Laboratory detection of *Neisseria meningitidis*- a case study

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**Abstract**

The diagnosis of bacterial meningitis remains a challenge to the clinician because of its rapid and lethal course lacking the consistency to particular clinical signs and symptoms. Currently, the diagnosis of bacterial meningitis is mostly done by Gram stain and culture. However, in many clinical settings, the use of rampant and short course antibiotic therapy prior to lumbar puncture reduces the chance of isolation of bacteria in cerebrospinal fluid culture making the diagnosis difficult. The present report examines a case in which conventional Gram stain and culture methods failed to identify the causative agent of bacterial meningitis that was subsequently identified by a Polymerase Chain Reaction (PCR) method. The importance of molecular methods for the rapid diagnosis of bacterial meningitis is also discussed. In a clinical setting, Gram stain and bacterial culture still remains the cornerstones of bacterial meningitis diagnosis.

**Key words:** *Neisseria meningitidis*, Polymerase Chain Reaction, CSF, bacterial meningitis, culture, tissue and organ procurement


**Introduction**

Invasive bacterial meningitis is a serious disease which can rapidly progress from a mild flu-like illness to death (1). In 2011, a total of 119 cases of meningococcal disease were notified in New Zealand, which equates to a rate of 2.7 per 100 000 population (1). Invasive meningococcal cases with fatality rates of 10 % to 15% have been previously reported and about 20% of the survivors suffer from a number of significant sequelae (2,3). Delays in diagnosis and treatment of meningococcal disease may contribute to its high morbidity and mortality (4). For this reason, administration of preadmission antibiotics to patients with suspected invasive meningococcal disease has been supported by some (5,6). However, after antimicrobial treatment is started, the rate of isolation of bacteria is strikingly reduced (7). In fact, prior antibiotic therapy 12 h or more before lumbar puncture can sterilize the cerebrospinal fluid (CSF) (8).

Once there is a suspicion of acute bacterial meningitis, blood samples must be taken for culture in addition to an immediate lumbar puncture to determine whether the CSF finding is consistent with the clinical diagnosis. The diagnosis is subsequently confirmed by microscopic detection and/or culture of the organism from the CSF (9). Diagnosis of bacterial meningitis based on direct microscopy is quick but lacking in sensitivity while the culture of CSF and blood takes at least 24 h and very often yields a negative result due to prior treatment with antibiotics (10).

In recent years, Polymerase Chain Reaction (PCR) based techniques have increasingly been used to amplify and detect microbial and viral deoxyribonucleic acid (DNA) in clinical samples. The use of PCR for rapid diagnosis of bacterial meningitis has the potential to overcome the poor sensitivity of culture when antibiotics have already been administered (10). The use of broad-range bacterial primers in the diagnosis of bacterial meningitis has been reported in earlier studies (9-11). At present, there is no definitive test that can confirm or exclude bacterial meningitis in patients with CSF findings that are consistent with a diagnosis of bacterial meningitis, but in whom the CSF Gram stain and culture results are negative. However, a combination of test results may permit an accurate prediction of the likelihood of bacterial versus viral meningitis.

The following case study reports the shortcomings of the Gram stain and culture methods in a bacterial meningitis case caused by *Neisseria meningitidis* that was subsequently identified using a PCR method. The advantages of applying PCR-based methods for the detection of bacterial meningitis, especially in post antibiotic samples, are also discussed.

**Case report**

An 18 years old female with no previous significant medical history was admitted to the intensive care unit (ICU) with a three day history of headaches, confusion, agitation, and vomiting. The illness started with a sore throat three days prior to hospital admission. The patient presented to the GP with aches and pains in her arms and legs a day prior to admission, and was given painkillers assuming a viral illness diagnosis. No skin rash was present.

Upon examination at the emergency department, the patient had cerebral irritation with a fluctuating Glasgow Coma Scale (GCS). Impressions of viral meningitis, encephalitis, or other intracranial pathology were suspected. Empirical treatment with IV ceftriaxone (2g) was administered and the patient was intubated to facilitate a computerised tomography head examination. Blood was collected and sent to the laboratory for analysis and culture before admission as an inpatient to ICU.

