Thinking Outside of the Box: The Ongoing Need for Microbiology Expertise in the Era of MALDI-TOF MS

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Learning Objectives

• Discuss the advantages and limitations of MALDI-TOF MS for identification of microorganisms recovered in culture
• Communicate the impact of MALDI-TOF MS on informing the clinical significance of emerging pathogens
• Review approaches to implementation of MALDI-TOF MS and result reporting in a routine clinical setting

Drivers of change in microbiology testing

Traditional Microbiology Culture Workflow

Sample Collection → Incubation → Identification → Antimicrobial Susceptibility Testing

Traditional Identification

Financial Disclosures

• Grant/Research Support:
  • Accelerate Diagnostics, Cepheid, bioMerieux, Aperture Bio, Theraovance, Luminex, CDC, AHRQ

• Salary/Consultant Fees:
  • Monsanto, Journal of Clinical Microbiology, Bone, Klaris, Clinical Microbiology Newsletter, Cepheid

• Board/Committee/Advisory Board Membership:
  • Thermo Fisher Scientific

• Speaker Fees:
  • BD, Accelerate Diagnostics, Medavera, Bruker Daltonics
MALDI-TOF MS Timeline

2010:
- MALDI-TOF MS for microorganism identification
- M-Matrix
- A-Assisted
- D-Desorption
- I-Ionization
- T-Time
- O-Order
- F-Flight
- M-Mass
- S-Spectrometry

MALDI-TOF MS Timeline

2011:
- MALDI-TOF MS in our Laboratory
- CAP Proficiency Testing (DHEC) - Testing MALDI TOF for Identification

MALDI-TOF MS – Overview

• Principle:
  - Identification of microorganisms by analyzing protein content
  - Separation of molecules based on the mass to charge ratio
  - Sample is ionized, ions are separated, detected
  - Data "fingerprint" is compared to a mass spectra database

Sample Preparation
Iontization
Detection
Data Analysis

MALDI-TOF MS for Microbial Identification

- Ionized sample accelerated over a short distance
- A molecular fingerprint is generated
- Primarily ribosomal proteins detected (relative abundance)
- Low reagent cost
- Identification is performed in minutes
- Potential to identify many organism types


Commercially Available Systems

Bruker MALDI Biotyper
bioMérieux VITEK MS

MALDI-TOF MS for Microbial Identification

2010:
- ECCMID
- MALDI-TOF MS for microorganism identification

2011:
- MALDI-TOF MS in our Laboratory
- CAP Proficiency Testing (DHEC) - Testing MALDI TOF for Identification

2010:

2011:
- Hsu and Burnham. 2014. Drug Micro Infect Dis. 73: 149-156.
“Challenging” Organism Identifications

Objective:
- Evaluate the analytical performance of MALDI-TOF MS for the most challenging microorganisms
- 174 bacterial isolates
  - 148 were sent out for identification (frozen stock)
  - 4-51 days
  - 26 required multiple methods to be identified (fresh)
- 85% of isolates identified to species level with MALDI-TOF MS
- Five of the isolates not identified by MALDI-TOF not identified by 16S rRNA gene sequencing (i.e. possible novel species!)


- MALDI-TOF MS vs. standard protocols, 12 week study
- Overall accuracy of MALDI-TOF MS identifications >98%
- Cost savings—consumables for identification
  - 54% reduction in cost with MALDI-TOF MS
- Time to identification: Average of 1.45 days earlier with MALDI-TOF MS compared to conventional methods


- 12 month retrospective analysis
  - 21,930 isolates collected between April 1, 2013 and March 31, 2014


MALDI-TOF vs Traditional: Cost Comparison


MALDI-TOF MS Timeline

**MALDI-TOF MS Timeline**

- **2009**: Ongoing optimization of MALDI-TOF in routine clinical use
- **2010**: Expanded databases, used more widely in clinical settings
- **2013**: Ongoing optimization of MALDI-TOF in routine clinical use
- **2016**: Expanded databases, used more widely in clinical settings
- **2019**: Improved resolution of organism identification

