae*, and *S. pneumoniae* (the same as with the FilmArray® ME panel)—in our laboratory diagnostic meningitis workflow. In this article, we describe our experience with this assay for diagnosing bacterial meningitis over 32 months (May 2016 to December 2018) at a tertiary-care university hospital in Italy. Based on our findings, we believe that the eazyplex® CSF direct panel assay could rationally and accurately facilitate the diagnosis of bacterial meningitis, particularly in resource-constrained settings with a moderate laboratory detection capacity.

Materials and methods

Patients and CSF samples

This study was conducted from 1 May 2016 through 31 December 2018 at the Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, which is a 1500-bed tertiary care university hospital in Rome, Italy. A central microbiology laboratory, which is open from 7:00 a.m. to 7:00 p.m. (Monday through Friday) and from 7:00 a.m. to 4:00 p.m. (Saturday), serves all hospital wards. As required, on Sunday/holidays as well as outside opening hours, a 24-h, on-call service from the microbiology laboratory personnel is available for urgent examination of appropriate patient samples (e.g. CSF samples). Furthermore, an inpatient infectious disease consultation team (IDCT), composed of four ID specialists, operates on case-by-case request. Notably, the laboratory diagnostic workflow always combines real-time results with IDCT notifications.

Non-repetitive CSF samples from adult or paediatric patients admitted to the hospital's emergency department and from neonates admitted to neonatal wards with a clinical suspicion of meningitis/encephalitis, subject to physician's discretion, were included in the study. All post-surgical meningitis cases were excluded. Patients were suspected of bacterial meningitis if some combination of headache, irritability, vomiting, lethargy, neck stiffness, or altered mental status plus 1 or more of the following were present: temperature > 38 °C, leucocytosis (white blood cell [WBC] count of > 10,000 cells/mm^3), positivity for C-reactive protein (CRP serum level of > 5 mg/l), hyperglycaemia (blood glucose level of > 110 mg/dl), or a petechial or purpuric rash [1]. Clinical conditions such as otitis media, sinusitis, CSF leak, alcoholism, human immunodeficiency virus infection, or other immunosuppressive conditions or medications were considered to be predisposing for bacterial meningitis [1]. Upon receipt, CSF samples were processed for microbiological analyses as described below. Data collected from patients' medical chart records included the following information: demographics; date and time of hospital admission; signs and/or symptoms of disease; blood and CSF laboratory findings; antimicrobial course (i.e. treatments either prior to/or after CSF sampling or after obtaining CSF sample's microbiological results); and 15-day outcome (i.e. assessed from the disease diagnosis until 15 days or death). We de-identified and securely stored data to preserve anonymity and confidentiality.

Microbiological testing of CSF samples

Bacterial culturing and Gram staining

We used each CSF sample's aliquots to perform bacterial culture and Gram staining, according to standard protocols [21].

Briefly, we plated CSF aliquots on either blood, chocolate, or MacConkey agar media (primary cultures) and inoculated them in thioglycollate broth tubes to obtain secondary cultures. All primary or secondary culture plates were incubated at 37 °C in 5% CO_2 for 24–48 h or up to bacterial growth detection (i.e. for up 4 to 7 days, as appropriate). Bacterial isolates that grew from positive cultures were identified using the MALDI BioTyper™ system (Bruker Daltonics, Bremen, Germany), and, in cases of unsuccessful identification, conventional phenotypic tests and/or PCR/sequencing of the 16S ribosomal RNA (rRNA) gene or the *rpoB* gene was performed [22]. We tested all the isolates for antimicrobial susceptibility using the VITEK® 2 system [23], and results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (versions 6.0, 7.1, 8.0, and 8.1, http://www.eucast.org/clinical_breakpoints/). In parallel, CSF aliquots were cytocentrifuged to prepare smears that were Gram-stained before microscopic examination.

