Clinical Pearls

Metagenomics-driven rapid diagnosis of an imported fatal case of rare amoebic meningoencephalitis

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Case presentation

A 59-year-old male resident of Riyadh, Saudi Arabia, visited Karachi, Pakistan in June 2019, and on the second day of arrival back to Saudi Arabia, he began to have a persistent fever. He was seen in the clinic in King Faisal Specialized Hospital in Riyadh and was prescribed antipyretics, which failed to improve his fever. His symptoms progressed, and he began to have continuous, nonprojectile vomiting associated with headaches.

During the patient’s travel to Karachi, he had no apparent contact with sick individuals either locally, in the airport, or on the flight. The patient did not report any history of upper respiratory tract infection (URTI) symptoms, ear problems, animal contact or swimming in any local river streams or bathing ponds in Pakistan.

In the emergency department, a cerebrospinal fluid (CSF) sample was taken, and the neurology team assessed him and noted that the patient was awake, confused and agitated. An hour later, the patient became drowsy, barely responsive to painful stimuli and was no longer protecting his airway; thus, he was intubated. He was admitted to the intensive care unit for further management with an impression of septic shock secondary to a primary central nervous system infection. The laboratory investigations upon arrival are summarized in Table S1, supplementary data are available at JTM online. Radiological imaging of the brain is illustrated in Figures 1A–D. Computed tomography (CT) brain angiogram showed no evidence of acute intracranial insult. Computed tomography angiography (CTA) demonstrated no significant stenosis or focal occlusion. CT of the brain without contrast on Day 1 showed new onset of diffuse brain oedema with moderate diffuse narrowing of the CSF spaces. Scattered hyperattenuating foci in the subarachnoid spaces concerned the leptomeningeal process.

The infectious disease team was consulted for antimicrobial treatment. The physical examination at this point showed the patient in a coma with fixed dilated pupils off sedation. His vital signs were stable on inotropic support. Other examinations were within normal limits. The patient was started on broad antimicrobial coverage of community-acquired meningitis (see Supplementary Materials). Lumbar puncture was performed, and the results are illustrated in Table S2, supplementary data are available at JTM online. Cultures remained negative and the remaining CSF sample was preserved for metagenomics-based diagnosis. The patient’s clinical condition worsened, and his antimicrobial treatment regimen was modified (see Supplementary Materials).

On Day 3 of the hospital course, a technetium-99 m HMPO brain perfusion scan confirmed brain death. His clinical and biochemical situation persisted and deteriorated, and on Day 9 of the hospital course, he passed away.

In the absence of any confirmed aetiology for the symptoms, we resorted to metagenomics-based analytical protocols. The CSF sample was subjected to retrospective metagenomic sequencing on an Illumina HiSeq 4000 platform, and we unambiguously identified the case as primary amoebic meningoencephalitis (PAM), with Naegleria fowleri as the aetiological agent. Unfortunately, the patient was already pronounced brain dead by that time. This prompted us to retrospectively develop a metagenomic next generation sequencing (mNGS)-based
Figure 1. Imaging and mNGS-based diagnosis pipeline. Brain CT without contrast (first day of admission) is shown in (A) and (B). CT without contrast (second day of admission) is shown in (C) and (D). (E) Schematic illustration of the mNGS-based Naegleria fowleri identification workflow. Patients with suspected PAM with recent travel history from N. fowleri high burden regions are recommended to go through a hypothesis-free (or unbiased) metagenomics pipeline for PAM diagnosis from CSF samples. The sample-to-report pipeline is expected to take \( \sim 24 \) h to confirm the aetiological agent(s) in our case. (F) Metagenome binning plot of the Illumina iSeq 100 simulation. Each contig is represented by grey (non-\( N. fowleri \)) or red (\( N. fowleri \)) coloured dots. The 18S rDNA sequence is highlighted with a cross. It should be noted that the Illumina technology used in this study could also be replaced by single molecule real-time sequencing protocols, such as the use of one of the Oxford Nanopore Technology (ONT) platforms, if a sufficient amount of starting DNA material could be accessible to the clinical NGS laboratory for PAM diagnosis.

protocol for the rapid diagnosis of PAM using Illumina iSeq 100 equipment. We demonstrated the effectiveness of the metagenomics approach in PAM diagnosis (Figure 1E and F). The patient developed the symptoms a day after returning from Karachi, which is a PAM burden area; hence, it is highly likely that the patient acquired the infection while in Karachi from an environmental source.

We also constructed the draft assembly of the first \( N. fowleri \) clinical isolate genome (Karachi-NF001) reconstituted directly from a patient CSF sample. The unique genetic features of the Karachi strain are worth exploring further to explain any link with the unique epidemiological characteristics of \( N. fowleri \), such as adaptation to saline waters in Karachi (see Supplementary Materials Figure S1 and Table S3).

Since PAM is a rare disease, it does not create a high index of suspicion in clinical microbiology laboratories. The visual detection of \( N. fowleri \) trophozoites in CSF or brain tissue is essential for the initial diagnosis. The amoebic trophozoites may be easily mistaken for macrophages or epithelial cells. Culture-based detection requires multiple days, which is not efficient due to the rapid progression of PAM. The PCR and immunohistochemistry-based methods that target \( N. fowleri \) require prior knowledge of suspected PAM, and the signs and symptoms of PAM are clinically similar to bacterial or viral meningitis, which lowers the index of suspicion for PAM. These multiple difficulties in diagnosing PAM increase the need to adopt accurate hypothesis-free diagnostic modalities, such as metagenomics.

Using our protocol, we showed the possibility of PAM detection within 24 h of receiving any clinical specimen. We demonstrated that the implementation of a mNGS-based diagnosis is a viable method for rapid and precise identification of PAM, particularly for cases where no definite aetiology is determined through routine laboratory diagnostic protocols within hours or days of patient admission. However, the main limitation for such a recommendation is access to NGS facilities and the resources to run such operations in local community hospitals. Thus, efforts must be made to establish such mNGS facilities in tertiary care hospitals and regional laboratories for directed diagnostics in case of clinical suspicion of PAM in patients with connections to high-burden countries.

Our study reveals that the mNGS-based hypothesis-free diagnosis method is a valuable tool for future implementation in clinical settings to aid in the rapid diagnosis of rare cases of PAM and clinical decision-making.

Data availability and ethics statement

The human reads-free Naegleria fowleri dataset is available at European Nucleotide Archive (ENA) under the study accession no. PRJEB44656 (ERP128727). The research protocol was
approved by the Institutional Review Board of King Faisal Specialist Hospital and Research Center (Riyadh, Saudi Arabia; Publication No. 2200085) and the Institutional Biosafety and Bioethics Committee of King Abdullah University of Science and Technology (Jeddah, Saudi Arabia; #17IBEC38).

Supplementary data
Supplementary data are available at JTM online.

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Authors’ Contributions
A.P. and R.S.A. conceived and supervised the study. All authors contributed either to research design (A.P., R.S.A. and Q.G.), and/or the clinical data acquisition (R.S.A., B.A. and M.H.) or genomics data acquisition (A.P., Q.G. and S.M.), analysis (Q.G.) or interpretation (all authors) of data. Q.G. and R.S.A. drafted the initial metagenomics and clinical parts of the manuscript, which was critically revised by A.P. and R.S.A. All authors approved the final version of the manuscript.

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