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Routine Testing for Anaerobic Bacteria in Cerebrospinal Fluid Cultures Improves Recovery of Clinically Significant Pathogens

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In North America, the widespread use of vaccines targeting *Haemophilus influenzae* type b and *Streptococcus pneumoniae* have dramatically altered the epidemiology of bacterial meningitis, while the methodology for culturing cerebrospinal fluid (CSF) specimens has remained largely unchanged. The aims of this study were 2-fold: to document the current epidemiology of bacterial meningitis at a tertiary care medical center and to assess the clinical utility of routinely querying for anaerobes in CSF cultures. To that end, we assessed CSF cultures submitted over a 2-year period. A brucella blood agar (BBA) plate, incubated anaerobically for 5 days, was included in the culture procedure for all CSF specimens during the second year of evaluation. In the pre- and postimplementation years, 2,353 and 2,302 CSF specimens were cultured, with 49 and 99 patients having positive culture results, respectively. The clinical and laboratory data for patients with positive cultures were reviewed. Anaerobic bacteria were isolated in the CSF samples from 33 patients post-BBA compared to two patients pre-BBA ($P = 0.01$). The anaerobic isolates included *Bacteroides thetaiotaomicron* ($n = 1$), *Propionibacterium* species ($n = 15$), and *Propionibacterium acnes* ($n = 19$) isolates; all of these isolates were recovered on the BBA. Eight of the 35 patients from whom anaerobic organisms were isolated received antimicrobial therapy. Although six of these patients had central nervous system hardware, two patients did not have a history of a neurosurgical procedure and had community-acquired anaerobic bacterial meningitis. This study demonstrates that the simple addition of an anaerobically incubated BBA to the culture of CSF specimens enhances the recovery of clinically significant anaerobic pathogens.

The epidemiology of bacterial meningitis in developed countries has changed dramatically over the last 3 decades. As recently as the 1980s in the United States, three pathogens, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis*, accounted for >80% of community-acquired bacterial meningitis cases (1, 2). With the introduction of vaccines directed at *H. influenzae* type b, *S. pneumoniae*, and *N. meningitis*, a dramatic decline has occurred in the rate of central nervous system infection caused by these organisms.

The contemporaneous emergence of immunocompromised patient populations and increasing antimicrobial resistance in bacterial pathogens has further contributed to the evolution of this disease. Patients with chronic diseases and/or chronic immunosuppression are living longer and are susceptible to infections caused by pathogens typically thought to be of low virulence (3, 4). Additionally, patients who undergo neurosurgical procedures or have ventriculoperitoneal shunts in place are at high risk for subsequent infection. The rates of infection are reported to be as high as 11 to 18% per patient after initial shunt placement (5, 6).

The protocols used by clinical microbiology laboratories for the culture of cerebrospinal fluid (CSF) specimens have been relatively static for many decades. Although attempts have been made to develop sophisticated assays to detect bacterial pathogens, these tests have not been shown to improve upon bacterial culture (7–10). The mainstay of CSF culture remains the inoculation of specimens to blood and chocolate agar plates and incubation at 35°C in an environment with 5% CO_2 (11). As a result of the altered landscape of bacterial meningitis, now is the ideal time for a reevaluation of culture methods for CSF specimens.

The objectives of this study were to evaluate the current epidemiology of pathogenic organisms recovered from CSF cultures at a tertiary care academic medical center and to evaluate the impact of adding a single piece of medium, incubated anaerobically, on the recovery of anaerobic organisms that are clinically relevant in the contemporary era of bacterial meningitis.

MATERIALS AND METHODS

Laboratory methods. Cytospin Gram stains were prepared on all CSF specimens submitted to the laboratory. If >2 ml of CSF was received by the laboratory, the specimen was centrifuged and the sediment was inoculated onto solid agar. Prior to 3 January 2012, all CSF specimens submitted to the Barnes-Jewish Hospital microbiology laboratory for bacterial culture were inoculated onto sheep blood agar (SBA) and chocolate agar (Remel, Lenexa, KS) and incubated in 5% CO_2 for a minimum of 48 h to observe for growth. After 3 January 2012, a prereduced brucella blood agar (BBA) plate (Hardy Diagnostics, Santa Maria, CA), incubated anaerobically, was added to the standard CSF specimen protocol. The BBA agar is routinely held for 48 h prior to being examined, and the anaerobic medium is held for a total of 5 days to observe for growth. Any organism recovered in culture was identified according to the laboratory standard operating procedures, typically involving a combination of Gram staining, spot tests, and phenotypic profiling.