eazyplex® CSF direct panel analysis

CSF samples were analyzed with the eazyplex® CSF direct panel (a Conformité Européene (CE)–marked in vitro diagnostic device), which was upgraded from the former eazyplex® CSF direct panel B version until it included all six bacterial targets available in 2017 (the eazyplex® CSF direct panel M version), according to the manufacturer's instructions (support@eazyplex.com). Briefly, after boiling a mixture of 125-μl CSF and 25-μl lysis solution, we mixed 125 μl of the lysed suspension with 125 μl of solution buffer. A 25-μl aliquot of the resulting suspension was then dispensed into the eazyplex® CSF strip, which consisted of tubes each containing a lyophilized master mix for bacterial species–specific LAMP (six tubes) and a LAMP-inhibition internal control (one tube). After a 30-min reaction in the instrument, the eazyplex® software automatically interpreted the results according to the real-time fluorescence detection of amplification products.

Reference molecular or additional analysis

For all study's CSF samples, with either positive or negative results obtained by abovementioned microbiological analyses, reference molecular detection of bacterial 16S rRNA was performed as previously described [24]. Furthermore, the CSF samples were routinely tested for the presence of herpes simplex virus (HSV)-1, HSV-2, varicella-zoster virus (VZV), or EV by real-time PCR-based methods, namely RealStar® alpha Herpesvirus PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) and Liferiver™ EnteroVirus Real Time RT-PCR Kit (Obelis S.A., Brussels, Belgium), respectively. Depending on exposure/risk factors/imaging, upon discussion with IDCT specialists, additional CSF testing included the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) to detect *Mycobacterium tuberculosis* DNA or the Remel™ Cryptococcus Antigen Test Kit assay (Thermo Fisher Scientific, Cleveland, OH, USA) to detect *C. neoformans* capsule polysaccharide antigen. Exceptionally, CSF samples' aliquots were submitted to further molecular testing (e.g. for cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, measles virus).

Data analysis

Demographic and clinical data were reported as count (percentage) or median (interquartile range) as appropriate. We assessed differences between patient (i.e. with or without bacterial meningitis) groups using the chi-square test for categorical variables and the Wilcoxon signed-rank test for continuous variables. Sensitivity, specificity, and positive and negative predictive values (with their 95% confidence interval) of any conventional (i.e. culture or microscopy) or molecular (i.e. the eazyplex® CSF direct panel) method were evaluated in comparison with the reference 16S rRNA PCR/sequencing method. Analysis was performed with the Stata software version 11.1 (StataCorp, College Station, TX, USA). Two-sided p values of < 0.05 were considered significant.

Results

Of 135 patients (70 males and 65 females) for whom CSF samples were analyzed, 44 (32.6%) had a diagnosis of bacterial meningitis as documented by any of microbiological methods used in this study (see below). Table 1 illustrates demographic and clinical characteristics for the 44 (30 adult, 10 paediatric, and four neonatal) patients compared with those of 91 (51 adult, 27 paediatric, and 13 neonatal) patients without diagnosis of bacterial meningitis. All peripheral blood parameters, including WBC count, neutrophil cell count, glycaemia, and CRP level, were significantly more abnormal in patients with bacterial meningitis than in patients without bacterial meningitis (p values, 0.003, 0.002, 0.001, and $<$ 0.001, respectively). Similarly, abnormalities in CSF parameters, including glucose CSF/blood ratio, protein concentration, WBC count, and neutrophil count, were significantly more frequent in patients with bacterial meningitis than in patients without bacterial meningitis (p values, < 0.001, 0.001, $<$ 0.001, and < 0.001, respectively). Notably, across 44 patients with bacterial meningitis (Table S1), 11 (25.0%) had fever, neck stiffness, and altered mental status, 6 (13.6%) had fever and neck stiffness, 14 (31.8%) had fever and altered mental status, and 13 (29.5%) only fever, if considering only the triad of "classic" signs/symptoms displayed by patients at admission; instead in the 13 patients, fever was accompanied with

Table 1 Demographic and clinical characteristics of 135 patients included in the study

