College of Science
Botany and Microbiology

# MIC (521)

## Summary of the practical article

# Diagnosis of pathogens causing bacterial meningitis using Nanopore sequencing in a resource-limited setting

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- **Introduction: Maha Alabdulkareem**

**1. Global Impact of Bacterial Infections:**
- Sepsis and meningitis are significant global health concerns.
- In 2017, sepsis contributed to 48.9 million cases and 11 million deaths, which accounted for 20% of global deaths.
- Meningitis alone caused approximately 250,000 deaths in 2019, primarily due to bacterial infections.

**2. Disproportionate Impact in Low- and Middle-Income Countries (LMICs):**
- LMICs are heavily affected by these infections due to limited healthcare infrastructure and diagnostic tools.
- High morbidity and mortality rates are common in these regions.

**3. Importance of Rapid and Accurate Diagnosis:**
- Bacterial meningitis and sepsis are medical emergencies that require immediate diagnosis and treatment.
- The gold standard for diagnosis—CSF and blood cultures—is time-consuming.
- Delayed diagnosis leads to increased mortality, prolonged hospital stays, and higher healthcare costs.

**4. Limitations of Conventional Culture Methods:**
- Standard cultures are often negative due to prior antibiotic use, low bacterial counts, or fastidious organisms.
- Molecular methods like PCR have shown higher sensitivity, detecting pathogens in 30-50% of culture-negative CSF cases.

**5. Advantages of Next-Generation Sequencing (NGS):**
- NGS technologies, especially 16S rRNA sequencing, have emerged as powerful tools for rapid pathogen detection.
- Oxford Nanopore's MinION sequencing platform is portable, cost-effective, and provides results within hours.
- This system allows rapid analysis in resource-limited settings, offering a solution to the limitations of traditional culture methods.

**6. Gaps in Current Research and Objective of the Study:**
- While Nanopore sequencing has shown promise in various applications, its use for diagnosing bacterial meningitis in clinical samples, especially in LMICs, is not well studied.
- This study aims to evaluate the effectiveness of 16S rRNA Nanopore sequencing compared to conventional culture methods for diagnosing bacterial meningitis in a resource-limited setting.
- The study uses the EPI2ME cloud platform to analyze data in real-time without requiring advanced bioinformatics expertise.

- **Material: Budoor Alhaiti**

Samples from cerebrospinal fluid (CSF) of patients suspected of bacterial meningitis were collected at the 108 Central Military Hospital in Vietnam between 2019 and 2020. The study included 30 patients.

| *Characteristics* | *CSF (n=30)* |
|---|---|
| *Age (years)* | 49 (19–82) |
| *Male* | 29 |
| *Female* | 1 |

- **Method: Budoor Alhaiti**

**Based on routine laboratory criteria :**

1-Bacterial species were diagnosed through microbiological cultures of cerebrospinal fluid (CSF).
2-Species identification was performed using the VITEK system.
3-Nucleic acids were isolated from 1 mL of CSF, and bacterial DNA was extracted using the SaMag system for 16S rRNA sequencing.
4-The quality and quantity of the extracted DNA were measured using a Qubit fluorometer.
5-PCR was used to amplify the 16S rRNA gene from the CSF sample.
6-The PCR amplicons were purified using AMPure XP beads and quantified again with the Qubit fluorometer.
7-Sequencing was performed using the MinION system for six hours, then the data were collected and analyzed.

- **Results : Layla Alharbi**

| Pathogen | Detected by CSF Culture | Detected by Nanopore Sequencing |
|---|---|---|
| *Streptococcus pneumoniae* | 4 | 4 |
| *Streptococcus suis* | 4 | 5 |
| *Streptococcus mitis* | 1 | 0 |
| *Pseudomonas aeruginosa* | 1 | 0 |
| *Aeromonas sobria* | 1 | 0 |

| | | |
|---|---|---|
| *Cryptococcus neoformans* (Fungal) | 1 | Not Detected (Fungal) |
| *Enterococcus hirae* | 0 | 1 |
| *Neisseria gonorrhoeae* | 0 | 1 |
| *Klebsiella pneumoniae* | 0 | 1 |
| *Aeromonas jandaei* | 0 | 1 |

| Detection Method | Results |
|---|---|
| **Total samples (n = 30)** | 30 |
| **CSF Culture Positive (40%)** | 12/30 |
| **Nanopore Sequencing Positive (43%)** | 13/30 |
| **Both Methods Concordant (70%)** | 21/30 |
| **Discordant Results (30%)** | 9/30 (5 false positive, 4 false negative) |
| **Nanopore Detected in Culture-Negative Samples** | 5/18 (additional detections using Nanopore sequencing) |
| **Culture Detected but Nanopore-Negative** | 2 samples (including *Cryptococcus neoformans*) |
| **Improved Positivity Rate** | 40% (culture alone) → 57% (with Nanopore) |

***Concordance: Indicates whether Nanopore sequencing and conventional methods (CSF and blood culture) produced matching results.**

Pathogen detections by each method, showing how Nanopore sequencing contributed additional diagnoses beyond culture results.

*At least about 200 reads per sample are sufficient in other reliable large scale sequencing studies.

-Reads: Number of Nanopore sequencing reads and percentage of predominant pathogen.

*Most common pathogens identified from the sample S. suis and S. pneumoniae by both methods.

Comparison of conventional microbiology diagnostic techniques and Nanopore sequencing:

*Nanopore sequencing was concordant with culture results in 21 of 30 samples.

*67% of culture-positive samples were detected using Nanopore sequencing.

*Additional pathogens including 5 culture-negative samples increased the total culture positive from 40% to 57%.

- **Discussion: Munirah Alsheddi**

This study demonstrated the ability of Nanopore 16S multiplex sequencing to rapidly and cost-effectively detect pathogenic bacteria in routine diagnostic methods. The predominant bacteria in CSF samples were ***S. pneumoniae and S. suis***. The results are consistent with previous studies, and demonstrate the superior performance of NGS-based detection method in detecting pathogens in patients with CNS infections. CSF and blood cultures are the gold standard for diagnosis, however, they are time-consuming. In contrast, using Nanopore EPI2ME, the detection time is within an hour. Rapid identification of bacterial pathogens in bloodstream infections within 6-12 hours can accelerate the initiation of appropriate treatment to improve clinical outcome.

- **Conclusion: Munirah Alsheddi**

This study demonstrated the ability of 16S multiplex nanopore sequencing to detect pathogens within 6 hours, which helps improve clinical management and outcome of severe infections.

- **References:**

Pallerla, S. R. et al. (2022) 'Diagnosis of pathogens causing bacterial meningitis using Nanopore sequencing in a resource-limited setting', Annals of Clinical Microbiology and Antimicrobials, 21(1), pp. 1–8. doi: 10.1186/s12941-022-00530-6.