Data collection. The microbiology laboratory database was queried for all CSF cultures submitted within 1 year prior to and 1 year following the introduction of BBA. All positive CSF cultures were linked with patient data collected from the electronic medical record, including patient

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history, presenting symptoms, culture results, patient outcome, and discharge diagnoses as enumerated in the physician discharge summary. Patients with more than one CSF culture submitted per visit had all positive culture results reviewed. For patients whose initial positive culture result matched those of the subsequent cultures, the initial culture result was utilized in statistical analyses. For the three patients whose subsequent culture results differed from those of the initial cultures ($n = 6$), the culture considered to be most clinically significant (see Results) was used in the statistical analysis.

The clinical significance of the recovery of an organism in culture was inferred from the decision of the clinicians to initiate or continue treatment with antimicrobials once the culture results became available. If empirical antimicrobials had been given but were stopped at the time of culture results, these patients were not considered to be treated for meningitis. As a separate but related measure of clinical significance, an infectious diseases specialist, blinded to actual treatment course, performed an independent review of presenting symptoms, medical comorbidities, CSF profile, and CSF culture results and gave a retrospective treatment recommendation. Thus, the clinical significance of the isolates recovered in culture was determined both prospectively and retrospectively by medical professionals.

Statistical analysis. Student's *t* test or Mann-Whitney U test for independent samples were used to compare the means and distributions of patient populations for the appropriate parametric data or nonparametric data, respectively. Chi-square or Fisher's exact tests were used to describe categorical differences between the patient populations and culture results. A kappa statistic was calculated for the agreement between the prospective and retrospective evaluations of clinical significance of the isolates recovered in culture. Statistical analyses were performed using SPSS Statistics version 20 software (IBM Corp., Armonk, NY).

RESULTS

Patient population. In the 12 months prior to the BBA addition, 2,352 CSF specimens were submitted to the laboratory for bacterial culture, resulting in 69 positive cultures (2.9%) from 49 patients. In the 12 months following the addition of the BBA, 2,302 CSF specimens were submitted for bacterial culture, resulting in 113 positive cultures (4.9%) from 99 patients, a statistically significant difference in positive culture results ($P < 0.001$). A sample from one patient was present in both the pre- and the post-BBA data, but all other patient samples were unique. The demographic and clinical characteristics of the 148 patients with positive CSF cultures are presented in Table 1. During both of the time periods evaluated, the patient populations were similar with regard to their presenting symptoms and discharge diagnoses. Additionally, the number of patients who were immunosuppressed (45 [30.2%] overall) and who had central nervous system hardware (39 [26.2%] overall) did not vary between the groups. Of interest, the patients in the pre-BBA year were more likely to have recently (within 90 days) had a surgical procedure (42.9%; $P = 0.004$), although the difference between those with neurosurgical and nonneurosurgical procedures was not statistically different ($P = 0.4$).

TABLE 1 Demographics and clinical characteristics of patients with positive CSF cultures