**CAP Proficiency Testing (DEX)**
- **2006**: 30% labs using MALDI-TOF for identification
- **2010**: 60% labs using MALDI-TOF for identification

**CLSI M58 Document: MALDI-TOF MS for Microbial Identification**

**MALDI-TOF MS Timeline**

**CAP Requirements for MALDI-TOF MS**

**MATRIX-ASSISTED LASER DESORPTION IONIZATION TIME-OF-FLIGHT (MALDI-TOF) MASS SPECTROMETRY**

This section applies to laboratories using MALDI-TOF systems to perform organism identification. Refer to the Test Method Validation section in the All Common Checklist for validation requirements pertinent to laboratory-developed tests.

- **INC: 15975**
  - **Instrument Operation**
  - Phase II
  - There are written procedures for the operation and calibration of the mass spectrometer.

- **INC: 16395**
  - **Mass Spectrometer Calibration**
  - Phase II

**Show of hands....**

- Who uses MALDI-TOF MS as the primary microorganism identification method in their laboratory?
Microbiology Expertise is Still Needed!!!

- Old name/new name (when getting it right has clinical implications)
- When getting it right has AST implications
- Biosafety
- When MALDI-TOF gets it wrong

Impact of MALDI-TOF MS on the Clinical Laboratory

- Clinical laboratory experience (Jan 2002 to Dec 2012)
  - French hospital
  - 500,179 bacterial identifications
  - Compared conventional phenotypic identification to MALDI-TOF MS identification

<table>
<thead>
<tr>
<th>Conventional Phenotype (91 Months)</th>
<th>MALDI-TOF (40 Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44 species identified annually</td>
<td>112 species identified annually</td>
</tr>
<tr>
<td>19 species/10,000 isolates</td>
<td>36 species/10,000 isolates</td>
</tr>
</tbody>
</table>


Case #1: Friend or foe?

- 34 year old woman from Mexico presents with granulomatous mastitis of the left breast
  - Painful, progressively worse over 14 months
  - Multiple biopsies and cultures
  - Incision and drainage revealed many pus-filled cavities
  - Surgical pathology demonstrated granulomatous inflammation
  - The patient was treated by multiple physicians and subspecialty services
  - Corynebacterium kroppenstedtii was recovered from cultures at multiple time points
  - Documented in medical record that "no need for antibiotics, Corynebacterium is just skin flora"
  - Patient was ultimately assessed by an ID provider and was treated with antibiotics for >2 months, abscesses resolved


"Diphtheroid-like" organisms in clinical specimens

- MALDI-TOF MS—rapid and accurate species level identification of Gram-positive bacilli (Reported as "coryneform" or "diphtheroids" in the past)
- "New" organisms with emerging disease associations
- Important to know disease associations so they are not dismissed as contaminants
- Many species are multi-drug resistant

“New” organisms with emerging disease associations

- Important to know disease associations so they are not dismissed as contaminants
- Many species are multi-drug resistant

Clinically Important Corynebacterium spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium diphtheriae</td>
<td>Diphtheria</td>
</tr>
<tr>
<td>Corynebacterium ulcerans, C. pseudotuberculosis</td>
<td>Kidney stones (urease activity)</td>
</tr>
<tr>
<td>Corynebacterium jeikeium</td>
<td>Nosocomial infection, line infection, multi-drug resistance</td>
</tr>
<tr>
<td>Corynebacterium macginleyi</td>
<td>Conjunctivitis</td>
</tr>
<tr>
<td>Corynebacterium kroppenstedtii</td>
<td>Granulomatous mastitis</td>
</tr>
<tr>
<td>Corynebacterium striatum</td>
<td>Device infection, blood stream infection, multi-drug resistant</td>
</tr>
<tr>
<td>Corynebacterium pseudodiphtheriticum</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>Turicella otitidis and Corynebacterium auris</td>
<td>Otitic media</td>
</tr>
</tbody>
</table>

**Candida auris:**
A drug-resistant germ that spreads in healthcare facilities

- Can be very resistant to antifungal agents
- Mortality is high in the setting of invasive infection
- Difficult for clinical laboratories to identify

Global Emergence of Candida auris

- 2009: First described in Japan, reported from 26 patients with otitis media in South Korea
- 2011: Bloodstream infection in South Korea
- 2012-2013: Outbreaks in India, South Africa, Kuwait
- 2013-2016: Emergence in USA
- 2015-2016: First European Outbreaks

Emerged independently and simultaneously in different parts of the world.

