There’s *What In My Brain?*

What We Can Learn from NGS Testing of Bacterial Meningitis Cases

Kara Mitchell, PhD

*NORTHEAST BRANCH—AMERICAN SOCIETY FOR MICROBIOLOGY*

*54TH ANNUAL REGION I MEETING*

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Bacterial meningitis is a serious and potentially deadly infection of the CNS

- Inflammation of the meninges
- Immediate diagnosis critical for patient care
- Sudden onset of fever, headache, stiff neck, altered mental status, nausea/vomiting
- Symptoms usually appear 3-7 days after exposure
- Children and older adults are the highest risk groups
Bacterial meningitis is a serious and potentially deadly infection of the CNS

- Most common causes of bacterial meningitis in the US are:
  - *Neisseria meningitidis*
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Group B Streptococcus*
  - *Listeria monocytogenes*

- Vaccines available for protection against *N. meningitidis, S. pneumoniae, and Haemophilus influenzae*
  - Serotyping performed to determine if it’s a vaccine preventable strain

- Identification of contacts important for prophylaxis and vaccination clinics in certain settings
Current testing algorithm for bacterial meningitis cases

**Neisseria meningitidis**
real-time PCR

- Positive Report positive; serogrouping performed
- Negative Report negative; 16S rDNA sequencing (upon request)

**Multiplex real-time PCR**

- Positive Report positive; serogrouping: H. influenzae or S. pneumoniae
- Negative Report negative; 16S rDNA sequencing (upon request)

**S. pneumoniae**
**H. influenzae**
**S. agalactiae (GBS)**
16S rDNA sequencing is commonly used for bacterial identification

- Universally found in all bacteria; highly conserved
- Allows for identification of fastidious organisms and culture-negative specimens
- Alternative testing method when unsure of the pathogenic bacteria
Meningitis testing in the Bacteriology Laboratory from 2015-2017

• Specimens tested:
  • Young children (ages 0-10): ~25%
  • Teenage/college-aged (ages 15-25): ~19%

• ~19% of specimens tested by real-time PCR were positive for targeted bacteria

• When 16S rDNA sequencing was requested: other bacteria identified in ~20% of specimens
  • Many specimens remain unidentified

• Can NGS can help resolve when organisms are not detected/identified?
In this study, we aimed to evaluate the performance of the Ion 16S™ Metagenomics Kit to identify bacteria in CSF in comparison to the current 16S Sanger sequencing method.

Increased identification = Better patient outcomes

Improve laboratory testing methods
Bacterial 16S rRNA gene: Primer targets of Ion 16S™ Metagenomics Kit

Workflow for sequencing using the Ion 16S™ Metagenomics Kit

Prepare samples, Epicentre DNA extraction → PCR setup → NGS

Library Prep (manual) → Templating → Sequencing, Analysis

Overview of the MasterPure™ Complete Kit protocol. © Epicentre
Setting an Analysis Threshold

**Appendix A: List of organisms found in negative aCSF controls across retrospective study runs 1-5; organisms recorded if Genus species was identified**

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>% of Total Reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aciditerrimonas</td>
<td>sp.</td>
<td>0.11</td>
</tr>
<tr>
<td>Agroccocus</td>
<td>jejuensis</td>
<td>0.02</td>
</tr>
<tr>
<td>Blastococcus</td>
<td>aggregatus</td>
<td>0.11</td>
</tr>
<tr>
<td>Blastococcus</td>
<td>saxobsidens</td>
<td>0.06</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>afermentans</td>
<td>0.12</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>tuberculostearicum</td>
<td>0.02*</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>sp.</td>
<td>0</td>
</tr>
<tr>
<td>Yimella</td>
<td>hitea</td>
<td>0.01</td>
</tr>
<tr>
<td>Dietzia</td>
<td>cercidiphylly</td>
<td>0</td>
</tr>
<tr>
<td>Dietzia</td>
<td>maris</td>
<td>0.01</td>
</tr>
<tr>
<td>Dietzia</td>
<td>natronolimnaea</td>
<td>0.05</td>
</tr>
<tr>
<td>Microbacterium</td>
<td>ginsengisoli</td>
<td>0.05</td>
</tr>
<tr>
<td>Kocuria</td>
<td>marina</td>
<td>0.04</td>
</tr>
<tr>
<td>Nocardoides</td>
<td>oleivorans</td>
<td>0.4</td>
</tr>
<tr>
<td>Prevotella</td>
<td>maculosa</td>
<td>0.07</td>
</tr>
</tbody>
</table>

- “Background bacteria” can be challenging
- Organisms in negative artificial CSF (aCSF) controls were identified and used to measure background/contamination
- Threshold to identify a bacterial species: ≥1.0% of total reads in the sample
Retrospective study

