Identification of a Novel *Moraxella* Species in a Culture-Negative CNS Shunt Infection Using Metagenomic Next-Generation Sequencing

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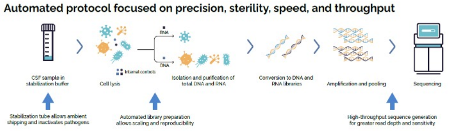
Background

- Metagenomic next-generation sequencing (mNGS) enables rapid, comprehensive, and culture-independent detection of pathogens, including atypical and novel species, directly from CSF, and has demonstrated clinical utility in challenging clinical scenarios.
- CNS shunt infections are often culture-negative due to fastidious pathogens, low microbial burden, or prior antimicrobial treatment.
- A case of a pediatric CNS shunt infection is described in which CSF gram stain was positive but multiple cultures and other tests were repeatedly negative; mNGS ultimately identified a potentially novel *Moraxella* species.

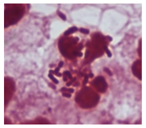
mNGS Analysis



- Test: Delve Detect CSF (Delve Bio), CLIA-certified/CAP-accredited laboratory- developed test (LDT)
- Sample: 1 mL CSF, DNA/RNA deep sequenced (>10M reads/library)
- Findings:
  - *Moraxella* bacteria was detected at subthreshold levels, despite the presence of high host background
  - Sequences aligned to multiple *Moraxella* species, making species-level identification challenging
  - Genomic coverage over the ribosomal RNA operon (RRN) was sufficient to build a consensus sequence and perform phylogenetic analysis
  - The closest phylogenetic matches by RRN were *Moraxella* sp. K1664 along with other Group I *Moraxella* species
  - Phylogenetic placement between these multiple species indicates a potentially novel *Moraxella* organism

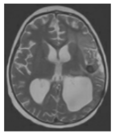
Findings

10 yo with h/o of astroblastoma s/p resection
Shunted hydrocephalus with revision 1 month ago
Presenting with AMS, fever, N/V

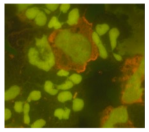


Gram stain
showing intracellular GNRs

HD 1 Meropenem initiated (21 day course)
HD 5 mNGS ordered
HD 10 Sample received
HD 11 mNGS result available



T2 MRI showing post-contrast enhancement of left cerebral hemisphere



Acridine Orange stain
showing intracellular bacilli

CSF	HD 1	HD 5	HD 10
WBC	43	23	4
% Neutrophils	81	7	
Glucose	<20	29	31
Gram stain	GNR	None seen	None seen
Culture	Neg	Neg	Neg

GNR = Gram negative rods, NOS = no organisms seen

delvedetect CEREBROSPINAL FLUID

Subthreshold Positive Result

- *Moraxella* species detected (below reporting threshold), closely aligning with *Moraxella bovis* and atypical species.
- Sample had high host DNA background, reducing sensitivity for detecting pathogens.

Species	rpM	rpM Ratio	Unique Reads	Biomass (fg)
Moraxella (declassified reads)	0.22991	0.22991	8	42.123
Moraxella bovis	2.21291	2.21291	66	405.434
Moraxella nonliquefaciens	0.31613	0.31613	9	57.9191
Moraxella bovoculi	0.17244	0.17244	6	31.5922
Moraxella haemolytica	0.08622	0.08622	1	15.7961
Moraxella ovis	0.02874	0.02874	1	5.26537
Moraxella nasibovis	0.02874	0.02874	1	5.26537
Moraxella genus (total)	3.07509	3.07509	92	563.39514

Table 1. Sequencing Metrics for *Moraxella* DNA Library Reads Detected by mNGS
Table shows reads per million (rpM), rpM ratio (normalized abundance relative to non-template control signal), number of unique reads (by exact sequence match), and microbial biomass (fg) for individual *Moraxella* species observed. *Moraxella* (species-declassified reads) indicates reads assigned to the genus level without confident species-level classification. *Moraxella* genus (total) indicates the sum across all *Moraxella* species and declassified reads. Elevated rpM and biomass were observed for *M. bovis* with lower contributions from several other *Moraxella* species, supporting the presence of a novel or divergent organism within the *Moraxella* genus.