High clonality within geographic clades, differences between geographic clades.

Why is Candida auris such a problem?

- Can cause serious infections
- Blood stream infections
- Mortality is high in the setting of invasive infection
- Can spread in healthcare settings
- Prolonged survival on surfaces
- Grows well at elevated temperatures (40 to 42 °C)
- Typically very resistant to antifungal agents
- Difficult for clinical laboratories to identify
  - May be misidentified or unidentified

Identification of Candida auris

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Likely to be identified as</th>
</tr>
</thead>
<tbody>
<tr>
<td>All methods</td>
<td>Candida haemulonii, Candida spp. not otherwise identified</td>
</tr>
<tr>
<td>API 20C</td>
<td>Rhodotorula glutinis, Candida sake, Unidentified</td>
</tr>
<tr>
<td>API Candida</td>
<td>Candida famata</td>
</tr>
<tr>
<td>BD Phoenix</td>
<td>Candida haemulonii, Candida tropicalis</td>
</tr>
<tr>
<td>bioMerieux VITEK 2 YST</td>
<td>Candida haemulonii, Candida lusitaniae, Candida famata, Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>MicroScan</td>
<td>Candida famata, Candida laevans, Candida guilliermondii, Candida parapsilosis, Canadila albicans, Candida tropicalis, Candida cattenuata</td>
</tr>
<tr>
<td>Sequencing (28S D1/D2 or ITS)</td>
<td>Candida auris</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>Not identified</td>
</tr>
</tbody>
</table>

CDC/FDA AR Isolate Bank Can Support Laboratory Validation Efforts

CDC/FDA Antimicrobial Resistance Isolate Bank

ARISOLATEBANK

Candida auris isolate panel

A panel of Candida auris isolates and other yeast species related to C. auris are commonly misidentified as C. auris.

Caution: Candida auris has been shown to be transmitted in healthcare settings. It is a good colonizer of skin and can live for up to four weeks on surfaces. Gloves and gowns should be worn when working with C. auris, and work in a hood or a biological safety cabinet is recommended to avoid laboratory contamination. As quaternary ammonium compounds may not be effective, 10% bleach should be used for cleaning the work area.

https://wwwn.cdc.gov/ARIsolateBank/Panel/AllIsolate

When the specific identification has implications for antimicrobial susceptibility testing

Staphylococcus intermedius group

- Member of oral, nasal, and skin microbiota in healthy dogs
- "The Staphylococcus aureus of dogs and cats"
  - Also pigeons, minks, horses, raccoons, goats
- The leading cause of skin and soft tissue infections in dogs (canine pyoderma)
  - Can also cause invasive disease
  - Colonization is a risk factor for infection


Staphylococcus intermedius group

- First report of human infection not associated with an animal bite in 1994
- Very little in the literature since then (until recently)
- True incidence of human infection is unknown because it has historically been misidentified as S. aureus


Human Clinical Microbiology Laboratory

Veterinary Clinical Microbiology Laboratory

Staphylococcus aureus

Staphylococcus intermedius group

Increasing Oxacillin Resistance in Isolates from Dogs

- Like S. aureus, attributed to meca
- On SCCmec (IV and V most common)

Multi-Center Study—AST for *S. intermedius* group

- Emory, UCLA, Washington University, Texas A&M College of Veterinary Medicine
- 115 isolates
- 45 isolates from human infections: 4 (9%) *mecA* positive
- 70 veterinary isolates: 33 (52%) *mecA* positive


Multi-Center Study—AST for *S. intermedius* group

- Cefoxitin was not at all predictive for *mecA*
  - 78% Very Major Errors (VME)
    - Call susceptible when really resistant
- Oxacillin disk diffusion or broth microdilution correlated with *mecA* status
  - No very major errors
  - 1 (1%) major error
    - Call resistant when really susceptible