Blood tests showed a raised white cell count (16.6 x10^9/L), a high neutrophil count (14.8x10^9/L), and elevated inflammatory marker level (CRP: 316mg/L). Additionally, the neutrophils displayed a left shift with toxic vacuolation. Two hours post initial CT head scan, the patient was noted to have fixed, dilated pupils. An urgent follow up CT scan showed significant diffuse cerebral oedema. A lumbar puncture (LP) had not been done at this point and was deferred given the signs of intracranial hypertension.

A LP was eventually performed later in the day (more than 8 h post antibiotic administration) at the request of the Transplant Coordinator to clarify the diagnosis (in particular, to rule out a viral illness). The LP recovered 4 mL of turbid CSF. Analysis performed on the fourth tube showed a white cell and red cell level (CRP: 316mg/L). Additionally, the neutrophils displayed a left shift with toxic vacuolation. Two hours post initial CT head scan, the patient was noted to have fixed, dilated pupils. An urgent follow up CT scan showed significant diffuse cerebral oedema. A lumbar puncture (LP) had not been done at this point and was deferred given the signs of intracranial hypertension.

Intravenous ceftriaxone (2g) and vancomycin (1g) were administered together with the treatment of the cerebral oedema.
with no subsequent neurologic improvement. The patient was confirmed brain dead after one day of admission into ICU. A family meeting of the patient agreed on organ donation with organs harvested the following day.

Consequently, the CSF sample was sent to LabPlus (Auckland) for PCR testing of: Herpes Simplex Virus, Varicella Zoster Virus, Enterovirus, Parechovirus, N. meningitidis, and Streptococcus pneumoniae. Using an in-house molecular diagnostics CSF PCR panel, the sample tested positive for N. meningitidis. Follow up results from ESR (Porirua) confirmed it as a group C N. meningitidis (siaD PCR test), subtype (PorA type) P1.5-1,10-8 (PorA type determined by DNA sequence analysis of the porA gene).

Discussion
The present case highlights the value of applying molecular-based methods in confirming the diagnosis of meningococcal disease, especially in post-antibiotic CSF samples. As stated in the Communicable Disease Control Manual (1), a positive nucleic acid test using PCR on CSF samples is an acceptable confirmatory test for the diagnosis of bacterial meningitis. In 2011, 28% (30/108) of meningococcal disease cases reported to the Ministry of Health were laboratory confirmed by the detection of meningococcal DNA by PCR (12). Therefore, culture-negative, PCR-positive cases deserve the same recognition as full culture positive cases.

From a clinical standpoint, this case displayed the classical signs and symptoms of a bacterial meningococcal disease with the exception of the negative Gram stain and culture results. Possible explanations for the observed differences in the detection rate between the Gram stain/culture method and the PCR assay could be: i) bacteria being below the limit of detection (bacterial load) of Gram stain and culture and/or ii) the ability of the PCR method to detect dead organisms. Both reasons are attributable to the effect of the antibiotic treatment, which inhibits the growth of N. meningitidis but does not interfere with PCR amplification of the organism’s DNA.

Gram stain examination of CSF permits a rapid, inexpensive, and accurate identification of the causative bacterium in 60-90% of patients with community acquired bacterial meningitis, and has a specificity of ≥97% (13). However, the likelihood of visualising the bacterium on the Gram stain relates to the concentration of bacteria in the CSF - concentrations of ≥105 colony-forming units (CFU)/mL associated with a positive Gram stain result 25% of the time; 103 to 105 CFU/mL yields a positive Gram stain result in 60% of patients, and CSF concentrations of 1,105 CFU/mL lead to positive microscopy results in 79% of cases (14). However, the yield of CSF Gram stain maybe 20% lower in patients who have received prior antimicrobial therapy (15). In addition, the likelihood of having a positive Gram stain result also depends on the specific bacterial pathogen causing meningitis (16): 90% of cases caused by S. pneumoniae, 86% of cases caused by Haemophilus influenzae, 75% of cases caused by N. meningitidis, and 50% of cases caused by other gram-negative bacilli.