	Patients with bacterial meningitis ($n = 44$)	Patients without bacterial meningitis ($n = 91$)	p value
Demographics			
Male gender	22 (50.0)	48 (52.7)	0.76
Adult patient	30 (68.2)	51 (56.1)	0.17
Non-adult patient[a]	14 (31.8)	40 (44.0)	0.17
Median age (IQR) (years)	51.5 (8–64.5)	31.0 (1–66.0)	0.31
Peripheral blood findings			
WBC count > 10,000/mm^3	38 (86.4)	56 (61.5)	0.003
Neutrophil cell count > 55%	44 (100.0)	73 (80.2)	0.002
Glycaemia > 110 mg/dl	35 (79.5)	45 (49.4)	0.001
C-reactive protein > 5 mg/l	44 (100.0)	64 (70.3)	< 0.001
CSF findings			
Glucose CSF/blood ratio < 0.66	43 (97.7)	48 (52.7)	< 0.001
Protein concentration > 40 mg/dl	42 (95.4)	64 (70.3)	0.001
WBC count > 5 cells/mm^3	44 (100.0)	33 (36.3)	< 0.001
Neutrophil cell predominance	44 (100.0)	17 (18.7)	< 0.001

Data are given as no. (%), unless otherwise specified. Thresholds for listed clinical findings are according to that reported previously [16]

WBC, white blood cell; *CSF*, cerebrospinal fluid; *WC*, white cell

[a] Paediatric or neonatal patient

one or more "non-classic" signs/symptoms (i.e. vomiting, headache, lethargy, or petechiae).

Table 2 summarizes the bacterial aetiologies for the 44 cases of meningitis identified, which were in decreasing order *S. pneumoniae* (21 cases), *N. meningitidis* (10 cases), *L. monocytogenes* (6 cases), *E. coli* (3 cases), *S. pyogenes* (2 cases), *S. agalactiae* (1 case), and *Citrobacter koseri* (1 case). Particularly, in 14 children (paediatric/neonatal patients), bacterial meningitis were due to *S. pneumoniae* (6 cases), *N. meningitidis* (4 cases), *L. monocytogenes* (1 case), *S. pyogenes* (1 case), *S. agalactiae* (1 case), or *C. koseri* (1 case). In the last case, the patient was a preterm neonate who developed a *C. koseri* meningitis with brain abscesses. Table S1 provides a case-by-case description of the microbiological results obtained. The eazyplex® CSF direct panel assay allowed identification in 40 (90.9%) of 44 cases. Of four not identified cases, three were caused by microorganisms not included in the panel (2 *S. pyogenes* and 1 *C. koseri*), and one was caused by a microorganism (*E. coli*) not included in the panel at the time of testing (i.e. before the release of an upgraded version of the method (eazyplex® CSF direct panel M). Twenty-eight (63.6%) of 44 cases had positive Gram stain microscopy results, whereas no bacterial forms (i.e. cocci or rods) were microscopically seen in the remaining 16 cases that tested positive for *N. meningitidis* (six cases), *S. pneumoniae* (six cases), or *L. monocytogenes* (four cases) by both the culture and the eazyplex® CSF direct panel methods. Thirty-two (72.7%) of 44 cases (including the four eazyplex® CSF negatives) had positive culture results, whereas no bacterial growth was seen in the remaining 12 cases that tested positive for *N. meningitidis* (six cases) or *S. pneumoniae* (six cases) by the eazyplex® CSF direct panel alone. Interestingly, combining both the microscopy and eazyplex® CSF direct panel methods allowed identification in 44 (100%) of 44 cases.

We confirmed all 135 positive or negative results by means of the 16S rRNA PCR/sequencing reference method. No false-positive results for each of the three methods used in the study occurred, resulting in overall 100% specificity. Excluding other bacterial aetiologies—no samples were positive for *M. tuberculosis*—11 (12.1%) of 91 negative samples had a positive molecular result for viral targets, such as HSV-1 (three cases), Epstein-Barr virus (two cases), VZV (two cases), cytomegalovirus (one case), EV (one case), human herpesvirus 6 (one case), and measles virus (one case). No sample yielded a positive result for cryptococcal antigen, leading to rule out *C. neoformans* meningitis in all 91 patients.

