Identification of *Streptococcus intermedius* Associated with Culture-Negative Brain Abscesses and Meningoencephalitis through CSF mNGS

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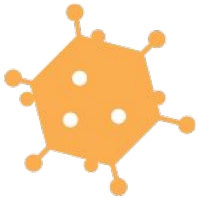
Disclosures

SD, SM: Employee, shareholder, Delve Bio

Meningitis and Encephalitis: Diagnostic and Stewardship Challenges

60%

of cases are due to infectious pathogens



50%

of cases remain undiagnosed

>100

different pathogens linked to ME

Empiric antimicrobial use

86% antibiotics

53% antivirals

8% antifungals

Cost per patient

$64k-260k

ICU level care

22%

Ellul M, *Lancet Infect Dis*, 2022
Glaser CA, *Clin Infect Dis*, 2006
Granerod J, *Lancet Infect Dis*, 2010
Mailles A, *Clin Infect Dis*, 2009
Vora NM, *Clin Infect Dis*, 2017

Role of CSF mNGS in CNS infections

- Traditional CSF diagnostics rely on culture, Gram stain, and targeted PCRs
  - May miss fastidious, unexpected, or low burden organisms
- Metagenomic next-generation sequencing (mNGS) provides unbiased detection of all microbial nucleic acids within a sample
- mNGS enables diagnosis of culture-negative CNS infections and supports precision antimicrobial management
- Applications include meningitis, encephalitis, ventriculitis, and brain abscesses with unclear etiology

mNGS Workflow: Laboratory + Bioinformatics

Sample Processing and Sequencing

Optimized for sensitivity, specificity, and contamination-free results

CSF sample
Stored in stabilization buffer

Lysis and Purification
DNA + RNA from all organisms

Target Enrichment
RNA & DNA viruses, bacteria, fungi, parasites

Library Prep
DNA and RNA libraries

Amplification and Pooling

Sequencing

Bioinformatics Pipeline (Delve D-CIDE)

Optimized for speed and removal of non-microbial background

Preprocessing

Host Background Removal

Microbial Identification

Pathogen Classification

Automated Reporting

Clinical Consultation

Clinical history

50YO male, previously healthy, limited contact with healthcare

- HPI: 2-week history of progressive altered mental status, ambulatory dysfunction, and two generalized tonic–clonic seizures
- No known immunosuppression or comorbidities
- Social history: Lives in rural Pennsylvania, works in construction, and frequently drinks untreated spring water from a local source
    - Also reports occasional consumption of unpasteurized milk from a nearby farm
- No history of IV drug use, recent dental procedures, or head trauma. No animal exposures or recent travel

Exam and workup

Exam: Afebrile (97.8°F), alert and oriented ×3, mild unsteadiness on gait testing; otherwise normal neurologic and systemic exam

- Labs: WBC 17.8 $\times 10^9$/L, normal renal and liver function, mildly low albumin.
- Imaging: MRI revealed multiple peripherally enhancing cystic lesions involving the right frontal and parietal lobes, consistent with brain abscesses
- Microbiology: Stereotactic aspirate showed **Gram-positive cocci**, but no growth on culture after 7 days
- CSF findings: WBC 867 (62% neutrophils, 31% lymphocytes), protein 112 mg/dL, normal glucose; Gram stain and culture negative

