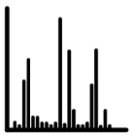
Thinking Outside of the Box: The Ongoing Need for Microbiology Expertise in the Era of MALDI-TOF MS



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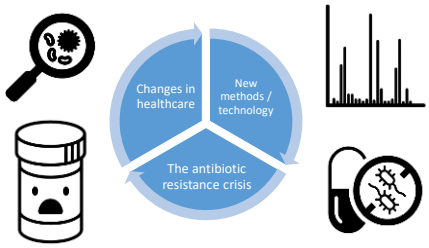
  - BD, Accelerate Diagnostics, Medavera, Bruker Daltonics

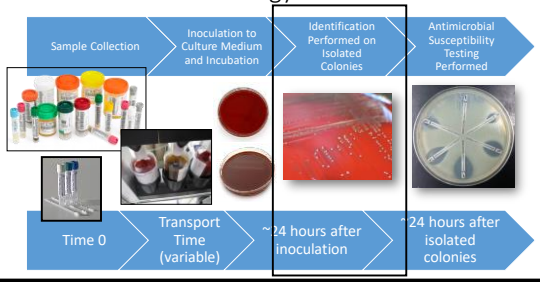
Learning Objectives

- 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



Traditional Identification

MALDI-TOF MS Timeline

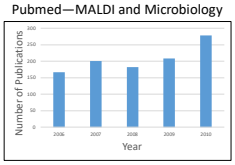
2010: ECCMID

MALDI-TOF MS for microorganism identification
- M-Matrix
- A-Assisted
- L-Laser
- D-Desorption
- I-Ionization

- T-Time
- O-Of
- F-Flight

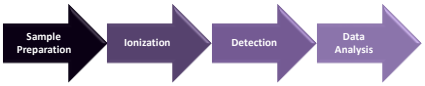
- M-Mass
- S-Spectrometry

Pubmed—MALDI and Microbiology

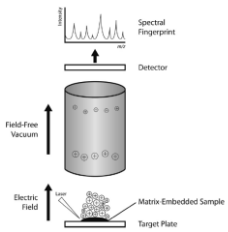


MALDI-TOF MS – Overview

- 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



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

Dingle and Butler-Wu. Clin Lab Med 2013. 33: 589-609.

Commercially Available Systems

Bruker MALDI Biotyper

bioMérieux VITEK MS

MALDI-TOF MS Timeline

2010: ECCMID

2011: MALDI-TOF MS in our Laboratory

CAP Proficiency Testing (DEX)—<1% labs using MALDI-TOF for identification

JCM

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Ior

Eur J Clin Microbiol Infect Dis (2014) 33:1703–1712
DOI 10.1007/s10096-014-2115-x

ARTICLE

Comparison and optimization of two MALDI-TOF MS platforms for the identification of medically relevant yeast species

M. A. Pence · E. McElvania TeKippe · M. A. Wallace · C.-A. D. Burnham

Yen-Michael S. Hsu [1], Carey-Ann D. Burnham *

Ford and Burnham. 2013. J Clin Microbiol. 51: 1412-1420.
McElvania TeKippe E et al. J Clin Microbiol. 2013. 51: 1421-1427.
Hsu and Burnham. 2014. Diag Micro Infect Dis. 79: 144-148. Pence et al. 2013. Eur J Clin Microbiol Infect Dis. 33: 17703-1712.

"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**!)

Erin McElvania

McElvania TeKippe and Burnham. 2014. Eur J Clin Microbiol Infect Dis. 33:2163-2171.

JCM

Prospective Evaluation of a Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System in a Hospital Clinical Microbiology Laboratory for Identification of Bacteria and Yeasts: a Bench-by-Bench Study for Assessing the Impact on Time to Identification and Cost-Effectiveness

K. E. Tan,[a] B. C. Ellis,[b] R. Lee,[b] P. D. Stamper,[a] S. X. Zhang,[a,b] and K. C. Carroll[a,b]
Division of Medical Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA,[a] and Microbiology Laboratory, Johns Hopkins Hospital, Baltimore, Maryland, USA[b]

- 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

Tan et al. J Clin Microbiol 2012. 50: 3301-3308.