Figure 1 depicts the microbiology laboratory diagnostic workflow with relative timing for all 135 CSF samples analyzed for bacterial meningitis diagnosis. According to this workflow, rapid and actionable (Gram stain/eazyplex® based) results as well as late but still actionable (culture based) results were available, with an average of 1.5 or 17.8 h of the CSF sample receipt in the laboratory, respectively. As detailed in Table 3, in the case of notification of rapid results, the ID physician immediately prompted to modify the empiric antibiotic treatment, which became appropriate in three patients

Table 2 Microbiological results for the 135 cerebrospinal fluid samples analyzed for bacterial meningitis diagnosis

	No. of samples with a positive result by the following method(s)			
	eazyplex® CSF panel	Microscopy	eazyplex® CSF panel plus microscopy	Culture
Microorganisms (no. of results)[a]				
S. pneumoniae (21)	21	15	21	15
N. meningitidis (10)	10	4	10	4
L. monocytogenes (6)	6	2	6	6
E. coli (3)	2[b]	3	3	3
S. pyogenes (2)	0[c]	2	2	2
C. koseri (1)	0[c]	1	1	1
S. agalactiae (1)	1	1	1	1
All (44)	40	28	44	32
None (91)	0	0	0	0
Method(s)' performance				
Sensitivity (95% CI), %	90.9 (78.3–97.5)	63.6 (47.8–77.6)	100.0 (92.0–100.0)	72.7 (92.0–100.0)
Specificity (95% CI), %	100.0 (96.0–100.0)	100.0 (96.0–100.0)	100.0 (96.0–100.0)	100.0 (96.0–100.0)
PPV (95% CI), %	100.0 (91.2–100.0)	100.0 (87.7–100.0)	100.0 (92.0–100.0)	100.0 (89.1–100.0)
NPV (95% CI), %	95.8 (89.6–98.8)	85.0 (76.9–91.2)	100.0 (96.0–100.0)	88.3 (80.5–93.8)

CI, confidence interval; *PPV*, positive predictive value; *NPV*, negative predictive value

[a] Cerebrospinal fluid (CSF) samples were also analyzed by 16S PCR/sequencing to confirm positive or negative results

[b] One sample tested as negative because the former eazyplex® CSF direct panel version (i.e. the eazyplex® CSF direct panel B version) did not contain *E. coli* as a target at the time of testing

[c] Sample(s) tested as negative because the current eazyplex® CSF direct panel version (i.e. the eazyplex® CSF direct panel M version) does not contain *S. pyogenes* or *C. koseri* as a target

(all positive for *L. monocytogenes*) and remained substantially appropriate in 37 other patients. When available—in 12 of 44 cases, the bacterial culture did not give any bacterial isolate—antimicrobial susceptibility testing results allowed to modify the antibiotic therapy for optimal treatment. Four patients could not benefit from any treatment modification because they precociously died following the disease. In 31 (77.5%) of 40 cases, patients were receiving ceftriaxone as specific antibiotic therapy, alone or in combination with vancomycin. In three patients infected by *L. monocytogenes* (1 empirically treated with ceftriaxone alone, 1 with ceftriaxone plus vancomycin, and 1 with meropenem plus vancomycin), therapy switched to ampicillin. In two patients infected by *S. pneumoniae* (1 empirically treated with ceftriaxone plus ampicillin and vancomycin and 1 with ceftriaxone alone), therapy switched to penicillin in one case and to linezolid in the other case. In one patient with *C. koseri* infection (empirically treated with ampicillin plus gentamicin), therapy switched to meropenem.