Prediction of Methicillin Resistance

<table>
<thead>
<tr>
<th>Staphylococcus aureus</th>
<th>Staphylococcus intermedius group</th>
</tr>
</thead>
</table>


SIG--Commercial Automated AST Systems

<table>
<thead>
<tr>
<th>System/Panel</th>
<th><em>S. aureus</em>/<em>S. lugdunensis</em> oxacillin breakpoint</th>
<th><em>S. intermedius</em> group oxacillin breakpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA (%)</td>
<td>No. (%) VME</td>
</tr>
<tr>
<td>BD Phoenix PMIC-8</td>
<td>90.4</td>
<td>11 (30)</td>
</tr>
<tr>
<td>bioMerieux Vitek2 AST GP71</td>
<td>93.0</td>
<td>8 (22)</td>
</tr>
<tr>
<td>Beckman Coulter MicroScan Pos MIC 29</td>
<td>95.7</td>
<td>5 (14)</td>
</tr>
</tbody>
</table>


Susceptibility Testing

- Jan 2016—CLSI M100—specific testing guidelines for *S. intermedius* group (SIG)
- Even though *S. aureus* and SIG share a high degree of phenotypic and genetic similarity, methods for detection of methicillin resistance are different
- Demonstrates the importance of species specific breakpoints for some organism/antimicrobial combinations

Detection of Methicillin Resistance in *Staphylococcus* spp.
Emerging Staphylococcal Species

- *Staphylococcus aureus*
  - Complex with 3 members
  - Staphylococcus argenteus
  - Predominantly human associated
  - Staphylococcus schweitzeri
  - Predominantly associated with wildlife
- *Staphylococcus argenteus* and *S. schweitzeri* in some MALDI databases
- All complex members can carry meca
- Misinterpretation of microbiology reports could have important patient care consequences
- Stay tuned!


Taxonomy and the CAP Microbiology Checklist

**MIC: 11375**

**Taxonomy Changes**

The laboratory incorporates taxonomic changes that potentially affect the choice of appropriate antimicrobials to report and for the interpretation breakpoints to use.

NOTE: The genus and/or species names of microorganisms may change as new methods are applied to their taxonomy. This can impact the antimicrobials that should be reported for that organism. It may also impact which breakpoints are used for reporting. For example, *Aeromonas hydrophila* was moved to the genus *Hyphomicrobiun* in 1993 and then to the new genus *Aggregatibacter* in 2006. The antimicrobials for *Hyphomicrobiun* species (CLSI M100, Table 3E) versus *Aggregatibacter* species (CLSI M100, Table 7). The laboratory should have a policy ensuring that clinically relevant taxonomic changes are incorporated into reporting patient and proficiency testing results even when commercial identification systems have not been updated.

CAP Accreditation Program Microbiology Checklist August 2017

Clinical Case #2: A wolf in sheep’s clothing

- 8 year old boy
- Presented to emergency room in Connecticut with fever, nausea, vomiting, body aches
- Traveled to Egypt with his family 3 months prior
- Blood culture was collected
  - Aerobic bottle: Coagulase-negative *Staphylococcus*
  - Anaerobic bottle: *Bacillus* species, not *Bacillus anthracis*
- One day later, another blood culture set was collected

Poonawala et al. 2018. J Clin Microbiol. 6: e00914-17

Biosafety

Second blood culture set...

- Aerobic bottle was positive after ~60 hours of incubation
- Small Gram-negative rods
- Growth on chocolate and blood agar after 18 h of incubation (no growth on MacConkey agar)
- Oxidase positive
- 99.9% *Ochrobactrum anthropi*
  - VITEK MS, FDA-cleared IVD database, “claimed” organism

Poonawala et al. 2018. J Clin Microbiol. 6: e00914-17

Thoughts?

- Consider all of the positive blood culture results contaminants?
- Perform additional testing?
- Collect more blood cultures?
- Treat the patient for *Ochrobactrum anthropi*?
- Other ideas?
Case #2 continued:

- The ID and GI teams considered all of the cultures to be contaminants
- Patient was discharged home
- Patient was re-admitted 3 days later with fever, fatigue, abdominal discomfort and diarrhea
  - Given empiric trimethoprim-sulfamethoxazole
- He was readmitted 4 days later with persistent fever, abdominal pain, nausea, vomiting, and diarrhea
  - MRI revealed lesions in skeleton, hepatosplenomegaly

Case #2 continued...