Patient characteristic[a]	Data by BBA year: Pre-BBA ($n = 49$ [33%])	Post-BBA ($n = 99$ [67%])	P[b]
Mean age (range) (yr)	54 (21–94)	50 (19–94)	0.22
Sex			0.11
Men	24 (49)	62 (63)	
Women	25 (51)	37 (37)	
Race			0.29[c]
White	27 (55)	64 (65)	
Black	21 (43)	34 (34)	
Other	1 (2)	1 (1)	
Presenting symptoms			
Headache	25 (51)	49 (49)	
Altered mental status	24 (49)	52 (53)	
Nausea/vomiting	10 (20)	20 (20)	
Fever	12 (24)	15 (15)	
Discharge diagnoses			
Bacterial meningitis	12 (24)	8 (8)	
Cryptococcal meningitis	8 (16)	9 (9)	
VP shunt malfunction	3 (6)	12 (12)	
Seizure disorder	1 (2)	11 (11)	
Mean no. of days in hospital (range)	16 (0–85)	11 (0–86)	**0.016**
CNS hardware placed	17 (35)	22 (22)	0.10
Recent (within past 90 days)	14	18	0.70
Remote (≥90 days ago)	3	5	
Surgery (past 90 days)	21 (43)	20 (20)	**0.004**[d]
Neurosurgery	19 (90)	16 (80)	0.4
Other	2 (10)	4 (20)	
Immunocompromised	17 (35)	28 (28)	0.43
HIV/AIDS	8 (16)	9 (9)	
Solid-organ transplant	0	3 (3)	
Chronic steroids	7 (14)	8 (8)	
Chemotherapy	4 (8)	10 (10)	

[a] Unless otherwise indicated, patient characteristic data are presented as the number (%) of subjects.
[b] *P* values in bold type are those that are statistically significant at a *P* value of <0.05.
[c] "Other" race category excluded from analysis.
[d] The proportions of patients who underwent an operation in the pre-and post-BBA years are significantly different, while the types of procedures between groups are not.

Culture results. One hundred fifty-one isolates were recovered over the study period from 148 patients (Table 2). Of these, only eight (0.5%) of the pathogens recovered were what would be considered "classic" pathogens in bacterial meningitis. One culture grew *H. influenzae*, one culture grew *N. meningitidis* (group B), and six cultures grew *S. pneumoniae*.

The majority of patients evaluated in this study had only one positive CSF culture result. Eighteen patients had more than one positive CSF culture result ($n = 52$) during a single period of illness. For 15 of these patients, the subsequent culture result matched the original culture result (46 total cultures), and therefore only the initial culture result is presented in Table 2. In the remaining three patients (2 cultures each), the original CSF culture did not match the subsequent CSF culture result. One patient had *Aspergillus fumigatus*, followed by coagulase-negative staphylococci (CoNS), one patient had CoNS followed by *Propionibacterium acnes*, and one patient had CoNS followed by *Staphylococcus aureus*. These six culture results are reflected in Table 2. The patient represented in both pre- and post-BBA periods grew *Cryptococcus neoformans* at both time points.

Additionally, 83 patients had blood cultures drawn during their hospital visit, with 25 patients having a positive blood culture

TABLE 2 Isolates recovered from CSF specimen cultures

Organism	No. of isolates by BBA year		P^a
	Pre-BBA ($n = 49$)	Post-BBA ($n = 102$)	
Aerobic	47	69	
Anaerobic	2	33	**0.01**
Bacillus spp., not *B. anthracis*	0	3	
Bacteroides thetaiotaomicron	0	1	
Citrobacter koseri	0	1	
Coagulase-negative *Staphylococcus* spp. ($n = 50$)			
CoNS not otherwise specified	18	23	
Staphylococcus capitis	0	1	
Staphylococcus epidermidis	1	5	
Staphylococcus hominis	0	1	
Staphylococcus warneri	0	1	
Corynebacterium spp.	1	3	
Enterobacter aerogenes	2	0	
Enterococcus faecalis	1	1	
Enterococcus faecium	2	0	
Escherichia coli	1	0	
Gram-negative bacilli	3	0	
Haemophilus influenzae	0	1	
Klebsiella pneumoniae	0	1	
Micrococcus spp.	0	3	
Mixed skin microorganisms	0	1	
Neisseria meningitidis, group B	0	1	
Propionibacterium acnes	2	17	**<0.001**
Propionibacterium spp.	0	15	
Pseudomonas aeruginosa	2	1	
Staphylococcus aureus	2	5	
Streptococcus mitis group	0	2	
Streptococcus pneumoniae	5	1	
Viridans group *Streptococcus*	0	1	
Yeast/fungus ($n = 22$)			
Aspergillus fumigatus	0	1	
Candida albicans	0	2	
Candida parapsilosis	1	0	
Candida spp.	0	1	
Cryptococcus neoformans	7	9	
Unspecified yeast	1	0	

