Next Generation Diagnosis of Pulmonary Infections

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Outline

- MALDI-TOF
- Polymerase Chain Reaction (PCR) & Amplification Based Techniques
- Sequencing Based Techniques

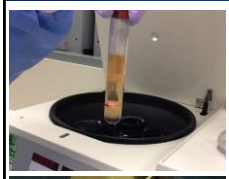
Matrix-assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry
(MALDI-TOF)

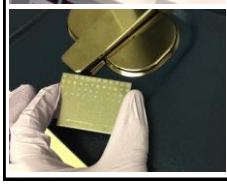
New technologies: MALDI-TOF Mass Spectrometry

- **Rapid identification of bacteria, yeast & fungi**
  - **Minimum of 24-96 h reduced turnaround time compared to conventional culture-based methods.**
  - **Even more dramatic results for certain organisms (i.e. *Legionella* and *Mycobacterium species*).**
- **Test directly from positive blood cultures**
  - **Bypasses time-consuming subculture steps.**
  - **Expanding to other sample types / sources**

Wimmer JL, *J Clin Microbiol.* 2012.
Perez, K. *Arch Pathol Lab Med.* 2012.

MALDI-TOF in our clinical laboratory





Identify Gram negative bacteria directly from positive blood culture media

- **Transfer organisms from positive blood culture media to the MALDI-TOF spotting plate.**
- **Immediately perform organism identification using the MALDI-TOF instrument.**
- **Concurrently perform antimicrobial susceptibility testing using the pellet.**

Gram-negative bacteremia causes significant morbidity and mortality

- **Account for 24% of nosocomial bloodstream infections.**
- **Survival is directly associated with timely and appropriate initiation of antibiotic therapy.**
- **For every hour that appropriate antibiotics are delayed, survival decreases by 7.6%**

Gaynes R. *Clin Infect Dis.* 2005.
Kang CI. *Antimicrob Agents Chemother.* 2005.
Kumar A. *Crit Care Med.* 2006.

Innovation plan:
MALDI-TOF & antimicrobial stewardship


Katherine Perez

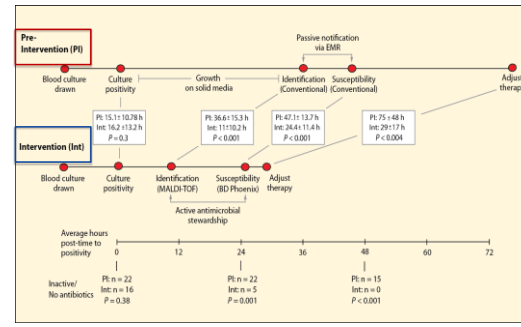
- **Rapid identification using MALDI-TOF and antibiotic susceptibility testing directly from blood cultures that recover a Gram-negative organism.**

Microbiology laboratory results are immediately reported to the infectious diseases pharmacist who is on-call 24/7.

Pharmacist interprets the patient/MALDI-TOF data, contacts the treating physician and recommends optimal therapy.

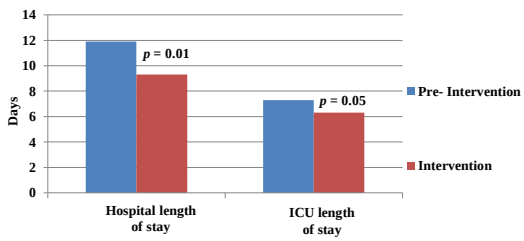
Perez, K. *Arch Pathol Lab Med.* 2012.

MALDI-TOF & antimicrobial stewardship significantly reduces turnaround times



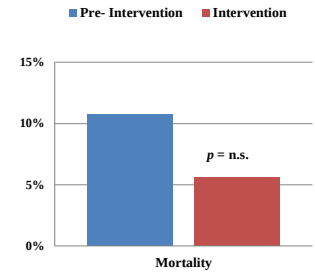
Perez, K. *Arch Pathol Lab Med.* 2012.

MALDI-TOF & antimicrobial stewardship significantly reduces length of stay



Perez, K. *Arch Pathol Lab Med.* 2012.

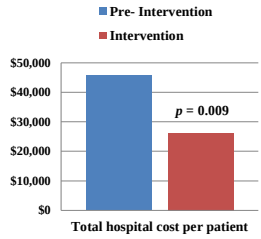
MALDI-TOF & antimicrobial stewardship decreases mortality



Perez, K. *Arch Pathol Lab Med.* 2012.