- Additional blood cultures were sent
- A Brucella serology from the first visit was resulted (IgG 1:1,280)
- Parents of child disclose exposure to sheep and consumption of unpasteurized milk while in Egypt
- After 4 days of incubation, blood cultures positive with Gram-negative coccobacilli
  - Sent to state laboratory; Brucella melitensis
- Re-testing of isolate reported as O. anthropi by state laboratory: Brucella melitensis

Case #3: Vacation Souvenir

- 65 year old man who suffered a myocardial infarction while on vacation in Thailand
  - Hospitalized for 7 days before returning to USA
  - Symptoms of UTI about 1 week later; prescribed nitrofurantoin
  - Symptoms did not improve; urine specimen was sent for culture
  - After 24 hours of incubation
    - Small colonies on blood agar plate, no growth on MacConkey
  - After 48 hours of incubation
    - Pure growth of a small gray colony; growing well on both blood agar and MacConkey agar
    - Oxidase positive

Case #3 continued

- MALDI-TOF MS (MALDI Biotyper):
  - Burkholderia thailandensis (Score 1.8)
- Thoughts?
  - Report as Burkholderia species?
  - Perform additional testing?
  - Refer to public health lab?

Case #3 continued

- Isolate referred to public health lab
  - Identified as Burkholderia pseudomallei
- 21 laboratory employees were exposed

MALDI-TOF MS and Potential BT Agents

- Select agents are absent from or scant in most of the MALDI-TOF MS databases
  - Each laboratory needs to understand what is (and is not) in the database
- Robust literature and guidance for biochemical testing, much less for MALDI-TOF MS
- May get no identification
  - Francisella
  - Brucella
- May get incorrect identification (usually highly related organism)
  - Burkholderia thailandensis
  - Bacillus cereus
  - Ochrobactrum anthropi
MALDI-TOF MS and Potential BT Agents

- Laboratory needs clear rule in/rule out procedures
- Gram negative coccobacilli that do not grow on MacConkey agar
- Microorganisms that do not identify
- Maintain a list of potential misidentifications that should raise alarm for potential select agents


When MALDI gets it wrong....

Clinical Case #4: Imposter Syndrome

- 8 year old girl
- History of interstitial lung disease and pulmonary hypertension being evaluated for lung transplantation
- As part of her evaluation, a tracheal aspirate specimen is submitted for culture
- Direct specimen Gram stain:
  - Rare polymorphonuclear leukocytes
  - No squamous epithelial cells
  - Rare Gram Negative Coccobacilli

Growth in Culture

- Growth on blood agar and chocolate agar
- No growth on MacConkey agar
- Isolate was submitted for MALDI-TOF MS

