

Challenge M244-4

February 2025

CSF: *Streptococcus pneumoniae*

HISTORY

A simulated brain abscess sample collected from a 1 year old with decreased level of consciousness was sent to category A laboratories. Participants were expected to isolate and report *Streptococcus pneumoniae* and perform susceptibility testing.

CMPT QA/QC/STATISTICS

All simulated CSF samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Streptococcus pneumoniae*.

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The challenge sample lot was confirmed to be homogeneous and stable for at least 14 days after shipment.

Organism identification and susceptibility profile was confirmed by a reference laboratory.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories

Identification: 12/12 (100%) labs reported *Streptococcus pneumoniae*; one lab does not process this type of sample.

Susceptibility: 11/12 (92%) labs reported penicillin susceptible (S), 1 lab reported penicillin resistant (R); 12/12 (100%) labs reported ceftriaxone or cefotaxime susceptible (S); 6/12 labs reported Vancomycin susceptible (S), 4 labs did not report, 2 labs indicated they would refer.

MAIN EDUCATIONAL POINTS from M244-4

1. Identification of infecting agents in CSF samples is one of the most important functions of the diagnostic microbiology laboratory.
2. *Streptococcus pneumoniae* is a major cause of meningitis, leading to high morbidity and mortality in pediatric and adult patients.
3. β -lactams are preferred agents to treat serious bacterial infections because they are bactericidal and well tolerated.

Participants

Identification: 46/46 (100%) reporting laboratories identified *Streptococcus pneumoniae*; 1 lab did not report; 4 labs reported sample not normally processed, refer. (Table 1)

Susceptibility: 39/40 (97%) testing laboratories reported the organism susceptible (S) to penicillin, 1 lab reported the organism resistant (R) to penicillin, 1 lab had no ID to report, 10 labs reported sample not normally processed; 40/40 (100%) testing laboratories reported the organism susceptible (S) to ceftriaxone or cefotaxime, 1 lab had no ID to report, 10 labs reported sample not normally processed; 28/28 (100%) testing laboratories reported the organism susceptible (S) to vancomycin, 9 labs did not report vancomycin, 1 lab had no ID to report, 13 labs reported sample not normally processed (Table 2A-C).

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

Organism identification and susceptibility to penicillin and ceftriaxone were correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and were therefore, determined to be suitable for grading. Susceptibility to vancomycin did not reach consensus and for this reason could not be graded.

Grading

Maximum grade: 12

Reporting *Streptococcus pneumoniae* was graded 4.

Reporting the isolate susceptible to penicillin and ceftriaxone was graded 4 for each agent.

Table 1. Identification results

Reported	Total	Grade
<i>Streptococcus pneumoniae</i>	46	4
no report	1	0
sample not normally processed, refer	4	ungraded
Total	51	

COMMENTS ON RESULTS

Identification:

The results were excellent with all laboratories that processed the sample being able to isolate and identify *Streptococcus pneumoniae*.

Susceptibility:

Laboratories at a minimum should report both penicillin and cefotaxime or ceftriaxone. The inclusion of vancomycin was not graded; however, this might be useful clinically had penicillin been reported as resistant.

ISOLATION AND IDENTIFICATION

Streptococcus pneumoniae is a bacterium with a classically described Gram stain of lancet-shaped pairs (diplococci) of Gram-positive cocci, although it may be found singly or in short chains as well. Encapsulated strains may exhibit a clear halo around the organism in Gram stain.

Colonies grow on nutritive media, including blood and chocolate agar, when incubated in 5-10% CO₂ at 35-37°C, normally within 24 hours. Colonies are alpha hemolytic, often with an umbilicated (depressed) center and may appear mucoid.

Isolates of *Streptococcus pneumoniae* are catalase negative, bile soluble (which may be tested by plate and tube methods, and susceptibility to optochin (zone of ≥14mm). These biochemical tests are more sensitive and specific than automated biochemical platforms, including the API 20 Strep and Vitek GPI card, which can struggle with identification of streptococcal species.^{15,16} Specific *S. pneumoniae* antigen tests, such as the Pneumostix latex agglutination test, continue to be available for *S. pneumoniae* identification. MALDI-TOF platforms, while originally documented to struggle with differentiating *S. pneumoniae* and other *S. mitis* group organisms, have now improved to the point where they can reliably identify *S. pneumoniae*.^{8,17}

Laboratory diagnosis

Diagnosis of any infection involving the CNS, including meningitis, is considered to be a medical emergency. A minimum of 0.5 mL of CSF should be sent to the microbiology laboratory in a sterile container for bacterial testing. Whenever clinically feasible, specimens for culture should be obtained prior to initiation of antimicrobial therapy.¹