[a] *P* values in bold type are those that are statistically significant at a *P* value of <0.05.

result (30.1%). Sixteen of these positive results (64.0%) confirmed the same isolate in both blood and CSF specimens: CoNS ($n = 1$), *C. neoformans* ($n = 6$), *Enterobacter aerogenes* ($n = 2$), *Enterococcus faecium* ($n = 1$), *Klebsiella pneumoniae* ($n = 1$), *S. aureus* ($n = 2$), and *S. pneumoniae* ($n = 3$). Nine patients had distinct isolates as determined from the blood and CSF methods, and these results are listed in Table 3.

Anaerobic culture results. Thirty-five isolates of anaerobic bacteria were recovered during the 2-year study period. In the pre-BBA year, 2 CSF cultures (4% of positive CSF cultures) resulted in the isolation of anaerobic bacteria, both from *P. acnes*. In the post-BBA year, 33 CSF cultures (32% of the positive CSF cultures) were positive for the growth of anaerobic bacteria ($P = 0.01$). These post-BBA isolates included 15 *Propionibacterium* spp. isolates, 17 *P. acnes* isolates, and one *B. thetaiotaomicron* isolate. Of note, the two *P. acnes* cultures from the pre-BBA year were plated to and isolated on a BBA plate because of high clinical suspicion due to the presence of a ventriculoperitoneal (VP) shunt in the respective patients. All 35 anaerobic isolates grew on the anaerobically incubated BBA. Only one isolate, a *P. acnes*, also grew as pinpoint colonies on the aerobically incubated chocolate agar.

TABLE 3 Patients with discrepant blood and CSF culture results[a]

Patient blood isolate	Patient CSF isolate
Acinetobacter calcoaceticus-A. baumannii complex	Coagulase-negative staphylococcal species
C. parapsilosis	*S. epidermidis*
CoNS sp. not otherwise specified	*S. aureus*
E. aerogenes	Gram-negative bacillus (Gram stain only)
P. aeruginosa	*Candida* sp., not *C. albicans*
P. aeruginosa	*Propionibacterium acnes*
Streptococcus agalactiae	CoNS sp.
S. agalactiae	CoNS sp.
S. mitis group	CoNS sp.

[a] The results from each culture are shown alongside each other (e.g., an isolate defined as *Acinetobacter calcoaceticus-A. baumannii* complex in blood culture was identified as coagulase-negative staphylococci species by CSF culture).

Aerobic medium is first examined after overnight incubation, but the BBA plate is typically not examined until it has been incubated for 2 days. The growth of aerobic isolates therefore became apparent within a median of 2 days (interquartile range [IQR], 1 to 3 days), while anaerobic isolates were noted at 3 days (IQR, 2 to 4; $P < 0.001$). Anaerobic isolates were recovered in a similar number of patients with and without central nervous system (CNS) hardware (22.0 versus 23.6% of positive cultures, respectively; $P = 0.827$) and in immunocompetent and immunocompromised patients (27.2 versus 13.3% of positive cultures, respectively; $P = 0.089$). On the other hand, patients who had undergone a neurosurgical operation within 90 days of CSF culture were less likely to have growth of anaerobic bacteria than those who had not had surgery (6.5 versus 33.7%, respectively; $P = 0.002$).

Clinical significance and antimicrobial therapy. Overall, patients were administered antimicrobial therapy for 46.4% ($n = 70$) of the isolates recovered. Of the 22 patient samples from which yeast or fungal organisms were cultured, 21 patients received treatment; one patient expired due to other causes prior to the CSF results becoming available. The majority of samples with yeast or fungal isolates were from immunocompromised patients (72.7%; $P < 0.001$).

In a comparison of the clinical decision to treat the patient at the time of culture with the retrospective recommendation of an infectious diseases specialist during chart review, the agreement was excellent ($\kappa = 0.94$).