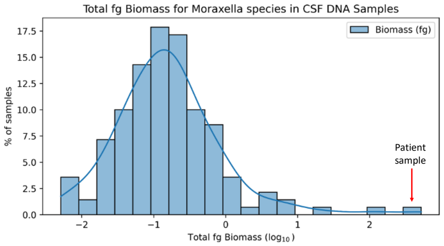


Figure 1. Elevated *Moraxella* Biomass in Patient Sample Compared to Historical mNGS Samples
Histogram of $\log_{10}$-transformed total microbial biomass (pg) for *Moraxella* species detected in CSF samples submitted for clinical mNGS testing. The patient sample (red arrow) demonstrates significantly higher organism biomass than the background level seen in historical clinical samples, supporting the presence of true infection rather than environmental contamination or incidental signal.

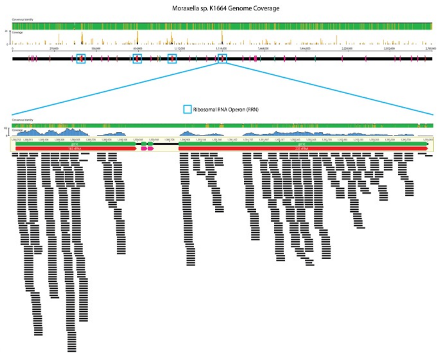


Figure 2. Read Alignment to *Moraxella* sp. K1664 Genome
Genome coverage plot showing mNGS read alignment to *Moraxella* sp. K1664 reference genome. Upper panel shows genome-wide consensus identity and read coverage at 1.7% of the genome. Lower panel shows zoomed-in coverage across the ribosomal RNA operon (RRN) which was used for phylogenetic analysis.

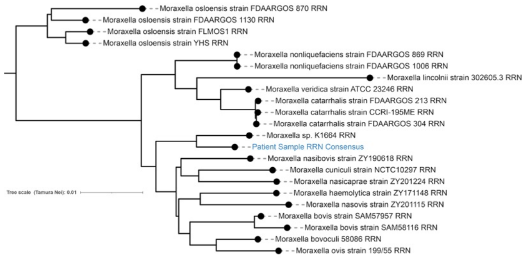


Figure 3. Phylogenetic Analysis of *Moraxella* Consensus Sequence
Maximum likelihood phylogenetic tree constructed from patient-derived ribosomal RNA operon (RRN) consensus sequence demonstrates that the patient sample (blue) clusters within Group I *Moraxella*. The patient sequence appears closely related to *Moraxella* sp. K1664, with approximately equivalent distance between several closely-related *Moraxella* species, supporting the presence of a potentially novel *Moraxella* species. (Scale: Tamura-Nei distance = 0.01)

Discussion

- Despite antibiotic pre-treatment, mNGS identified a pathogen consistent with CSF Gram stain result in a case repeatedly negative by culture and multiple other tests
- mNGS provided definitive diagnosis and could have enabled narrowing of antibiotics
- CNS shunt-associated infections often involve fastidious or biofilm-forming organisms that can evade culture, particularly with prior antimicrobial exposure
- *Moraxella* is an uncommon CNS pathogen and rarely reported in shunt infections; its identification here underscores:
  - Diagnostic limits of culture-based microbiology
  - mNGS value in high-risk, culture-negative infections
- Phylogenetic analysis supports a potentially uncharacterized *Moraxella* species.

Conclusions

- Delve Detect mNGS enabled the diagnosis of a culture-negative CNS shunt infection caused by a potentially novel *Moraxella* species.
- This case underscores the clinical utility of mNGS in complex infections and the ability to detect emerging or atypical pathogens missed by conventional diagnostics.
- Together with findings from other studies, this case supports broader integration of mNGS into the diagnostic workup for unexplained or culture-negative CNS infections, particularly in scenarios of CSF-associated shunts, devices, or other hardware