Imaging findings

MRI Brain: Multifocal Rim-Enhancing Lesions Consistent with Brain Abscesses

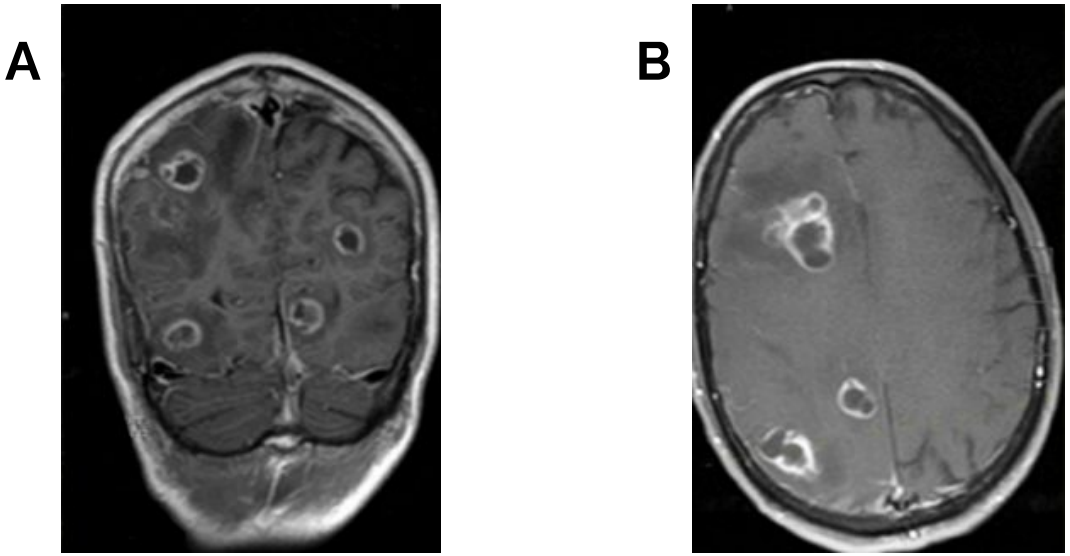


Figure 1. (A) Axial T1 post-contrast MRI showing multiple rim-enhancing cystic lesions involving the right frontal and parietal lobes. (B) Coronal T1 post-contrast MRI demonstrating multifocal abscesses with surrounding vasogenic edema and mass effect.

Workup (continued)

Differential diagnosis:

- Septic emboli
- Nocardiosis
- *Listeria monocytogenes* (history of raw milk/spring water)
- Parasitic CNS infections (cysticercosis, toxoplasmosis)

Broad infectious workup negative: HIV, TB Quantiferon, toxoplasmosis, cysticercosis serologies, and transthoracic echocardiogram (no vegetations)

- Started on broad empiric antibiotics to cover Gram positive, Gram negative, and anaerobic bacteria; anticonvulsants initiated for seizure control

Given persistent culture negativity, CSF was submitted for Delve Detect CSF mNGS testing to investigate fastidious or unculturable pathogens

mNGS results

Table A - Organism Result Summary

Organism	Total reads	Unique reads	rpM	rpM ratio	Biomass (fg)
Streptococcus intermedius (anginosus group)	21	17	0.96	0.96	1.256×10^3

Table B - Sequencing & Host Background Metrics

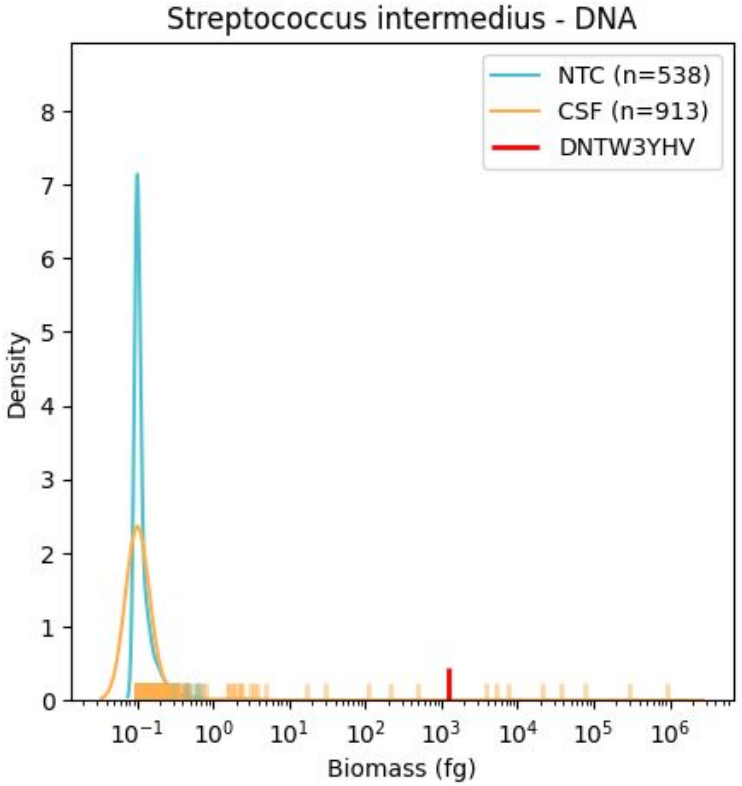
Library	Reads PF	% Human	Total biomass (fg)	Control biomass (fg)	Human biomass (fg)
DNA	21,863,961	99.9456	1.308×10^9	6.244×10^4	1.307×10^9
RNA	35,213,154	99.7029	1.118×10^9	2.577×10^4	1.114×10^9