Discussion

Despite being obliged—only at the end of 2018, we opted for the FilmArray® ME panel in clinical routine—the choice of using the eazyplex® CSF panel in 32 months until then has been fruitful. First, we show that our real-life experience with the eazyplex® CSF panel, which currently detects six (including *E. coli*) most common bacterial agents of meningitis, was successful. Excluding four cases due to species not included in the panel (three species in total), 40 (100%) of 40 diagnosable cases had a specific aetiology identified by the molecular assay. Nonetheless, all four cases that the assay apparently failed to identify, had a positive Gram stain microscopy result, and this allowed a rapid—eazyplex/microscopy—result to be released in 44 (100%) of 44 cases. In all four cases, a microorganism grew in culture (2 *S. pyogenes*, 1 *C. koseri*, and 1 *E. coli*)—the last species is now included in a current version of the eazyplex® CSF panel, by which part of the study's samples (33/135) were tested. Only 77.5% (31/40) of eazyplex® CSF panel positive cases had a culture positive result, whereas none of 91 cases with a negative result by any—eazyplex/microscopy/culture—methods gave a positive 16S rRNA PCR/sequencing result. Second, our experience gives us the cue for pointing out some diagnostic issues related to bacterial meningitis.

In a recent narrative review, Vetter et al. [19] suggested that clinical or laboratory CSF findings (i.e. purulent vs clear aspect, high neutrophil vs lymphocyte count, low vs normal-to-low glucose) should guide the first-line microbiological