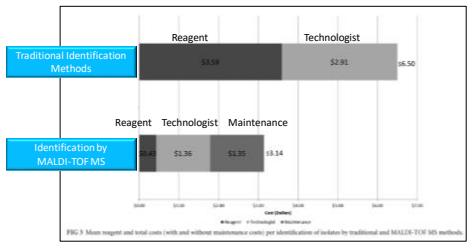
Cost Savings Realized by Implementation of Routine Microbiological Identification by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Anthony Tran,[a,c] Kevin Alby,[a,c] Alan Kerr,[a] Melissa Jones,[a] Peter H. Gilligan[a,b]
Clinical Microbiology-Immunology Laboratories, University of North Carolina Health Care,[a] and Departments of Pathology-Laboratory Medicine and Microbiology-Immunology,[b] Chapel Hill, North Carolina, USA

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

Tran et al. J Clin Microbiol 2015. 53: 2473-2479.

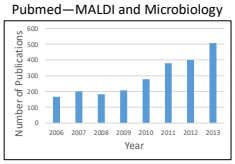
MALDI-TOF vs Traditional: Cost Comparison



Tran et al. J Clin Microbiol 2015. 53: 2473-2479.

MALDI-TOF MS Timeline

- 2010: ECCMID
- 2011: MALDI-TOF MS in our Laboratory
- 2013: Ongoing optimization MALDI-TOF in routine clinical use
- CAP Proficiency Testing (DEX)—<1% labs using MALDI-TOF for identification
- CAP Proficiency Testing (DEX)—5% labs using MALDI-TOF for identification

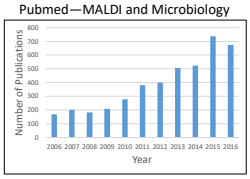


MALDI-TOF MS Timeline

- 2010: ECCMID
- 2011: MALDI-TOF MS in our Laboratory
- 2013: Ongoing optimization of MALDI-TOF in routine clinical use
- 2016: Expanded databases, Used more widely in clinical settings
- CAP Proficiency Testing (DEX)—30% labs using MALDI-TOF for identification

MALDI-TOF MS Timeline

Pubmed—MALDI and Microbiology



2013: Ongoing optimization of MALDI-TOF in routine clinical use

2016: Expanded databases, Used more widely in clinical settings

CAP Proficiency Testing (DEX)—30% labs using MALDI-TOF for identification

CAP Requirements for MALDI-TOF MS

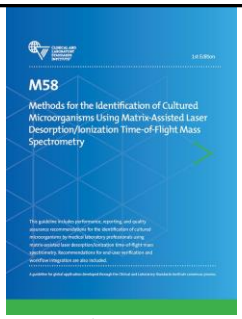
MATRIX-ASSISTED LASER DESORPTION IONIZATION TIME-OF-FLIGHT (MALDI-TOF) MASS SPECTROMETRY

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.

MIC.16575	**Instrument Operation**	**Phase II**
	There are written procedures for the operation and calibration of the mass spectrometer.	
MIC.16595	**Mass Spectrometer Calibration**	**Phase II**

CAP Accreditation Program Microbiology Checklist August 2017

CLSI M58 Document: MALDI-TOF MS for Microbial Identification



CLSI M58. 2017. *Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry*

MALDI-TOF MS Timeline

2010: ECCMID

2011: MALDI-TOF MS in our Laboratory

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)—30% labs using MALDI-TOF for identification

CAP Proficiency Testing (DEX)—60% labs using MALDI-TOF for identification

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

Old name/new name (when getting it right has clinical implications)

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

Conventional Phenotype (91 Months)	MALDI-TOF (40 Months)
44 species identified annually	112 species identified annually
19 species/10,000 isolates	36 species/10,000 isolates

Seng et al. Journal Clin Microbiol 2013. 51: 2182-2194.