PCR based assays, on the other hand, have consistently shown high sensitivities and specificities (>90%) in comparison to Gram stain and culture (4, 17-19). PCR methods have been utilised to amplify bacterial DNA from patients with meningitis caused by the common meningococcal pathogens (N. meningitidis, S. pneumoniae, H. influenzae type b, Streptococcus agalactiae, and Listeria monocytogenes) (13). Investigators in the United Kingdom have dramatically increased the sensitivity of diagnosis with the routine use of a PCR based assay (5). They have been able to confirm 56% more cases of invasive meningococcal disease with PCR than with culture. Moreover, Richardson and colleagues (4) compared the results of Gram staining and culture of CSF to results obtained with a rapid PCR based assay for the diagnosis of meningococcal meningitis in 281 cases of suspected bacterial meningitis. They reported sensitivity and specificity values of 97% and 99.6% respectively for the PCR based assay compared to a sensitivity of 55% for culture.

The increase in sensitivity of PCR-based assays can be linked to ability of these techniques in the detection of dead bacteria (20). PCR methods are able to detect the presence of organisms as long as the target DNA sequence is not modified or destroyed by the antibiotic treatment. PCR methods are able to detect the presence of organisms even when the cell is viable, inactive or dead (21). Consequently, PCR techniques are evidently more sensitive than culture; nonetheless, they lack the ability to distinguish active cells from dead cells unless supplementary methods, such as viability assays, are used (22). Hence, given the use of IV ceftriaxone treatment in the present case, it should be no surprise that the Gram stain and subsequent culture were both negative. It is important to note that even if organisms were seen in the Gram stain, the culture may not yield bacterial growth due to the inhibitory effect of the antimicrobial agent.

A recommendation for improved laboratory diagnosis of bacterial meningitis is high speed centrifugation (1000 x g for 10 minutes). While the probability of visualising bacteria on a Gram stain can be increased up to 100-fold by using cytospin techniques (23), high speed centrifugation of the CSF, if a volume of more than 1mL is available, and using the pellet for microscopic examination and culture increases the bacterial load for both Gram stain, culture, and PCR methods (4,24).

In regards to organ donation, while a donor with bacterial meningitis is often considered to be controversial for organ retrieval; many high quality published studies have established that organ transplantation using donors with bacterial meningitis is an acceptable strategy as long as proper antimicrobial treatment of the donor and the recipient is done (25,26). Authors of a single center 20 year follow up study concluded that given the shortage of organs, the use of grafts from donors with bacterial meningitis (N. meningitides, S. pneumoniae, and H. influenzae) for heart-lung transplantation seems appropriate if sufficient antibiotic therapy and careful clinical management is instituted for both the donors and the recipient (25).

It is important that clinicians applying PCR-based assays be fully aware of the practical aspects involving the diagnosis of central nervous system infections. If bacterial meningitis is suspected in a patient, antimicrobial therapy should be commenced rapidly. Therefore, decisions regarding the initial treatment in these patients must always be made before obtaining the results of the PCR assay. For the time being, Gram staining and bacterial culture remain the cornerstones of bacterial meningitides diagnosis in a clinical setting.

Conclusion
This case demonstrates that routine microbiological Gram stain and culture methods can occasionally be substandard in the detection of N. meningitides and potentially other causative agents of bacterial meningitis due to low bacterial load and/or non-viable organisms as a result of antimicrobial treatment prior to CSF collection. For these reasons, it is wise to introduce PCR-based assays as adjuncts to conventional bacterial culture methods in establishing the bacterial aetiology in meningitis. For the time being, Gram stain and culture remain the cornerstones of laboratory detection of the organism causing bacterial meningitis. Additionally, the inclusion of a high speed centrifugation step before performing the Gram stain and culture maybe of diagnostic value. In this particular clinical case, no difference in clinical outcome would have resulted if the isolate was seen in the Gram stain and/or isolated in culture; however, the accurate and timely detection of the causative agent is paramount in any clinical case.

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