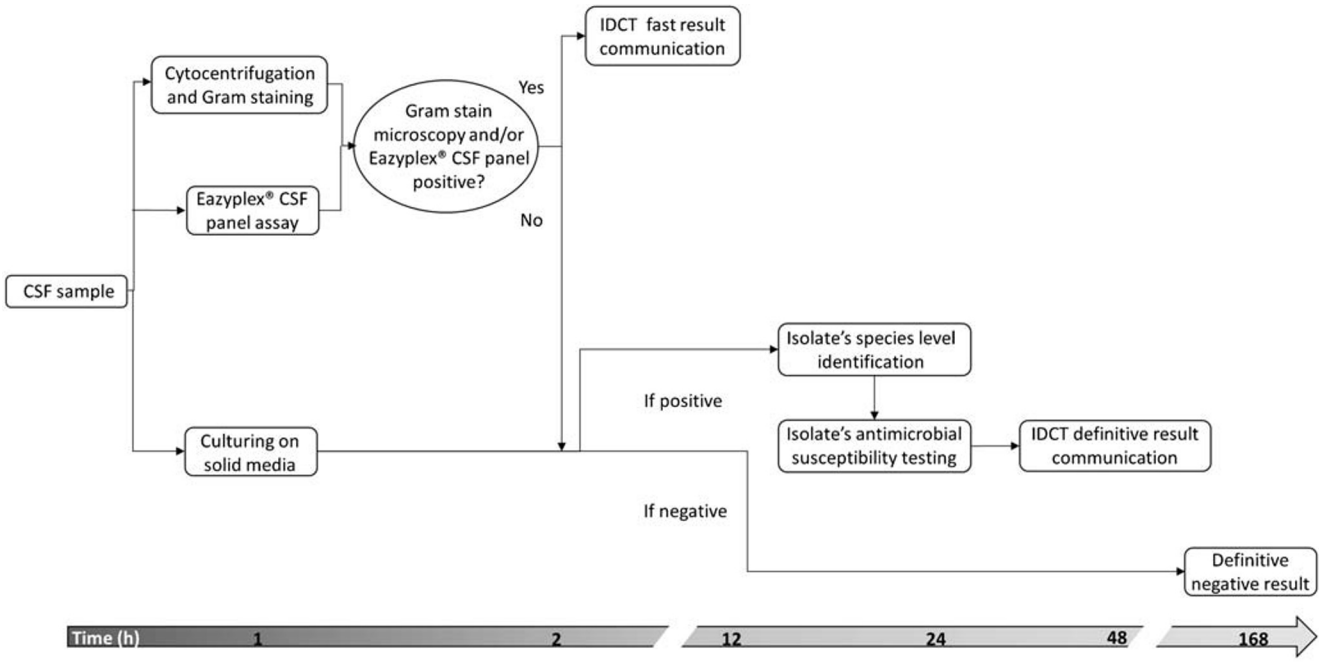

Fig. 1 Laboratory diagnostic workflow for bacterial meningitis. It consists in submitting cerebrospinal fluid (CSF) samples to both Gram stain microscopy and eazyplex® CSF panel assay and, in parallel, to culture. In one way, a patient's sample result was rapidly available and, in the other way, the result was subordinate to the potential growth of causative microorganism. Fast or definitive (including antimicrobial susceptibility testing) results were immediately actionable to the infectious disease consultancy team (IDCT)

testing. In our study, of 44 patients diagnosed with bacterial meningitis, 44 (100%) had CSF samples with > 5 WBC/mm^3 and predominant neutrophils, and 43 (97.7%) had CSF samples with low glucose. Conversely, of 91 patients undiagnosed with bacterial meningitis, 48 (52.7%), 33 (36.3%), and 17 (18.7%) had CSF samples with low glucose, > 5 WBC/mm^3 or predominant neutrophils, respectively. If we would have applied the strategy proposed by Vetter et al. [19], it would have led to test only 119 (88.1%) of 135 patients who presented with at least one altered CSF parameter (44/44 and 75/91 patients). However, such testing strategy for bacterial meningitis may be hazardous especially in the context of meningitis due to *L. monocytogenes*. Although a leucocyte count of ≥ 1000 cells/mm^3 with a neutrophilic predominance is strongly indicative of bacterial meningitis [17], a leucocyte count of < 1000 cells/mm^3, which may be lymphocytic (mimicking viral meningitis) [17], occurs in approximately 60% of listerial meningitis cases [25].

Economic reasons prompted Pfefferle et al. [26] to adopt a CSF sample selection strategy for rationalizing the use of FilmArray® ME panel assay—the cost of the test per patient is 120 € vs 50 € of the eazyplex® CSF panel. Contrarily to us—we performed eazyplex® CSF panel analysis on all 135 CSF samples for which a microbiological examination was necessary—the authors in that study limited the FilmArray® ME panel analysis to 171 of 4623 CSF samples (1601 individuals) that matched their risk criteria (i.e. had a high pre-test probability of infectious meningitis) during a 18-month period. In that study [26], 44/54 (81.5%) within the subset of samples selected upon request by physicians as well as 44/116 (37.9%) within the subset of samples selected for leucocytes seen at Gram stain microscopy examination had positive FilmArray® ME results. In our study, all 135 samples were from patients with a moderate/high suspicion of bacterial meningitis, thus resulting in a positivity rate of 32.6% (44/135 samples). Excluding 11 (8.1%) of 135 samples with a documented viral aetiology, a negative molecular assay, as restricted to few microbial targets as with the eazyplex® CSF panel, did not definitively rule out an infection. However, we excluded either tuberculous or cryptococcal meningitis, which are both well-known causes of non-viral aseptic meningitis in adults [1].

This study shows that *S. pneumoniae*, *N. meningitidis*, and *L. monocytogenes* were the first, second, and third most common causes of meningitis in our clinical setting, respectively. Ceftriaxone, alone or in combination with other antibiotics, was the most administered antimicrobial agent for empiric treatment (before or after performing lumbar puncture for CSF sampling) in our patients, and this was essentially in accordance with the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines [15, 18]. While some patients were receiving vancomycin—which is recommended when the likelihood of pneumococcal meningitis is high—unfortunately, only three of six patients with listerial meningitis were receiving an antimicrobial treatment covering *L. monocytogenes* (e.g. penicillin or ampicillin) [4]. Eight (18.2%) of 44 patients with bacterial meningitis

Table 3 Details about antibiotic treatments for 44 patients with microbiologically documented bacterial meningitis