Results from MALDI-TOF MS

Result Overview

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Organism (best match)</th>
<th>Score Value</th>
<th>Organism (second-best match)</th>
<th>Score Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT (+++) (A)</td>
<td>BTS (BTS)</td>
<td>Enterobacteriaceae</td>
<td>2.01</td>
<td>Enterobacteriaceae</td>
<td>2.11</td>
</tr>
<tr>
<td>BT (++) (A)</td>
<td>6392 (control)</td>
<td>No Organism Identification Possible</td>
<td>1.95</td>
<td>No Organism Identification Possible</td>
<td>1.92</td>
</tr>
<tr>
<td>BT (+++) (A)</td>
<td>NM2 (mock)</td>
<td>Neisseria meningitidis</td>
<td>2.10</td>
<td>Neisseria meningitidis</td>
<td>2.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rank (Quality)</th>
<th>Matched Pattern</th>
<th>Score Value</th>
<th>Match IDentifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (++)</td>
<td>Neisseria meningitidis / CCAG-0003 / CCAG</td>
<td>2.19</td>
<td>A211</td>
</tr>
<tr>
<td>2 (++)</td>
<td>Neisseria meningitidis / CCAG-0004 / CCAG</td>
<td>2.03</td>
<td>A211</td>
</tr>
<tr>
<td>3 (++)</td>
<td>Neisseria meningitidis / CCAG-0005 / CCAG</td>
<td>2.11</td>
<td>A211</td>
</tr>
<tr>
<td>4 (++)</td>
<td>Neisseria meningitidis / CCAG-0006 / CCAG</td>
<td>2.11</td>
<td>A211</td>
</tr>
<tr>
<td>5 (++)</td>
<td>Neisseria meningitidis / CCAG-0007 / CCAG</td>
<td>2.11</td>
<td>A211</td>
</tr>
<tr>
<td>6 (++)</td>
<td>Neisseria meningitidis / CCAG-0008 / CCAG</td>
<td>2.11</td>
<td>A211</td>
</tr>
<tr>
<td>7 (++)</td>
<td>Neisseria meningitidis / CCAG-0009 / CCAG</td>
<td>2.11</td>
<td>A211</td>
</tr>
<tr>
<td>8 (++)</td>
<td>Neisseria meningitidis / CCAG-0010 / CCAG</td>
<td>2.11</td>
<td>A211</td>
</tr>
<tr>
<td>9 (++)</td>
<td>Neisseria meningitidis / CCAG-0011 / CCAG</td>
<td>2.11</td>
<td>A211</td>
</tr>
</tbody>
</table>
What should we do next?

- Report as Neisseria meningitidis?
  - Send to state public health laboratory
  - Call infection prevention
- Report as normal upper respiratory flora?
- Perform additional testing?
- Something else?

Growth on: Blood Agar

Growth on: Chocolate Agar

Isolate was ultimately identified as *N. polysaccharea*

Neisseria meningitidis

- Utilizes glucose and maltose
- 13 serotypes
  - Most common serogroups: A, B, C, Y, W135
  - US: Most disease B, C, Y
  - Serogroup W and nongroupable strains—small portion of disease
- Up to 30% of people are asymptptomatically colonized in respiratory tract
- Transmission via respiratory droplets
- Outbreaks can occur in crowded conditions
  - Military
  - College dormitories

Meningitis, blood stream infection
- ~10-15% of infected individuals will die, even with treatment
- ~20% of survivors will have long-term complications (such as loss of limb(s), deafness, nervous system problems, brain damage)

- Pneumonia/isolated respiratory infection very rare

[https://www.cdc.gov/meningococcal/about/diagnosis-treatment.html](https://www.cdc.gov/meningococcal/about/diagnosis-treatment.html)


*Neisseria meningitidis*

- Utilizes glucose and maltose
- 13 serotypes
  - Most common serogroups: A, B, C, Y, W135
  - US: Most disease B, C, Y
  - Serogroup W and nongroupable strains—small portion of disease
- Up to 30% of people are asymptptomatically colonized in respiratory tract
- Transmission via respiratory droplets
- Outbreaks can occur in crowded conditions
  - Military
  - College dormitories
Neisseria polysaccharea

- Described in 1983
- Utilizes glucose, maltose
- Produces polysaccharide from sucrose
- Stains dark blue-purple to black with iodine
- Previously (mis)identified as nontypable strains of N. meningitidis
- Not pathogenic

https://www.cdc.gov/std/gonorrhea/lab/npol.htm

Neisseria spp. and MALDI-TOF MS

- MALDI-TOF MS does not always accurately identify Neisseria species
- Commonly misidentified:
  - N. cinerea, N. polysaccharea, N. meningitidis, N. subflava, N. mucosa...
  - N. polysaccharea frequently misidentified as N. meningitidis
- Result can be a cascade of unnecessary actions
  - Public health, patient care, management of exposure for laboratory personnel and close contacts of the patient

https://www.cdc.gov/std/gonorrhea/lab/tests/polysac.htm


### Differentiation of N. meningitidis and N. polysaccharea

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Neisseria meningitidis</th>
<th>Neisseria polysaccharea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>Gram-negative diplococcus</td>
<td>Gram-negative diplococcus</td>
</tr>
<tr>
<td>Oxidase</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Acid production from glucose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Acid production from maltose</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Polysaccharide from sucrose test</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Nitrate reduction test</td>
<td>Nitrate-negative</td>
<td>Nitrate-negative</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Non-pigmented</td>
<td>Non-pigmented</td>
</tr>
<tr>
<td>Colistin</td>
<td>Resistant</td>
<td>Usually susceptible</td>
</tr>
</tbody>
</table>