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

Johnson et al. 2016. J Clin Microbiol. 54: 1938-1941.

"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

Bernard et al. 2012. J Clin Micro. 50: 3152-3158. Leal et al. 2016. J Clin Micro. 54: 2928-2936. Johnson et al. 2016. J Clin Micro. 54: 1938-1941. McMullen et al. 2017. AAC. 61(11). pii: e01111-17.

Clinically Important *Corynebacterium* spp.

Species	Disease Association
Corynebacterium diphtheriae, C. ulcerans, C. pseudotuberculosis	Diphtheria
Corynebacterium urealyticum	Kidney stones (urease activity)
Corynebacterium jeikeium	Nosocomial infection, line infection, multi-drug resistance
Corynebacterium macginleyi	Conjunctivitis
Corynebacterium kroppenstedtii	Granulomatous mastitis
Corynebacterium striatum	Device infection, blood stream infection, multi-drug resistant
Corynebacterium pseudodiphtheriticum	Pneumonia
Turicella otitidis and *Corynebacterium auris*	Otitis media

Bernard et al. 2012. J Clin Micro. 50: 3152-3158. Leal et al. 2016. J Clin Micro. 54: 2928-2936. Johnson et al. 2016. J Clin Micro. 54: 1938-1941. McMullen et al. 2017. AAC. 61(11). pii: e01111-17.

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

Candida auris (also called *C. auris*) is a fungus that causes serious infections. Patients with *C. auris* infection, their family members and other close contacts, public health officials, laboratory staff, and healthcare workers can all help stop it from spreading.

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

https://www.cdc.gov/fungal/diseases/candidiasis/pdf/Candida_auris_508.pdf

Global Emergence of *Candida auris*

- **2009:** First described in from the ear of a patient in Japan. Reported from 15 patients with otitis media in South Korea
- **2011:** Blood stream infection in South Korea. Subsequent outbreaks in India, South Africa, Kuwait
- **2012-2013:** Outbreaks in South America
- **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

Jeffery-Smith et al. 2018. Clin Micro Revs. 31: e00029-17. Spivak et al. 2017. J Clin Microbiol. 56. 2: e01588-17.
Lockhart et al. 2017. Clin Micro Newsletter. 39-99-103. Chowdhary et al. 2017. PLOS Pathogens.

DEADLY GERMS, LOST CURES

A Mysterious Infection, Spanning the Globe in a Climate of Secrecy

The rise of Candida auris embodies a serious and growing public health threat: drug-resistant germs.

What You Need to Know About Candida Auris

C. auris is a mysterious and dangerous fungal infection that is among a growing number of germs that have evolved defenses against common medicines. Here are some basic facts about it.

April 6, 2019

https://www.nytimes.com/2019/04/06/health/drug-resistant-candida-auris.html

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**

https://www.cdc.gov/fungal/diseases/candidiasis/pdf/Candida_auris_508.pdf
Lockhart et al. 2017. Clin Micro Newsletter. 39-99-103.
Spivak et al. 2017. J Clin Microbiol. 56. 2: e01588-17.

Identification of *Candida auris*

Methodology	Likely to be identified as…..
All methods	***Candida haemulonii***, *Candida* spp. not otherwise identified
API 20C	***Rhodotorula glutinis***, *Candida sake*, Unidentified
API Candida	*Candida famata*
BD Phoenix	***Candida haemulonii***, *Candida catenulata*
bioMerieux VITEK 2 YST	***Candida haemulonii***, *Candida duobushaemulonii*, *Candida lusitaniae*, *Candida famata*, ***Saccharomyces cerevisiae***
MicroScan	*Candida famata*, *Candida lusitaniae*, *Candida guilliermondii*, *Candida parapsilosis*, *Candida albicans*, *Candida tropicalis*, *Candida catenulata*
Sequencing (28S D1/D2 or ITS)	*Candida auris*
MALDI-TOF MS	*Candida auris* Not identified

Mizusawa et al. 2017. J Clin Microbiol. 55: 638-640. Jeffery-Smith et al. 2018. Clin Micro Revs. 31: e00029-17.
Spivak et al. 2017. J Clin Microbiol. 56. 2: e01588-17. Bao et al. 2018. J Clin Microbiol. 4: e01700-17.
Ambaraghassi et al. 2019. J Clin Microbiol. PMID: 31413079.