Tested archived CSF specimens that had been tested previously

68 total specimens tested
- 15 known positives
- 53 “unknowns”
  - No prior 16S sequencing performed
  - Not positive for any other real-time PCR targets
  - Not tested by another laboratory at Wadsworth
### Targeted 16S NGS Results:
- **15/15**: Identification of meningitis positive samples correlated
- **15/53**: Samples initially negative by PCR were found to be positive for at least one bacterial organism (28%)
- **38/53**: Samples previously determined negative by PCR were negative by NGS

### 16S Sanger Sequencing Results:
- **10/15**: Identification of meningitis positive samples correlated
- **3/53**: Samples initially negative by PCR were found to be positive for at least one bacterial organism (6%)
- **50/53**: Samples previously determined negative by PCR were negative by 16S sanger sequencing
## Retrospective study results: breakdown of Ion Torrent NGS Positives

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ion Torrent NGS</th>
<th>16S Sanger Sequencing</th>
<th>Other Real-time PCR</th>
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<tbody>
<tr>
<td>17</td>
<td><em>Streptococcus anginosus, Streptococcus intermedius,</em> Fusobacterium necrophorum</td>
<td>NBD*</td>
<td><em>Streptococcus anginosus</em></td>
</tr>
<tr>
<td>18</td>
<td><em>Streptococcus salivarius</em></td>
<td>NBD*</td>
<td><em>Streptococcus pyogenes</em></td>
</tr>
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<td>23</td>
<td><em>Clostridium septicum, Klebsiella pneumoniae, Klebsiella variicola</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td><em>Staphylococcus auricularis</em></td>
<td>NBD*</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td><em>Prevotella maculosa, Prevotella oris</em></td>
<td>NBD*</td>
<td><em>Streptococcus constellatus</em></td>
</tr>
<tr>
<td>43</td>
<td><em>Streptococcus pasteurianus</em></td>
<td>NBD*</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td><em>Streptococcus salivarius</em></td>
<td>NBD*</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td><em>Diaphorobacter oryzae</em></td>
<td>NBD*</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>NBD*</td>
<td><em>Klebsiella sp.</em></td>
</tr>
<tr>
<td>55</td>
<td><em>Bacteroides caccae, Bacteroides dorei, Prevotella, Prevotella sp., Lactobacillus gasseri, Ruminococcus gnavus</em></td>
<td>NBD*</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td><em>Streptococcus salivarius</em></td>
<td></td>
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</tr>
<tr>
<td>65</td>
<td><em>Corynebacterium sp., Cloacibacterium normanense,</em> Enterococcus cecorum</td>
<td>NBD*</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td><em>Nocardioides sp., Propionibacterium acnes</em></td>
<td>NBD*</td>
<td></td>
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*NBD: no bacterial DNA detected*
59 year-old female
Suspected meningitis – culture negative at hospital laboratory
CSF sent to Wadsworth for meningitis testing
With NGS *Legionella pneumophila* was identified
Confirmed result with lab developed real-time PCR
- *Streptococcus salivarius* identified in 3 samples
- Normally found in the oral cavity, and is an uncommon cause of invasive infections.

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• **Streptococcus salivarius** identified in 3 samples
• Normally found in the oral cavity, and is an uncommon cause of invasive infections.
• Has been associated with meningitis in past cases

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5 Samples had organisms identified that we currently have real-time PCR assays developed for:
- **Streptococcus anginosus group**
- **Klebsiella pneumoniae**
- **Streptococcus pyogenes**
- **in two cases we identified the pathogen and confirmed with real-time prior to setting our threshold cutoffs.**

Other organisms were identified in these samples that could not be confirmed by real-time PCR – no current assays:
- **Fusobacterium necrophorum**
- **Prevotella sp.**
November 18, 2019

• 5 Samples had organisms identified that we currently have real-time PCR assays developed for
  • **Streptococcus anginosus group**
  • **Klebsiella pneumoniae**
  • **Steptococcus pyogenes**
  • ** in two cases we identified a pathogen and confirmed with real-time prior to setting our threshold cutoffs.**

• Other organisms were identified in these samples that could were not confirmed by real-time PCR – no current assays
  • **Fusobacterium necrophorum**
  • **Prevotella sp.**

### Results

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• Additional samples:
  *Staphylococcus auricularis*
  *Streptococcus pasteurianus*

• Can cause opportunistic infections
• Rarely associated with infection or meningitis

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*Streptococcus gallopyticus* Subspecies *pasteurianus* (Biotype II/2), a Newly Reported Cause of Adult Meningitis

Amy S. Sturt,1,2,* Lijing Yang,2 Kuldeep Sandhu,3 Zhiheng Pei,2,3 Nicholas Cassal,3 and Martin J. Blaser2,3

*Author information* • *Article notes* • *Copyright and License information* *Disclaimer*
Numerous samples (17, 23, 55, and 65) had multiple organisms identified, unlikely mixed infections
  - Could represent contamination from specimen collection or the laboratory