MALDI-TOF & antimicrobial stewardship significantly reduces costs

- **The MALDI-TOF antimicrobial stewardship program saved our hospital 1.97 million dollars during the study period.**
- **The program is estimated to save our hospital $18 million dollars annually.**



Perez, K. *Arch Pathol Lab Med.* 2012.

Polymerase Chain Reaction (PCR) & Amplification Based Techniques

Respiratory Pathogen PCR vs. Viral Culture

- Traditional viral culture method
  - Shell vials for Respiratory viruses, Enterovirus, *Chlamydia*.
    - Slow growth (up to 5 days)
    - Labor intensive
    - Supply chain difficulties
    - Sensitivity not fantastic

- Multiplex Respiratory Pathogen Panel
  - Single assay for 20 pathogens (17 viral, 3 bacterial)
    - Direct specimen
    - Automated extraction, interpretation
    - Rapid (4 hour TAT)

Respiratory pathogens panel

Detects 20 respiratory pathogens

Adenovirus
Bordetella pertussis
Chlamydophila pneumoniae
Coronavirus (4 types)
Influenza A virus (subtypes)
Influenza B virus
Human metapneumovirus
Mycoplasma pneumoniae
Parainfluenza viruses 1-4
Respiratory syncytial virus
Rhinovirus/enterovirus

BioFire Film Array instrument

Running almost 100 per day at height of respiratory virus season

Available 24/7

Goal: Report all results within 4 hours of receiving the specimen in the lab.

Respiratory Pathogen PCR vs. Viral Culture

- Benefits
  - Clinicians love the rapid TAT
  - Can help guide antibiotic and antiviral prescribing
  - Lab techs love the ease of use
  - Allowed lab to eliminate most shell vial culture
  - Allows detection of difficult to culture organisms
- Cons
  - Maintaining TAT during seasonal peaks difficult
  - Lacks some desirable organisms (*S. pneumo*, MRSA)
  - Detection of nucleic acid doesn't necessarily mean viable organism

Respiratory Pathogen PCR vs. Viral Culture

- The Case for a Multitest Platform
  - BioFireDX has two other FDA cleared panels
    - GI panel (22 targets: bacteria, parasites, viruses)
    - Blood culture identification (24 targets, 3 resistance genes)
  - Multiple tests can aid your financial justification
  - Versatility may guard against obsolescence
  - Technologist familiarity with platform aids training
    - Be cautious of potential for confusion / human error

Sequencing Based Techniques

Traditional Sanger Sequencing

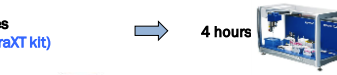
- Traditional Sanger Sequencing
  - Targeted
  - 16S rRNA (identification)
  - Low-resolution epidemiology
    - Multilocus sequence typing (MLST)
    - Allele typing (ie. *Spa* typing in *S. aureus*)
  - Capillary electrophoresis, dye-terminator chemistry

"Next generation" Whole Genome Sequencing

- Whole Genome Sequencing (WGS)
  - Parallel sequencing of millions of short DNA fragments that can be assembled/mapped to the whole genome
  - Identification of organism, resistance genes, plasmids
  - High-resolution epidemiology
  - Sequencing by synthesis (reversible terminators)
- Whole genome sequences of bacteria can be generated in a few days for less than $50 each
  - *A $10 genome is a realistic goal*
  - *Same day turnaround is also obtainable*

Typical workflow for whole genome sequencing of microorganisms

Extract genomic DNA (Automated, Qiagen)	4 hours
Prepare sequencing libraries (Automated, Illumina NexteraXT kit)	4 hours
Perform sequencing reaction (Illumina MiSeq)	6-39 hours (overnight)
Analyze data (Custom bioinformatics process)	Hours - Days



Whole genome sequencing in our clinical laboratory

- Species assignment for slow growing, difficult to cultivate or difficult to identify organisms.
- Near real-time investigation of nosocomial infections and outbreaks.
- Understand bacteria strain genotype – patient disease phenotype relationships.
- Study the molecular basis of severe, unusual, or interesting infections.

A laboratory technologist setting up a whole genome sequencing run on our Ion Torrent Personal Genome Machine.