CDC/FDA AR Isolate Bank Can Support Laboratory Validation Efforts

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

Candida auris isolate panel

Candida auris (21)

A panel of Candida auris isolates and other yeast species that are related to C. auris or 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 fomites. 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 ammonia 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

Lainhart et al. 2018. J Clin Microbiol. 56: e00839-17.

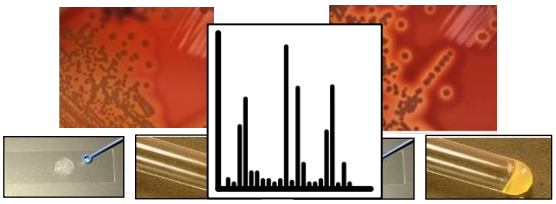
Staphylococcus intermedius group

- 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*

Lainhart et al. 2018. J Clin Microbiol. 56: e00839-17.
Yarbrough et al. 2018. J Clin Microbiol. 56: pii: e01788-17.

Human Clinical Microbiology Laboratory

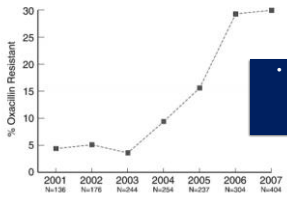
Veterinary Clinical Microbiology Laboratory



Staphylococcus aureus

Staphylococcus intermedius group

Increasing Oxacillin Resistance in Isolates from Dogs



- Like *S. aureus*, attributed to *mecA*
  - On SCC*mec* (IV and V most common)

Figure 2. Frequencies of oxacillin resistance among *Staphylococcus pseudintermedius* isolates from dogs from 2001 to 2007. Disk diffusion method; resistant: ≤17 mm, susceptible: ≥18 mm.

Bemis et al. 2009. J Vet Diagn Invest. 21: 53-58. Knoxville, TN

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

Lars Westblade

Wu et al. 2016. J Clin Microbiol. 54:535-542.

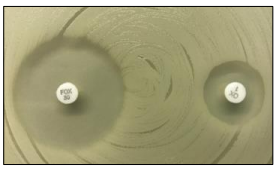
Multi-Center Study—AST for *S. intermedius* group

- **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

Wu et al. 2016. J Clin Microbiol. 54:535-542.

Prediction of Methicillin Resistance

Staphylococcus aureus	*Staphylococcus intermedius group*



Lainhart et al. 2018. J Clin Microbiol. 56: e00839-17.

SIG--Commercial Automated AST Systems

System/Panel	*S. aureus/S. lugdunensis* oxacillin breakpoint		*S. intermedius* group oxacillin breakpoint	
	CA (%)	No. (%) VME	CA (%)	No. (%) VME
BD Phoenix PMIC-8	90.4	11 (30)	95.7	4 (11)
bioMerieux Vitek2 AST-GP71	93.0	8 (22)	98.3	1 (3)
Beckman Coulter MicroScan Pos MIC 29	95.7	5 (14)	99.1	0 (0)

0/37 *mecA* positive isolates were cefoxitin resistant on ANY of the commercial systems

CA-Category Agreement
VME-Very Major Error (categorized as susceptible when resistant)

Wu et al. 2016. J Clin Microbiol. 54:535-542.

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.

Detection of oxacillin resistance in staphylococci is achieved by using specific methods as listed in Table 2C and further described in Table 3E.