Patient	Infecting species	Empiric treatment		Targeted treatment with[a]
		Before CSF collection with	After CSF collection with	
1	*S. pneumoniae*	Ceftriaxone	Ampicillin, ceftriaxone, vancomycin	Ceftriaxone
2†	*L. monocytogenes*	Ceftriaxone	Ampicillin, amikacin	Ampicillin
3	*L. monocytogenes*	Ceftriaxone	Ceftriaxone	Ampicillin
4	*S. pneumoniae*	None	Ceftriaxone	Ceftriaxone
5	*S. pneumoniae*	None	Ceftriaxone	Ceftriaxone
6	*S. pyogenes*	None	Ceftriaxone	Ceftriaxone
7	*S. pneumoniae*	None	Ampicillin, ceftriaxone, vancomycin	Ceftriaxone, vancomycin
8	*S. pneumoniae*	None	Ampicillin, ceftriaxone	Ceftriaxone
9†	*E. coli*	None	Ceftriaxone	Ceftriaxone
10†	*S. pneumoniae*	None	Ceftriaxone	Ceftriaxone
11	*N. meningitidis*	None	Ceftriaxone	Ceftriaxone
12	*S. pneumoniae*	Ceftriaxone	Ceftriaxone	Ceftriaxone
13	*N. meningitidis*	Ceftriaxone	Ampicillin, ceftriaxone	Ceftriaxone
14†	*S. agalactiae*	None	Ampicillin, amikacin	–
15†	*S. pneumoniae*	Ceftriaxone	Ceftriaxone	Ceftriaxone, vancomycin
16	*N. meningitidis*	Ceftriaxone	Ampicillin, ceftriaxone, vancomycin	Ceftriaxone
17	*C. koseri*	None	Ampicillin, gentamycin	Meropenem
18	*S. pneumoniae*	None	Ceftriaxone, vancomycin	Ceftriaxone
19	*S. pneumoniae*	None	Ampicillin, ceftriaxone, vancomycin	Penicillin
20	*S. pneumoniae*	Ciprofloxacin	Ampicillin, ceftriaxone, vancomycin	Ceftriaxone
21†	*E. coli*	None	Ceftriaxone	–
22	*N. meningitidis*	Ceftriaxone	Ceftriaxone	Ceftriaxone
23	*S. pneumoniae*	None	Ampicillin, ceftriaxone	Ceftriaxone
24	*S. pneumoniae*	None	Ceftriaxone	Linezolid
25	*S. pneumoniae*	Amoxicillin-clavulanic acid	Ampicillin, ceftriaxone, vancomycin	Ceftriaxone
26	*N. meningitidis*	None	Ceftriaxone	Ceftriaxone
27	*N. meningitidis*	Amoxicillin-clavulanic acid	Ceftriaxone	Ceftriaxone
28†	*S. pneumoniae*	None	Ceftriaxone	–
29†	*S. pyogenes*	None	Ceftriaxone	–
30	*S. pneumoniae*	Ceftriaxone	Ampicillin, ceftriaxone	Ceftriaxone
31	*N. meningitidis*	Ceftriaxone	Ceftriaxone	Ceftriaxone
32	*N. meningitidis*	Ceftriaxone	Ampicillin, ceftriaxone, vancomycin	Ceftriaxone
33	*L. monocytogenes*	None	Ampicillin, ceftriaxone, vancomycin	Ampicillin
34	*L. monocytogenes*	None	Ampicillin, ceftriaxone, rifampin	Ampicillin
35	*E. coli*	None	Ceftriaxone	Ceftriaxone
36	*S. pneumoniae*	Ceftriaxone	Ceftriaxone	Ceftriaxone
37	*L. monocytogenes*	None	Meropenem, vancomycin	Ampicillin
38	*N. meningitidis*	None	Ampicillin, ceftriaxone	Ceftriaxone
39	*L. monocytogenes*	None	Ceftriaxone, vancomycin	Ampicillin
40	*S. pneumoniae*	None	Ceftriaxone	Ceftriaxone
41	*S. pneumoniae*	None	Ceftriaxone	Ceftriaxone
42	*S. pneumoniae*	None	Ceftriaxone	Ceftriaxone
43	*S. pneumoniae*	None	Ceftriaxone	Ceftriaxone
44	*N. meningitidis*	None	Ceftriaxone	Ceftriaxone

