

## Unbiased metagenomics for determining etiologies of idiopathic meningitis

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## Background

The burden of meningitis in low-and-middle-income countries remains significant despite the introductions of *Haemophilus influenzae* type b (Hib) and pneumococcal conjugate vaccines (PCV). The infectious causes of these remaining cases of meningitis are largely unknown, and there has been little development in evidence-based diagnostics for implementation in LMICs. This lack of data on etiology of meningitis impede institution of evidence-based treatment and prevention decisions. We conducted a validation and application study of unbiased metagenomic next-generation sequencing (mNGS) study to elucidate etiologies of pediatric meningitis in Bangladesh.

## Methods

This RNA mNGS study was performed on cerebrospinal fluid (CSF) specimens from patients admitted in the largest pediatric hospital, a World Health Organization sentinel site, with known neurologic infections (n=36), idiopathic meningitis (n=25), and with no infection (n=30), and six environmental samples, collected between 2012-2018. The conventional laboratory tests used to select samples for this mNGS study included culture, antigen tests and qPCR. The results of the mNGS data were analysed using the open-access bioinformatics pipeline IDseq and machine learning that distinguished potentially pathogenic microbes from environmental and commensal flora. The results were confirmed through qPCR and Sanger sequencing and cases were followed-up through phone/home-visits. A direct-PCR method was optimized to test additional CSF samples for the presence of Chikungunya virus.

## Results

In samples with known etiology and without infections, there was 83% concordance between mNGS and conventional testing. In idiopathic cases, mNGS identified a potential bacterial or viral etiology in 40%. There were three instances of neuroinvasive Chikungunya virus (CHIKV), whose genomes were >99%

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identical to each other and to a Bangladeshi strain previously shown to cause febrile illness in 2017. CHIKV-specific qPCR of all remaining stored CSF samples from children who presented with idiopathic meningitis in 2017 (n=472) revealed 17 additional CHIKV meningitis cases exposing an unrecognized meningitis outbreak. Orthogonal molecular confirmation, case-based clinical data, and patient follow-up substantiated the findings.

### **Conclusions**

Unbiased metagenomic studies, guided by careful selection of positive and negative controls, can facilitate attribution of etiology to meningitis cases where traditional techniques have failed. Inclusion of machine learning techniques facilitate analysis of metagenomic data, and complement methods independent of nucleic acid, such as direct antigen testing, or serology. While we do not foresee administration of metagenomics for everyday diagnosis in LMICs in the near future, or it as a replacement of all techniques, it can act as a complementary tool that can be used in established surveillance platforms in endemic regions, in both outbreak and non-outbreak situations. In this study, CHIKV RNA was detected using metagenomics and the outbreak of CHIKV meningitis was subsequently revealed by a low-cost qPCR technique guided by the findings of metagenomics. Ultimately, unbiased pathogen identification methods like mNGS can complement traditional surveillance methods to facilitate identification of etiologies that eludes standard laboratory testing. These improved patient and population-level data can inform better health policy decisions, including but not limited to vaccine deployment, antibiotic stewardship, vector control and pandemic preparedness.