Organism	Acceptable Methods				
	Cefoxitin MIC	Cefoxitin disk diffusion	Oxacillin MIC	Oxacillin disk diffusion	Oxacillin salt agar
S. aureus	Yes	Yes	Yes	No	Yes
S. lugdunensis	Yes	Yes	Yes	No	No
S. epidermidis	**No**	**Yes**	**Yes**	**Yes**	**No**
S. pseudintermedius	No	No	Yes	Yes	No
S. schleiferi	No	No	Yes	Yes	No
Other *Staphylococcus* spp. (not listed above)	No	Yes	Yes[1]	No	No

[1] For **other *Staphylococcus* spp.** with oxacillin MICs between 0.5–2 µg/mL, see comment (17) for recommendations on testing for *mecA* or for PBP2a.

CLSI M100-S29. 2019. Table 2C *Staphylococcus*.

Emerging Staphylococcal Species

Staphylococcus argenteus

- *Staphylococcus aureus*
  - Complex with 3 members
  - *Staphylococcus argenteus*
    - Southeast Asia, Australia, the Amazon
    - Predominantly human associated
  - *Staphylococcus schweitzeri*
    - Africa
    - Predominantly associated with wildlife
  - *S. 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!

Chantratita et al. 2016. Clin Microbiol Infect. 22: 458.e11-458.e19.
Becker et al. 2019. Clin Microbiol Infect. 25(9):1064-1070.

Taxonomy and the CAP Microbiology Checklist

MIC.11375 **Taxonomy Changes** **Phase I**

The laboratory incorporates taxonomic changes that potentially affect the choice of appropriate antimicrobials to report and/or the interpretative 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, Actinobacillus actinomycetemcomitans was moved to the genus Haemophilus in 1985 and then to the new genus Aggregatibacter in 2006. The antimicrobials differ for Haemophilus species (CLSI M100, Table 2E) versus Aggregatibacter species (CLSI M45, 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

Biosafety

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

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

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

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*

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

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

Dingle et al. 2014. J Clin Microbiol. 52:3490-3491.

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?

Dingle et al. 2014. J Clin Microbiol. 52:3490-3491.

Case #3 continued

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

Dingle et al. 2014. J Clin Microbiol. 52:3490-3491.

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*

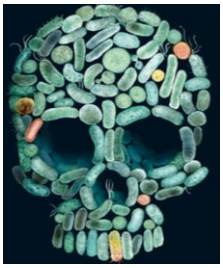
Cunningham et al. 2013. J Clin Microbiol. 51: 1639-1640.
Poonawala et al. 2018. J Clin Microbiol. 6: e00914-17. Dingle et al. 2014. J Clin Microbiol. 52:3490-3491.

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

Cunningham et al. 2013. J Clin Microbiol. 51: 1639-1640.
Poonawala et al. 2018. J Clin Microbiol. 6: e00914-17. Dingle et al. 2014. J Clin Microbiol. 52:3490-3491.

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

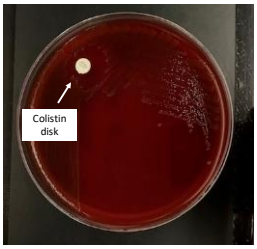
Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
B7 (+++) (A)	BTS (BTS)	Escherichia coli	2.31	Escherichia coli	2.31
B8 (-) (C)	6392 (standard)	**No Organism Identification Possible**	1.35	**No Organism Identification Possible**	1.27
B9 (+++) (A)	NM2 (standard)	Neisseria meningitidis	2.38	Neisseria meningitidis	2.35

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Neisseria meningitidis CCUG 63283 CCUG	2.38	487
2 (+++)	Neisseria meningitidis 24086406 MLD	2.35	487
3 (+++)	Neisseria meningitidis DSM 25942 DSM	2.31	487
4 (+++)	Neisseria meningitidis CCUG 8661 CCUG	2.31	487
5 (+++)	Neisseria meningitidis Serogroup W135 BRL	2.29	487
6 (+++)	Neisseria meningitidis DSM 15464 DSM	2.29	487
7 (+++)	Neisseria meningitidis Serogroup A BRL	2.28	487
8 (+++)	Neisseria meningitidis Serogroup X BRL	2.27	487
9	Neisseria meningitidis Serogroup Y BRL	2.27	487

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



Chocolate Agar

Growth on: Blood Agar

Chocolate Agar

Isolate was ultimately identified as *N. polysaccharea*

Neisseria meningitidis

- 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
Winstead et al. 2000. Clin Infect Dis. 30: 87-94.