[a] Targeted treatment was based on each patient's microbiology laboratory results (including antimicrobial susceptibility testing results, when available), as described in the text

†Death of the patient

–The patient could not receive any targeted treatment because he/she died

died, and four before further antibiotic therapy was directed at the causative pathogen. Notably, death occurred not only in one patient with *L. monocytogenes* infection—who did receive empiric treatment with ampicillin—but also in patients with *S. pneumoniae* (three patients), *E. coli* (two patients), *S. agalactiae* (one patient), or *S. pyogenes* (one patient) infection.

False-negative results may occur due to interfering antibiotics in patients under a treatment initiated before CSF collection [17]. This procedure applied to 16 our patients, which is

unfortunate given that only four of 16 patients had CSF samples positive in culture (3 for *L. monocytogenes* and 1 for *S. pneumoniae*). Without eazyplex® CSF panel analysis, 12 meningitis cases (6 due to *S. pneumoniae* and 6 due to *N. meningitidis*) would have been undiagnosed, also because the CSF samples in all cases were Gram stain negative. It is difficult to believe in misleading positive results because sensitivity rates equate to an approximate limit of detection of 10^4 CFU/ml for Gram stain and of 10^2 to 10^3 CFU/ml for culture, which are above the limit of detection of many NAATs [17]. However, we cannot exclude contamination at the time of collection or laboratory testing to cause false positivity in the 12 samples with an eazyplex® CSF panel positive result.

While infectious disease diagnostics are rapidly evolving [27] and new direct pathogen detection methods are upcoming in clinical routine [28], the diagnosis of meningitis remains a challenge, particularly using NAATs as "stand-alone" diagnostic assays [19]. Although it is beyond the study's scope, we report data of 106 CSF samples tested for suspected meningitis using the FilmArray® ME panel assay during the May 2018 to February 2020 laboratory activity (unpublished data). Because of partially overlapped testing period (i.e. May 2018 to December 2018), some samples underwent concomitant analysis with the eazyplex® CSF panel assay. Interestingly, 28 (26.4%) samples provided a positive FilmArray® ME panel result, 14 for bacterial aetiologies, 13 for viral aetiologies, and one for *C. neoformans*/*C. gattii* aetiology. For six positive samples tested with both assays, the FilmArray® ME panel result was concordant with that of the eazyplex® CSF panel—3 *L. monocytogenes*, 2 *N. meningitidis*, and 1 *S. pneumoniae*. One sample culture positive for *E. coli* was false negative with the FilmArray® ME panel (but positive with the eazyplex® CSF panel) because the isolate was not an O1K1-serotype strain, which is the only strain detectable with the FilmArray® ME panel assay.

In conclusion, our study proved that eazyplex® CSF panel assay performed better than culture for most common bacteria causing meningitis, especially in patients with prior antibiotic therapy. Notably, used in combination with Gram stain, the assay accelerated the bacterial meningitis diagnosis, thus allowing a timely initiation or continuation of appropriate therapy for patients. Meanwhile, negative eazyplex® CSF panel results allowed patients treated empirically or observed based on the level of clinical suspicion to withhold antibiotic therapy and/or direct to additional testing in case of high suspicion of infection (data not shown). Finally, the eazyplex® CSF panel assay is easy to perform and not necessitating much expertise, which often lacks in laboratories that routinely handle a relatively small number of clinical samples. However, more experience will be necessary before widely implementing the eazyplex® CSF panel assay in routine laboratory diagnostic workflows.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval and informed consent The Ethics Committee of our institution approved the study (approval number 34253/17), and informed consent was waived because of testing patients' CSF samples that were collected as part of routine clinical care.

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