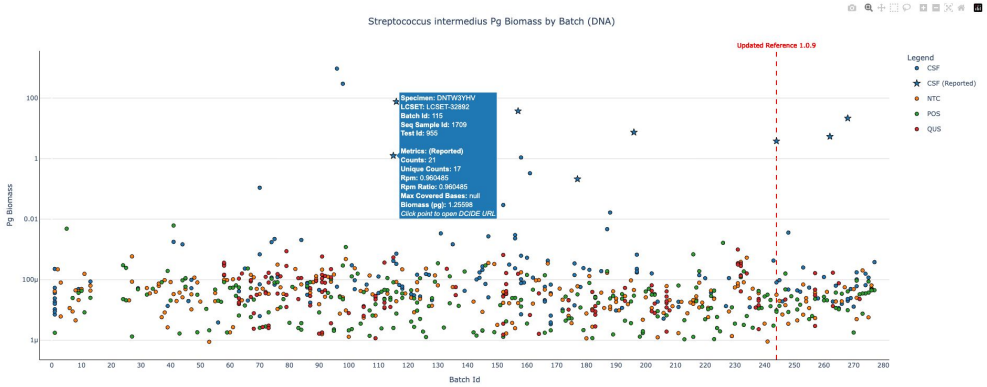
Sequencing metrics (hidden)

Show raw

Total Biomass (pg)	Control Biomass (pg)	Human Biomass (pg)	Organism Biomass (pg)	Unaligned Biomass (pg)	Unclassified Biomass (pg)
1,307,650.00	62.44	1,306,940.00	58.37	590.07	0.84
11,175.50	25.77	11,142.30	3.31	3.04	1.08

Organism biomass





BLAST summary (top matches)

Rank	Organism (hit)	Accession	Max score	Query cover	E-value	% identity
1	Streptococcus intermedius B196, complete genome	CP003857.1	198	100%	1.00E-46	99.09%
2	Streptococcus intermedius JTH08, complete genome	AP010969.1	198	100%	1.00E-46	99.09%
3	Streptococcus intermedius NCTC11324, chromosome 1	LS483436.1	198	100%	1.00E-46	99.09%
4	Streptococcus intermedius FDAARGOS_769, complete genome	CP053999.1	198	100%	1.00E-46	99.09%
5	Streptococcus intermedius C270, complete genome	CP003858.1	198	100%	1.00E-46	99.09%

Interpretation: Multiple top hits map to *S. intermedius* with 99.09% identity and 1e-46 E-values, supporting species-level identification within the anginosus group

Clinical impact

- mNGS confirmed *Streptococcus intermedius* etiology of culture-negative CNS abscess
  - Supported a monomicrobial etiology without evidence for a mixed infection
- Diagnosis achieved directly from CSF without tissue testing
- Enabled de-escalation to ceftriaxone monotherapy and discontinuation of vancomycin and metronidazole
- Reduced exposure to unnecessary antimicrobials and supported shorter hospitalization

Key takeways

- CSF mNGS can identify fastidious pathogens in culture-negative CNS infections
  - Interpreting mNGS in high host-background samples requires understanding how relative signal strength reflects organism biomass and confidence of detection
- Provides actionable information for antimicrobial optimization
- Less invasive than brain tissue sequencing; supports precision infectious disease diagnosis
- Integration of mNGS into clinical workflows can reduce morbidity from delayed or indeterminate diagnoses

Thank you!

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Delve Detect CSF: Test Overview and Controls

Test Description

- Delve Detect CSF is an mNGS assay for qualitative pathogen detection in CSF from patients with suspected meningitis or encephalitis
- Detects organisms by sequence homology of nucleic acid (DNA & RNA libraries)
- Sequencing on Illumina platform
- Proprietary bioinformatic pipeline for read assignment
- Reports reviewed by board-certified physicians with interpretation and comments
- Results should be considered in the context of clinical history, labs, and imaging

Controls

- Negative control: Synthetic CSF matrix
- Positive control: Synthetic CSF with 7 representative non-pathogenic organisms (DNA & RNA viruses, GN / GP bacteria, fungi, parasites)
- Internal control: DNA and RNA phage spiked into each sample prior to extraction

Clinical use cases for CSF mNGS testing

Expedited Diagnosis in High-Risk Patients	• Critically-ill, ICU • Immunocompromised hosts • Pediatric patients
Resolve Atypical Clinical Presentations	• Suspected atypical or fastidious pathogen • High clinical suspicion with negative conventional testing
Optimize Treatment Decisions	• Antimicrobial de-escalation • Initiation of immunosuppression • Tissue-localized brain infections
Public Health and Geographic Medicine	• Unusual pathogens • Complex exposure history • Outbreak detection and PH investigations