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 **asymptomatically colonized** in respiratory tract
- Transmission via respiratory droplets
- Outbreaks can occur in crowded conditions
  - Military
  - College dormitories

College Outbreaks

Rutgers University – New Brunswick

Rutgers University – New Brunswick is experiencing an outbreak of serogroup B meningococcal disease. The New Jersey Department of Health and Rutgers University recommend vaccination for at-risk populations at Rutgers University – New Brunswick. Learn more from Rutgers University.

Columbia University

Columbia University's School of International and Public Affairs (SIPA) is experiencing an outbreak of serogroup B meningococcal disease. Columbia Health recommends vaccination for students enrolled in SIPA. Learn more from Columbia University.

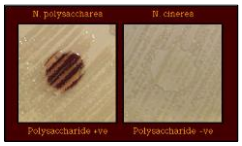
San Diego State University

San Diego State University (SDSU) is experiencing an outbreak of serogroup B meningococcal disease. San Diego County's public health officer recommends vaccination for all SDSU undergraduate students 23 years old and younger. Learn more from SDSU.

https://www.cdc.gov/meningococcal/index.html; October 2019

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

Cunningham et al. 2014. J Clin Microbiol. 52: 2270-2271. Deak et al. 2014. J Clin Microbiol. 52: 3496. Hong et al. 2019. Clin Micro Infect. 25: 717-722. Buchanan et al. 2016. Clin Micro Infect. 22: 815.e5-815.e7.

Differentiation of *N. meningitidis* and *N. polysaccharea*

Characteristic	*Neisseria meningitidis*	*Neisseria polysaccharea*
Gram stain	Gram-negative diplococcus	Gram-negative diplococcus
Oxidase	Positive	Positive
Acid production from glucose	Positive	Positive
Acid production from maltose	Positive	Positive
Polysaccharide from sucrose test	**Negative**	**Positive**
Nitrate reduction test	Nitrate negative	Nitrate negative
Pigmentation	Non-pigmented	Non-pigmented
Colistin	**Resistant**	**Usually susceptible**

Cunningham et al. 2014. J Clin Microbiol. 52: 2270-2271. Deak et al. 2014. J Clin Microbiol. 52: 3496.
https://www.cdc.gov/std/gonorrhea/lab/npol.htm

Neisseria spp. and MALDI-TOF MS

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- 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 reactions
    - 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

Cunningham et al. 2014. J Clin Microbiol. 52: 2270-2271. Deak et al. 2014. J Clin Microbiol. 52: 3496. Hong et al. 2019. Clin Micro Infect. 25: 717-722. Buchanan et al. 2016. Clin Micro Infect. 22: 815.e5-815.e7.

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 commensal Neisseria species for identification verification purposes.

This panel can be supplemented with additional *Neisseria gonorrhoeae strains :*
https://wwwn.cdc.gov/ARIsolateBank/Panel/PanelDetail?ID=1158

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

Limitations Associated with MALDI-TOF MS

Organism or Group	Limitation/Pitfall	Possible Approach to Resolution
E. coli and *Shigella* spp.	MALDI-TOF MS cannot resolve these organisms	Will vary by specimen type and local epidemiology. Supplemental biochemical testing.
Streptococcus pneumoniae vs. *Streptococcus mitis* group	Highly similar, MALDI-TOF MS may misidentify	Be aware of manufacturer specific claims Supplemental testing (optochin, bile solubility)
Achromobacter spp.	Difficulty resolving to species level	Consider reporting to genus-level, if appropriate Additional biochemical and/or molecular testing if needed
Enterobacter cloacae complex	Large complex of closely related species; specific clinical significance and/or accuracy of identification within the complex not well defined	Consider reporting as *Enterobacter cloacae* complex
Neisseria meningitidis	May misidentify *N. cinerea* and *N. polysaccharea*	Supplemental testing as needed

Adapted from CLSI M58 1st ed. 2017.