Factors involved in decision to treat. For the cultures with bacterial growth, 49 patients (38%) were considered to have bacterial meningitis and were treated with antimicrobials accordingly. Treatment was more likely to be recommended if the patient presented with a fever (60.0 versus 34.0%; $P = 0.028$), had a recent neurosurgical procedure (87.1 versus 22.1%; $P < 0.001$), or possessed CNS hardware (72.2 versus 24.7%; $P < 0.001$). Treatment was less likely, however, if the patient presented with seizure activity (21.1 versus 50.4%; $P = 0.025$). The median CSF nucleated

TABLE 4 Analysis of CSF specimens with bacterial growth

CSF analyte	Median (IQR) values by antimicrobial treatment status		P^b
	Treated[a]	Untreated	
Nucleated cells (cells/μl)	704 (25, 1,607)	0 (0, 6)	<0.001
Neutrophils (%)	78 (21, 88)	2 (0, 17)	<0.001
Lymphocytes (%)	8 (2, 18)	65 (33, 81)	<0.001
Protein (mg/dl)	111 (46, 376)	68 (29, 69)	<0.001
Glucose (mg/dl)	23 (20, 68)	68 (55, 77)	<0.001

[a] Treated, indicates that these patients were treated with appropriate antimicrobials after the culture results had been finalized.
[b] All *P* values are significant at <0.05.

cell count was significantly higher in the group that received antimicrobial therapy (704 cells/μl [IQR, 25 to 1,607 cells/μl]) than in the untreated group (0 cells/μl [IQR, 0 to 6 cells/μl]; $P < 0.001$), as was the CSF protein concentration (111 mg/dl [IQR, 46 to 376 mg/dl] versus 42 mg/dl [IQR, 29 to 69 mg/dl], respectively; $P < 0.001$) (Table 4).

For all 23 isolates (17.8%) for which an organism was observed on direct Gram stain of the specimen, antimicrobial treatment was initiated in the patients. Of the 69 culture-positive specimens (53.5%) with negative specimen Gram stain (i.e., no organisms, no polymorphonuclear cells [PMN] observed), only 7 patients were treated ($P < 0.001$). The remaining 37 positive specimens (28.7%) had PMN cells but no organisms seen on specimen Gram stain. These cases were almost evenly split, with 19 patients (51.4%) receiving antimicrobial therapy and 18 patients (48.6%) not receiving therapy. For the 49 patients from whom an organism was isolated in the CSF culture, therapy did not differ between immunocompromised and immunocompetent patients (41.4 versus 37.1%, respectively; $P = 0.678$).

Treatment of anaerobic bacteria. Eight patients (22.9%) with CSF cultures growing anaerobic isolates received antimicrobial therapy. Table 5 highlights the differences between the patients with anaerobic isolates who received antimicrobials and those who did not receive therapy. Patients were more likely to receive treatment if they had CNS hardware (75.0 versus 11.1%; $P = 0.001$) or if they had a positive specimen Gram stain with either an organism or PMN cells (87.5 versus 22.2%; $P = 0.002$). An increased proportion of neutrophils in the CSF cell count (median, 53 versus 0% neutrophils; $P < 0.001$) was also associated with the decision to treat the patient. Although clinically significant isolates seem to grow slightly faster than isolates for which patients did not receive antimicrobial therapy (median, 2.5 versus 3 days), the difference was not statistically significant ($P = 0.08$). Other parameters, such as immunocompromised state, recent surgical procedure, or CSF chemistries did not play a consistent role in the decision to treat.

Of note, two patients who received treatment did not have CNS hardware. One patient was a man who presented with altered mental status and was found to have a pilonidal cyst. Brain magnetic resonance imaging (MRI) showed leptomeningeal enhancement, and the patient's CSF sample had an elevated protein (60 mg/dl) and nucleated cell count (111 cells/μl), with a neutrophilic predominance. The specimen Gram stain demonstrated polymorphonuclear cells and Gram-negative bacilli, and the culture grew *B. thetaiotaomicron*; growth was observed on BBA in just 2 days, and metronidazole was initiated in the patient as soon as an anaerobic pathogen was suspected. After this change in his antimicrobial therapy, his infection cleared.