Neisseria spp. and MALDI-TOF MS

- MALDI-TOF MS does not always accurately identify Neisseria species
- Commonly misidentified:
  - N. cinerea, N. polysaccharea, N. meningitidis, N. subflava, N. mucosa...
  - N. polysaccharea frequently misidentified as N. meningitidis
- Result can be a cascade of unnecessary actions
  - Public health, patient care, management of exposure for laboratory personnel and close contacts of the patient
  - Laboratory safety—caution needed when working with any suspected Neisseria strain
- Misidentification of N. gonorrhoeae less common but does occur


### CDC/FDA Strain Bank

Neisseria species MALDI-TOF Verification (30)

This panel contains a representative number of Neisseria species including 6 N. gonorrhoeae, 5 N. meningitidis, 17 other Neisseria species, 1 Kingella denitrificans, and 2 Moraxella catarrhalis. This will allow PHLS to have access to a rare collection of commercial Neisseria species for identification verification purposes.

This panel can be supplemented with additional Neisseria gonorrhoeae strains:

[https://www.cdc.gov/AtRiskBank/Panel/PanelDetail?ID=1158](https://www.cdc.gov/AtRiskBank/Panel/PanelDetail?ID=1158)

[https://www.cdc.gov/AtRiskBank/Panel/AtRiskBank](https://www.cdc.gov/AtRiskBank/Panel/AtRiskBank)

### Limitations Associated with MALDI-TOF MS

<table>
<thead>
<tr>
<th>Organism/Culture</th>
<th>MalDia-TOF MS Limitation/Pitfall</th>
<th>Possible Approach to Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli and Shigella spp.</td>
<td>MALDI-TOF MS cannot resolve these organisms</td>
<td>WISH with specimen type and local epidemiology, Supplemental biochemical testing.</td>
</tr>
<tr>
<td>Streptococcus pneumoniae, Streptococcus mitis group</td>
<td>Highly similar, MALDI-TOF MS may misidentify</td>
<td>Be aware of manufacturer specific claims, Supplemental testing (optochin, bile solubility).</td>
</tr>
<tr>
<td>Achromobacter spp.</td>
<td>Difficultly resolving to species level</td>
<td>Consider reporting to genus level, if appropriate. Additional biochemical and/or molecular testing if needed.</td>
</tr>
<tr>
<td>Enterobacter cloacae complex</td>
<td>Large complex of closely related species, specific clinical significance and accuracy of identification complex not well defined</td>
<td>Consider reporting as Enterobacter cloacae complex.</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>May misidentify N. cinerea and N. polysaccharea</td>
<td>Supplemental testing as needed.</td>
</tr>
</tbody>
</table>

Adapted from CLSI M35 1st ed. 2017.
Reporting Considerations

- Level of resolution for reporting
  - Single positive blood cultures with coagulase negative staphylococci, coryneforms, etc.
  - "Group B Streptococcus" vs. *Streptococcus agalactiae*
  - Unusual identifications
  - Group/complex level identifications

Examples of Microorganisms Reported to Species or Subspecies

<table>
<thead>
<tr>
<th>Organism</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus gallolyticus subsp. gallolyticus</em></td>
<td>Established association with gastrointestinal malignancies</td>
</tr>
<tr>
<td><em>Streptococcus anginosus</em> (group <em>Streptococcus anginosus</em>)</td>
<td>Associated with nasal cavities when found in urine specimens</td>
</tr>
<tr>
<td><em>Corynebacterium ulcerans</em></td>
<td>Pathogenic potential</td>
</tr>
<tr>
<td><em>Corynebacterium diphtheriae</em></td>
<td>Public health importance; pathogenic potential</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Established association with gastrointestinal malignancies</td>
</tr>
<tr>
<td><em>Staphylococcus lugdunensis</em></td>
<td>Pathogenic potential</td>
</tr>
<tr>
<td><em>Staphylococcus pseudintermedius</em></td>
<td>Pathogenic potential</td>
</tr>
</tbody>
</table>