Reporting Considerations

- Level of resolution for reporting
  - Single positive blood cultures with coagulase negative staphylococci, coryneforms, etc.
    - More information is not always better
  - "Group B Streptococcus" vs. *Streptococcus agalactiae*
  - Unusual identifications
  - Group/complex level identifications

Examples of Microorganisms Reported to Species or Subspecies

Table 6. Examples of Microorganisms That Should Always Be Reported to the Species or Subspecies Level

Organism	Rationale
Streptococcus gallolyticus subsp. *gallolyticus*	Established association with gastrointestinal neoplasm
Trueperella pyogenes (Arcanobacterium pyogenes)	Pathogenic potential
Corynebacterium urealyticum from urine	Associated with renal calculi when found in urine specimens
Corynebacterium ulcerans	Pathogenic potential
Corynebacterium diphtheriae	Public health importance, pathogenic potential
Clostridium septicum from blood	Established association with gastrointestinal neoplasm
Staphylococcus lugdunensis	Pathogenic potential
Staphylococcus pseudintermedius	Pathogenic potential

CLSI M58 1st ed. 2017.

Reporting Considerations

- Level of resolution for reporting
  - Single positive blood cultures with coagulase negative staphylococci, coryneforms, etc.
    - More information is not always better
  - "Group B Streptococcus" vs. *Streptococcus agalactiae*
  - Unusual identifications
  - Group/complex level identifications
- "Trusted list"

"Trusted List"

Bruker MALDI-TOF Result:	Report:	Additional Notes	Cerner Code	Acceptable Score
Finegoldia magna	Finegoldia magna		FMAG	≥ 1.8
Flavobacterium lindanitolerans	Flavobacterium species		FLAV	≥ 2.0
Flavonifractor plautii	Flavonifractor species		FLAVON	≥ 1.8
Fusobacterium necrophorum	Fusobacterium necrophorum		FNEC	≥ 1.8
Fusobacterium gonidaformans	Fusobacterium gonidaformans		FGON	≥ 1.8
Fusobacterium mortiferum	Fusobacterium mortiferum		FMOR	≥ 1.8
Fusobacterium nucleatum	Fusobacterium nucleatum		FNUC	≥ 1.8
Gemella haemolysans	Gemella haemolysans		GHAE	≥ 1.8
Gemella morbillorum	Gemella morbillorum		GMOR	≥ 1.8
Gemella sanguinis	Gemella sanguinis		GSANG	≥ 1.8
Gemella spp. (other than species on the trusted list)	Gemella species		GEMI	≥ 1.8
Geotrichum capitatum	Geotrichum capitatum		GCAP	≥ 1.7
Gordonia spp.	Gordonia species		GORD	≥ 1.7
Granulicatella adiacens	Granulicatella adiacens		GRAD	≥ 1.8
Haemophilus influenzae	Haemophilus influenzae	**Send to the State Public Health Lab for serotyping if from an invasive site**	HINF	≥ 2.0
Lactobacillus acidophilus	Lactobacillus acidophilus		LACI	≥ 1.8
Lactobacillus casei	Lactobacillus casei		LCAS	≥ 1.8
Lactobacillus paracasei	Lactobacillus paracasei		LPAR	≥ 1.8
Lactobacillus rhamnosus	Lactobacillus rhamnosus		LRHA	≥ 1.8
Lactobacillus spp. (other than species on the trusted list)	Lactobacillus species		LACTO	≥ 1.8
Lactococcus garvieae	Lactococcus garvieae		LGAR	≥ 1.8
Lactococcus lactis	Lactococcus lactis		LLAC	≥ 1.8
Lautropia mirabilis	Lautropia species		LAUT	≥ 1.8
Leclercia adecarboxylata	Leclercia adecarboxylata		LADE	≥ 1.8
Legionella longbeachae	Legionella longbeachae	**FREEZE all isolates**	LLON	≥ 2.0
Legionella pneumophilia	Legionella pneumophilia	**FREEZE all isolates Send all isolates to the State Public Health Lab**	LEGI	≥ 2.0
Leuconostoc mesenteroides	Leuconostoc species		LEUC	≥ 1.8
Leuconostoc pseudomesenteroides	Leuconostoc species		LEUC	≥ 1.8