TABLE 5 Evaluation of patients with isolation of anaerobic isolates who did or did not receive antimicrobial therapy

Clinical characteristic	Data by antimicrobial treatment status		P^a
	Treated ($n = 8$)	Untreated ($n = 27$)	
Fever (*n* [%])	1 (12.5)	1 (3.7)	0.41
CNS hardware (*n* [%])	6 (75.0)	3 (11.1)	**0.001**
Immunocompromised (*n* [%])	1 (12.5)	5 (18.5)	>0.9
Neurosurgery within 90 days (*n* [%])	2 (25.0)	1 (3.7)	0.124
No. with specimen Gram stain			**0.002**[b]
Organism detected	5	0	
PMN (no organism)	2	6	
Negative (no organism, no PMN)	1	21	
No. of CSF nucleated cells/μl (median [IQR])	58 (0, 364)	0 (0, 5)	0.088
% neutrophils (median [IQR])	53 (22, 91)	0 (0, 5)	**<0.001**
CSF protein (median [IQR]) (mg/dl)	70 (25, 176)	36 (25, 56)	0.323
CSF Glucose (median [IQR]) (mg/dl)	20 (11, 76)	65 (60, 73)	0.096
Single isolate growth	7 (87.5)	25 (92.6)	0.553
Days of incubation for detection (median [IQR])	2.5 (0.8–4.3)	3.0 (1–5)	0.08

[a] *P* values in bold type are those that are statistically significant at a *P* value of <0.05.
[b] *P* value calculated for negative Gram stain versus organism/PMN on Gram stain.

The second patient was a man with a history of melanoma who presented with altered mental status, nausea, vomiting, and headache. A head computed tomography (CT) scan showed only dilated ventricles, and his CSF had elevated protein (518 mg/dl) and nucleated cells (145 cells/μl). A specimen Gram stain showed the presence of polymorphonuclear cells, and 3 consecutive CSF specimen cultures received over a 7-day period grew *P. acnes*. Despite appropriate antimicrobial therapy, the patient was also found to have metastatic cancer, and he eventually died as a result of this disease (11).

DISCUSSION

The contemporary patterns of bacterial meningitis in the United States are markedly different from those 30 years ago. As a result of this epidemiologic change, clinical microbiology laboratories must be prepared to isolate bacterial pathogens that may not historically be considered common causes of bacterial meningitis. In this study, we demonstrate that bacterial pathogens classically associated with meningitis now comprise a minority of pathogens isolated at a tertiary care medical center. Additionally, we demonstrate that anaerobic organisms may be clinically significant pathogens in bacterial meningitis. By adding a single piece of medium, incubated anaerobically, to our standard laboratory procedure for CSF cultures, we were able to capture these clinically relevant organisms.

Although anaerobes are often considered contaminants because they are normal flora on human skin and mucosal surfaces, numerous case reports detail the existence and relevance of anaerobic organisms in bacterial meningitis (12–21). These reports highlight some of the predisposing factors for anaerobic menin-

gitis, such as infection (otitis media or upper respiratory infections), trauma, a recent surgical procedure, or the presence of CNS hardware, in particular a ventriculoperitoneal shunt (14). These reports also emphasize the fact that in a symptomatic patient with a CSF pure culture of the organism, it is unlikely that the anaerobic organism is a mere contaminant (13). Still, because anaerobes require specific growth conditions not routinely provided for CSF specimens, the true incidence of anaerobic bacterial meningitis remains unknown.

In our study population, approximately one-third of all organisms isolated from CSF specimens after the addition of the brucella blood agar were anaerobic bacteria. A subset of these anaerobes were considered clinically significant, and the patients were treated with antimicrobial therapy plus shunt revision where appropriate. In patients with a CNS shunt, the presence of anaerobic bacteria in the CSF has been associated with low-grade infection that resolves upon exchange of the shunt (18, 22). Additionally, early recognition of shunt infection and subsequent replacement can decrease expensive repeat hospitalizations, infectious workup, and unnecessarily broad antimicrobial treatment.