"Trusted List"

Ongoing Microbiology Expertise: Strategies

Biochemical of the week

- Select a biochemical, demo, explain principle, prepare a 1 page handout
- 2 to 5 minute presentation/discussion
- Include a picture of the reaction or results
- Create a library of the documents

Catalase Test

Purpose: To differentiate between genera of many Gram-positive bacteria.

Principle: This test detects the presence of catalase in bacterial cells. Catalase decomposes hydrogen peroxide, a byproduct of aerobic carbohydrate metabolism, into oxygen and water.

\[
2H_2O_2 \rightarrow 2H_2O + O_2
\]

Procedure:

1. Place a small amount of a bacterial colony (18–24 hour aerobe; 24–72 hour anaerobe) onto a glass slide. Add a drop of H2O2 onto the organism and watch for bubble formation.

Aerobic bacteria: 3% H2O2
Anaerobic bacteria: 15% H2O2

Interpretation:

Positive Reaction: Immediate production of bubbles
Negative Reaction: No bubbles or few bubbles after 20–30 seconds

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Catalase Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus spp.</td>
<td>Positive</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>Negative</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>Positive</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>Positive</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>Positive</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Positive</td>
</tr>
<tr>
<td>Erysipelothrix spp.</td>
<td>Positive</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>Positive</td>
</tr>
<tr>
<td>Actinomyces</td>
<td>Positive</td>
</tr>
<tr>
<td>Arcanobacterium haemolyticum</td>
<td>Positive</td>
</tr>
<tr>
<td>Arcanobacterium pyogenes</td>
<td>Positive</td>
</tr>
<tr>
<td>Propionibacterium</td>
<td>Positive</td>
</tr>
<tr>
<td>Actinomyces</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Limitations:

False positive reactions may be caused by carry over from colonies picked on blood agar or using wire loops.
False negative reactions may occur with older growth (>24 hours) or from colonies picked from subculture subculturing media.
Lipase and Lecithinase

Lipase

Bacterial lipases hydrolyze the breakdown of triglycerides into glycerol and free fatty acids. Fatty acids are insoluble for the most part, and their presence causes opacity on egg yolk agar. This can be visualized by an iridescent sheen on lipase producing bacterial colonies grown on egg yolk agar. Lipase is not diffusible and the reaction occurs only on the surface of the agar in the immediate vicinity of the colony.

Lecithinase

Lecithinases are enzymes released by certain bacteria that aid in the destruction of tissue. Lecithin is a normal component of egg yolks. When grown on egg yolk agar, lecithinase producing bacteria split lecithovitellin (saline extract of egg yolks) into phosphorylcholine + insoluble diglyceride. Insoluble diglyceride forms a precipitate in the medium which appears as a white, opaque halo. Lecithinase is diffusible in agar causing the opaque, white color to extend beyond the boundaries of the lecithinase producing bacterial colony or streak.

Pocket Bugs

Organism:
Gram-stain:
Key rapid biochemicals (eg catalase for Gram pos, oxidase for Gram neg):
Growth medium/incubation conditions/atmosphere:
Colonial morphology:
Key susceptibility traits:
Clinical significance:
Other (if needed):

Tell us about the microorganism in 20 words or less!

Microbiology expertise is essential!

- MALDI-TOF MS has revolutionized the practice of clinical microbiology
- Informing the clinical significance/biology of microorganisms
- Ongoing microbiology expertise is essential!
- Correlating MALDI-TOF MS with colony morphology, Gram stain, etc.
- Recognizing and reporting “new” microorganisms
- Communicating the significance of these “new” microorganisms to the healthcare team
- Recognizing AST implications
- Recognizing and responding when MALDI-TOF gets it wrong
- Biosafety

Thank you to the Barnes-Jewish Hospital Microbiology Team!!