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 the enzyme catalase in bacteria. Catalase decomposes hydrogen peroxide, a byproduct of aerobic carbohydrate metabolism, into oxygen and water.

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

Procedures: Place a small amount of a bacterial colony (18-24 hour old aerobe; 24-72 hour old anaerobe) onto a glass slide. Add a drop of H_2O_2 onto the organism and watch for bubble formation.

Aerobic bacteria: 3% H_2O_2
Anaerobic bacteria: 15% H_2O_2

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

Limitations:

False positive reactions may be caused by carry-over from colonies picked on blood agar or using wire loops.

False negatives may occur with older growth (>24 hours) or from colonies picked from carbohydrate-containing media.

Positive	Negative
Micrococcus spp.	*Streptococcus spp.*
Staphylococcus spp.	
Bacillus spp.	*Clostridium spp.*
Listeria monocytogenes	*Erysipelothrix spp.*
Corynebacterium spp.	*Arcanobacterium haemolyticum*
	Arcanobacterium pyogenes
Propionibacterium	*Actinomyces*

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 lechithinase producing bacterial colony or streak.

Procedure:
Subculture isolate on egg yolk agar and incubate aerobically or anaerobically for 48-72 hours at 35-37°C.

	Lipase positive	Lipase negative
Differentiation of *Clostridium* spp.	*Clostridium sporanges* *Clostridium novyi A*	Other *Clostridium* spp.
Fusobacterium spp.	*F. necrophorum*	*F. nucleatum*

	Lecithinase positive	Lecithinase negative
Differentiation of spore-forming, Gram positive rods	*B. anthracis* and *B. cereus* group (*B. cereus, B. thuringiensis,* and *B. mycoides*)	Other *Bacillus* spp.
	C. perfringens *C. novyi A* *C. bifermentans* *C. sordellii*	Other *Clostridium* spp.

Pocket Bugs

Organism:

Gram-stain:

Key rapid biochemicals (eg catalase for Gram pos, oxidase for Gram neg):

Growth medium/incubation conditions/atmosphere:

Colony morphology:

Key susceptibility traits:

Clinical significance:

Other (if needed):

Tell us about the microorganism in 20 words or less!

MALDI-TOF MS Timeline

- **2010:** ECCMID
- **2011:** MALDI-TOF MS in our Laboratory
- **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
- **Future:** Resistance prediction; Improved strain resolution; Automation

Microbiology expertise is essential!

- MALDI-TOF MS has revolutionized the practice of clinical microbiology
  - Laboratory work up and work flow
  - 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!!


Neil Anderson, MD


Melanie Yarbrough, PhD

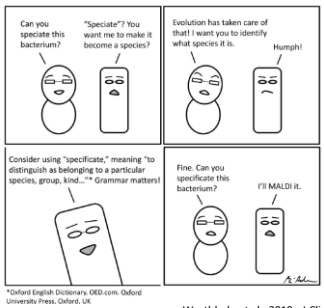

Crystal Squires


Kaitlin Mitchell, PhD


Eric Ransom, PhD


Meghan Wallace

Washington University in St. Louis



Westblade et al. 2019. J Clin Microbiol. 7: e00541-19.