Anaerobic meningitis is not limited to patients with CNS hardware, and two of our study patients with CSF anaerobic isolates did not have a shunt or a history of a recent neurosurgical procedure. Although these represent only two cases of community-acquired anaerobic meningitis from a total of 2,302 cultures, the importance of recovering anaerobes from CSF specimens cannot be overstated. Prompt recovery of a pathogen, if present, can expedite appropriate treatment and minimize additional medical interventions and/or diagnostic studies. Anaerobic bacteria remain highly susceptible to antimicrobials, meaning that discovery of an anaerobic pathogen can allow for streamlining of antimicrobial therapy (14).

The purchase of BBA and an increase in labor time are laboratory costs that were considered with the institution of this protocol. The addition of a BBA plate can be quite easily incorporated into the existing laboratory procedure for the culture of CSF specimens. The cost of the additional medium is approximately $4.00 per specimen, making this an inexpensive intervention. Due to the relatively low growth rate of anaerobic bacteria, an extended incubation time for the BBA is necessary; in this study, 80% of all anaerobes recovered had been isolated within the first 4 days. No plates were incubated for >6 days, and thus we lack data to promote or refute prolonged incubation of CSF cultures. Although increased incubation times may marginally increase laboratory costs, this cost may be outweighed by cost savings at a hospital level due to timely pathogen identification and appropriate antimicrobial therapy, not to mention the benefit to the patient. At a minimum, we recommend that patients with CNS hardware have an anaerobic component to their CSF culture, although the community-acquired anaerobic meningitis cases highlight the importance of taking an unbiased approach to the addition of the BBA medium.

The anaerobic isolates in our population were found to be *Propionibacterium* spp. or *P. acnes*, with the exception of a single *B. thetaiotaomicron* isolate. It is well known that *Propionibacterium* is a component of the normal skin flora and that *P. acnes* is especially prevalent on areas of skin with a high density of sebaceous glands, such as the scalp (23). For this reason, teasing out true infection versus a contaminated culture can be a difficult task for clinicians. As this study shows, the decision to treat a patient with antimicrobials is complex and based only in part on the result of a CSF culture. Clinicians take into account patient history and presenting symptoms, with special emphasis placed on the results of CSF studies, including direct specimen Gram stain, protein and glucose concentrations, nucleated cell count, and the relative percentage of neutrophils identified (12, 13).

This study has several strengths. By evaluating 2 years worth of laboratory and clinical data, we were able to present a detailed view of the isolates attributed to bacterial meningitis in the post-vaccination era at an academic medical center. The clinical data collected on each patient were extensive, and the inclusion of an infectious diseases specialist on our study team allowed for the most accurate evaluation possible of the clinical significance of each isolate recovered from the CSF specimens. That being said, this study is not without limitations. As with any retrospective study, the data collected may be incomplete, and it is difficult to evaluate the clinical significance of any one laboratory isolate with a chart review. Because separate physician orders for aerobic and anaerobic bacterial CSF cultures do not exist within our system, we cannot comment on clinical suspicion of anaerobic meningitis prior to the routine addition of BBA to CSF cultures. This study was conducted at a large tertiary care academic medical center and thus may not be reflective of other care settings. Additionally, our laboratory serves an adult population only. Children, and neonates especially, have a distinct set of risk factors that alter the microbes implicated in pediatric bacterial meningitis, and our data cannot be extrapolated to the pediatric population. Finally, although we had large numbers of CSF specimens submitted for culture, the number of positive results over the 2-year study period remained relatively small.

The results of this study highlight the change in the contemporary epidemiology of bacterial meningitis in the United States. Our results demonstrate that the simple inexpensive addition of an anaerobically incubated BBA plate to the CSF culture protocol aids in the recovery of clinically significant anaerobic isolates from CSF specimens. The patients with meningitis from anaerobic bacteria are not limited to patients with CNS hardware or to immunosuppressed patients. In 1998, Sabeen Askari and Charles Cartwright noted (24):

> The clinical consequences of bacterial infection of the central nervous system guarantee that the diagnosis of such infections will remain a high priority for clinicians and laboratorians alike. In the face of a disease whose epidemiology continues to change, it seems likely that periodic reassessments of how best to make a diagnosis of bacterial meningitis will be necessary.

This observation still holds true today.

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