Whole genome sequencing in the clinical laboratory

"A day in the life of the clinical microbiology laboratory"

- 130 samples collected from 116 patient cultures on a single day.
- Isolated colonies and mixed samples.
- Aerobic bacteria, anaerobic bacteria, acid-fast bacilli and fungi.

Long, et al, *J. Clin Microbiol*, 2013

Results: A day in the life of the clinical microbiology laboratory

Whole genome sequencing identified:

- Most of organisms sequenced
  - 88.5% concordance with reference method
- *Mycobacterium species* in two samples
  - 10 days before the conventional method
- Multiple pathogens present in several mixed samples

Whole genome sequencing in the clinical laboratory

"A year in the life of the microbiology laboratory"

First clinical run as a validated test: June 26, 2013

- ~400 genome sequences analyzed in the validation study
- Bacteria, fungi and influenza A virus
- >350 genomes generated as an ordered test

First organism to be identified by our clinical laboratory

Mycobacterium abscessus subsp. massiliense

- Single gene sequencing assays can not distinguish members of the *M. abscessus* group
  - This organism has higher treatment response rates to combination therapy since it usually lacks inducible resistance to clarithromycin
  - Genomic data can guide patient care decisions

Whole genome sequencing of microorganisms in the clinical lab

Routine cases

- Organisms that can not be identified using MALDI-TOF
  - *Mycobacterium species*
  - Fungus
  - Uncommon pathogens not represented in the database
- Organisms requiring further classification for optimal patient care or public health efforts
  - Serotyping *Salmonella enterica* and *Neisseria meningitidis*

Whole genome sequencing of microorganisms in the clinical lab

Special cases

- Organisms suspected of comprising an outbreak
  - Investigated a cluster of invasive group A Streptococcus infections in a small rural community (Brown, et al., *Emerg Infect Dis*, 2014)
  - Investigated a perceived outbreak of multiple-drug resistant *Klebsiella pneumoniae* infections in our hospital

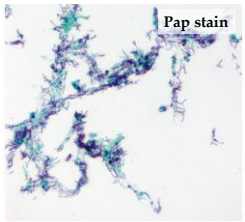
Whole genome sequencing of microorganisms in the clinical lab

Special cases

- Organisms with unusual antimicrobial resistance
  - Ceftaroline resistant MRSA (MIC > 32mg/L) (Long, et al., *Antimicrob Agents Chemother*, 2014)
- Organisms causing unusually severe or unexpected clinical presentations
  - Investigated a fatal anthrax-like infection (Wright, et al., *Arch Pathol Lab Med*, 2011)

Previously healthy male with severe pneumonia and death within 72 h

BAL on presentation
with extremely high numbers of rod-shaped bacteria



Blood culture
pure culture of *Bacillus cereus* group organisms

Wright, et al., *Arch Pathol Lab Med*, 2011

The *Bacillus cereus* group troika

Bacillus anthracis
Bacillus cereus
Bacillus thuringiensis

- Very closely related
- Difficult to unambiguously classify using conventional microbiology techniques
- Mobile genetic elements (plasmids) are crucial for virulence

We performed whole genome sequencing to investigate the molecular basis of this severe, rapidly fatal infection.

Whole genome sequencing identified the causative agent: *B. cereus*

- Identified etiologic agent of the fatal Anthrax-like infection
  - *B. cereus* with anthrax toxin genes, not *B. anthracis*
- Ruled out the likelihood of bioterrorism
  - No foreign DNA or evidence of other genetic manipulation
- Guided infection control response
  - No cases of secondary transmission
- Gained new insight to molecular pathogenesis
  - Launched a new line of investigation

Summary: Benefits of whole genome sequencing

- Identify unknown organisms
- Investigate suspected outbreaks
- Understand the molecular basis of interesting infections
- Rapid and cost effective testing to guide patient care
- New lines of investigation

Summary of next generation diagnosis of pulmonary infections

- Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry
- PCR based assays
- Whole genome sequencing of microorganisms

- Rapid identification of pathogenic organisms
- Detection from patient specimens or primary cultures
- Rapid inferred antimicrobial susceptibility and virulence

Implementation of new technologies may rapidly inform treatment decisions to improve patient care and decrease costs

Acknowledgements

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Questions & Contact Information

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