Table 2. Susceptibility results

2A Penicillin	Total	Grade
S	39	4
R	1	1
n/a, (no report)	1	ungraded
sample not normally processed, refer	10	ungraded
Total	51	
2B Ceftriaxone	Total	Grade
Ceftriaxone S	33	4
Cefotaxime S	7	4
n/a, no ID report	1	ungraded
sample not normally processed, refer	10	ungraded
Total	51	
2C Vancomycin	Total	Grade
S	28	ungraded
no report	9	ungraded
n/a, no ID report	1	ungraded
sample not normally processed, refer	13	ungraded
Total	51	

The cell count and biochemical parameters of CSF may be helpful in determining a bacterial etiology: in particular, the CSF WBC count is normally elevated with a neutrophilic predominance, the CSF glucose is decreased, and the CSF protein is increased.² The Gram stain may facilitate a rapid presumptive diagnosis in as many as 60-90% of patients with bacterial meningitis, especially when sufficient volume is submitted to permit examination of a concentrated (Cytospin) slide.

CSF culture remains the most common diagnostic modality of bacterial meningitis, and performs well as long as the sample is collected prior to antibiotics, with a sensitivity of 80-95%, depending on the pathogen. This yield decreases with antibiotic pre-treatment, to 62-70%.¹⁰

PCR in CSF for the diagnosis of bacterial meningitis is thus particularly useful in the context of antibiotic administration prior to CSF collection. In general, the sensitivity of PCR assays when performed directly on CSF specimens is higher than that of culture; however, PCR is hardly a monolithic entity as there are a huge variety of primers, assays, and differences in test implementation that make comparison between PCR studies difficult. Their accessibility in different laboratories and regions of the culture vary, but this is certainly a diagnostic option particularly in situations where culture is negative.

ANTIMICROBIAL SUSCEPTIBILITY

In the CLSI M100-Ed35:2025 antimicrobial susceptibility testing guidelines, there are no disc diffusion breakpoints for any of the commonly used beta-lactam antibiotics, including penicillin, ceftriaxone, cefotaxime, and meropenem. Thus, testing for any of these critical antimicrobials must be performed using a minimal inhibitory concentration (MIC) method. In particular, for *S. pneumoniae* isolates from CSF, it recommends that penicillin, cefotaxime/ceftriaxone, and/or meropenem should be tested by a reliable minimal inhibitory concentration method.¹² While the oxacillin disc screen for penicillin susceptibility can be used for most isolates of *S. pneumoniae*, it is not recommended for meningitis isolates.

Laboratories may choose to consider using broth microdilution or referring certain isolates for reference testing when testing close to or on a breakpoint. Additionally, although ceftriaxone and cefotaxime are often considered synonymous, they may show differences in susceptibility with *S. pneumoniae*. In penicillin-resistant isolates, MICs to cefotaxime may be higher than those of ceftriaxone, and it may be important to test the drug being used for treatment.¹⁴

Finally, laboratories are reminded that for penicillin and ceftriaxone/cefotaxime, among others, there are separate breakpoints for meningitis and non-meningitis disease, corresponding with the pharmacologic levels considered achievable in the CSF, which are in general lower than serum levels. While both meningitis and non-meningitis interpretations should be reported in the case of positive blood cultures, where the clinical situation is often unclear to the laboratory, only the meningitis interpretations should be reported in the case of a CSF isolate. Microbiologically, a positive CSF bacterial culture is by definition meningitis, and reporting a non-meningitis result can be at best confusing and at worst harmful, if the clinician is encouraged to use an inappropriate antimicrobial.

CLINICAL RELEVANCE

Streptococcus pneumoniae is a common commensal of the human oropharynx, but is likewise frequently identified as an etiologic agent in patients with respiratory infections including community-acquired pneumonia, sinusitis, meningitis, and otitis media. In the very young, the elderly, and in patients with serious underlying illnesses or immunocompromised, *S. pneumoniae* remains an important cause of invasive infections, including meningitis.¹⁴

Signs and symptoms of *S. pneumoniae* meningitis are largely the same as all bacterial meningitides: fever and altered mental status are cardinal symptoms, and often accompanied by neck stiffness, meningismus, headache, and nausea/vomiting. These are less frequent in younger infants, where the presentation may be more subtle and atypical.¹⁰

The incidence of bacterial meningitis in infants and children has decreased since the routine use of conjugated vaccines target-

ing *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis*. However, pneumococcal meningitis continues to be associated with high rates of mortality and long-term neurological sequelae, including deafness and other cranial nerve palsies.

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