For many of these bacteria, meningitis reported in rare cases

Important to consider whole clinical picture!
Summary of major findings

• Targeted 16S rDNA NGS shows increased sensitivity for detection of gram-positive and gram-negative bacteria

• Targeted 16S rDNA NGS identified other bacteria in previously negative clinical CSF specimens
  • 28% of specimens vs 6% by 16S Sanger sequencing

• Public health impact:
  • Results could lead to implementation of new assays (ex: real time PCR for S. salivarius)
  • Integration of existing assays into current testing algorithm (ex: S. pyogenes)
  • Improved testing methods = better patient and community health outcomes
Challenges of NGS for Bacterial ID

- NGS most cost-effective when sequencing volume is high
- Increased sensitivity can lead to issues with result interpretation
  - Background
  - Contamination
- Lack of standardization
  - NGS platforms
  - Bioinformatics
- Limitations of 16S rDNA sequencing
Conclusions and future directions

• NGS can be a valuable tool for sensitive identification of bacteria in clinical CSF specimens

• Future studies
  • Illumina MiSeq
  • Oxford Nanopore Technologies’ MinION
  • Continued retrospective testing of clinical specimens
  • Expand to other specimen sources, including whole blood
  • Clinical Validation of Targeted 16S NGS assay
Acknowledgements

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Anna Kidney

Tanya Halse

Elizabeth Nazarian
Kailee Cummings
Daryl Lamson
Dr. Linda Styer
Dr. Bill Lee
Applied Genomic Technologies Core

Bacteriology Laboratory
Calculated costs of Ion Torrent NGS vs 16S Sanger sequencing and Real-time PCR

<table>
<thead>
<tr>
<th></th>
<th>Ion Torrent NGS(^a)</th>
<th>16S Sanger Sequencing(^b)</th>
<th>Real-time PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per sample</td>
<td>$412.71</td>
<td>$26.65</td>
<td>$15</td>
</tr>
<tr>
<td>TAT (days)</td>
<td>4</td>
<td>Standard: 7-10</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Priority: 2</td>
<td></td>
</tr>
</tbody>
</table>

- Ion Torrent NGS: High reagent costs, high cost/sample, labor intensive (manual library prep)
- Bacterial identification using Ion 16S™ Metagenomics Kit not feasible for routine use in the Bacteriology Lab
## DISTINCTIONS BETWEEN MEDICINE & PUBLIC HEALTH

<table>
<thead>
<tr>
<th></th>
<th>Public Health</th>
<th>Medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Focus</strong></td>
<td>Population/Entire Community</td>
<td>Individual</td>
</tr>
<tr>
<td><strong>Emphasis</strong></td>
<td>Disease prevention and health promotion for the whole community</td>
<td>Disease diagnosis, treatment, and care for the individual patient</td>
</tr>
<tr>
<td><strong>Paradigm</strong></td>
<td>Interventions aimed at the environment, human behavior and lifestyle, and medical care</td>
<td>Places predominant emphasis on medical care</td>
</tr>
<tr>
<td><strong>Specializations</strong></td>
<td>Analytical method (epidemiology, toxicology)</td>
<td>Organ system (cardiology, neurology)</td>
</tr>
<tr>
<td></td>
<td>Setting and Population (occupational health, international health)</td>
<td>Patient group (obstetrics, pediatrics)</td>
</tr>
<tr>
<td></td>
<td>Substantive health problem (environmental health, nutrition)</td>
<td>Etiology and pathophysiology (infectious disease, oncology)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>technical skill (radiology, surgery)</td>
</tr>
</tbody>
</table>
Levels of Specimen Testing

- **Patient specimen**
  - Clinical Microbiology Laboratory
    - Hospital (Initial Testing/Diagnosis)
      - Patient management
      - Infection control
  - State Public Health Laboratory (Confirmation/ additional testing)
    - Surveillance
    - Characterization
    - Epidemiology
    - Investigation
  - Federal Public Health Laboratory (Confirmation/ additional testing)
    - Monitoring
    - National trends

Short TAT is critical
Wadsworth Center

Laboratories in four scientific divisions:
- Environmental Health
- Infectious Disease
- Genetics
- Translational Medicine

Division of Infectious Diseases

Viral Diseases
- Virology
- Viral Encephalitis
- Enteric Virus

Bacterial Diseases
- Bacteriology
- Mycobacteriology
- Biodefense

Bloodborne Diseases
- Bloodborne Virus
- Diagnostic Immunology

Mycotic & Parasitic Diseases
- Parasitology
- Mycology

Viral Replication and Vector Biology
- Arbovirology
- Rabies

Research Laboratories
PI- grant funded programs
Bacterial meningitis is a serious and potentially deadly infection of the CNS

• Immediate diagnosis critical for patient care
• Most common causes of bacterial meningitis in the US are: *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Group B Streptococcus*, *Listeria monocytogenes*
• Children are the highest risk group
• Vaccines for protection against *N. meningitidis*, *S. pneumoniae*, and *Haemophilus influenzae*