Molecular Diagnostics and it’s Role in Infection Prevention

Dr. Sanchita Das, University of Chicago
A Webber Training Teleclass

Molecular Diagnostics and it’s Role in Infection Prevention

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The Changing Laboratory

Late 1800's  Early 2000's  2013


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The Challenging Laboratory

Use of Molecular Assays in Infection Prevention

- Early diagnosis that can impact management
- Antimicrobial stewardship
- Screening and surveillance for Infection Prevention
- Choice of testing algorithms and platforms that improve infection control
- Implementation of new technologies (NGS): are we there yet?
Early Diagnosis?

Well Now
Wasn’t That
A No Brainer!!

Case

Lung Abscess in a Young Adult

- 37YO female former smoker presents on 11/17 with hemoptysis for 1 month
- Undergoing outpatient management of lung abscess
- Initially started on augmentin, later switched to clindamycin
- Bilateral chest pains worse with deep inspiration
- Rare coughing
- Generalized malaise, weakness and occasional nausea
- Underwent bronchoscopy on 10/27
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Bronchoscopy Lab Results (10/27)

Culture Results:
- Streptococcus millis group
  - 1+ rare
- Streptococcus salivarius group
  - 1+ rare
- Pasteurella multocida
  - 1+ rare

Susceptibility Testing:

<table>
<thead>
<tr>
<th></th>
<th>R. miciliagenes</th>
<th>S. millis</th>
<th>S. salivarius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Penicillin G</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S</td>
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<td>R</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Cytopathology

Final Diagnosis:
Lung, right lower lobe (superior segment), transbronchial biopsy
- Non-necrotizing granulomatous inflammation.
- Acute and chronic inflammation.
- No dysplasia or malignancy present.

Admission and Subsequent Management

- Admitted, started to vancomycin and piperacillin/tazobactam
- ID and Pulmonology consulted
- Chest X-ray findings:
  - Large right perihilar mass similar in size to previous study.
  - Left apical consolidation that may be due to pneumonia is smaller but still present. Heart and pulmonary vessels are normal in size. No pleural effusion.
Other Lab Results and Clinical Impression

- Fungal and vasculitis studies negative
- TB quantiferon gold negative
- Non-TB mycobacteria possible
- Vancomycin intolerant
- Changed to linezolid and ertapenem

Lung abscess not responding to antibiotics
Recommend surgery

VATS Laboratory Results (11/22)

DIRECT SMEAR

Smear Result: Positive Acid fast bacilli 3+(10-90/field)

HISTOPATHOLOGY

Right lower lobe of lung, wedge resection;
- Necrotizing granulomatous inflammation. See comment and laboratory data.

COMMENT:

Sections taken from the right lower lobe of lung confirm the presence of the necrotizing granulomatous inflammatory process. An AFB stain is positive for numerous acid fast bacteria. Cultures were obtained from the lung parenchyma and AFB stains on that tissue are also positive. A silver methenamine stain is negative for fungal elements.

TBPCR POSITIVE
Culture and Susceptibility (12/28)

**Culture Results:**
M. tuberculosis complex

**Susceptibility Testing:**

<table>
<thead>
<tr>
<th>Medication</th>
<th>S</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>INH (L,A,H)</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Pnnonamide</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Ethionamide</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>

MDRTB! Total time to Identification and Susceptibility ~2 months

Timeline of Diagnosis and Management

- Patient started on augmentin
- Therapy changed to broader spectrum
- Presented with Symptoms Bronchoscopy performed
- Culture results with 3 different bacteria
- Hemoptysis Admission to floor
- AFB on direct smear TB PCR Positive
- 10/27
- 10/31
- 11/17
- 11/22
- TBPCR
- XPERT TB/RIF
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WHO-RECOMMENDED DIAGNOSTIC TOOLS

RECOMMENDED FOR USE (detailed policy guidance: http://www.who.int/mediacentre/policies-statements/en/)
- LED microscopy: For use at all laboratory levels as replacement of conventional fluorochrome and light microscopy.
- Commercial liquid culture and DST systems: For use at central/regional reference laboratory level, as current reference standard.
- Rapid speciation strip technology: For use with conventional culture and DST at central/regional reference laboratory level, to identify Mycobacterium tuberculosis.
- Automated real-time nucleic acid amplification - Xpert MTB/RIF system: For rapid detection of pulmonary and extrapulmonary TB and rifampicin resistance in both adults and children at decentralised laboratory and healthcare centres.
- Lateral flow urine lipoarabinomannan (LF-LAM) assay may be used to assist in the diagnosis of TB in HIV positive patients with signs and symptoms of TB (pulmonary and/or extrapulmonary) who have a CD4 cell count less than or equal to 100 cells/μL, or HIV positive patients who are seriously ill regardless of CD4 count or with unknown CD4 count.
- Loop-mediated isothermal amplification test kit for TB (LB-LAMP): Manual molecular assay to replace microscopy to diagnose TB in settings where automated molecular tests cannot be used.
- Line probe assay (LPA) as a rapid diagnostic test for detection of rifampicin and isoniazid resistance. The WHO recommended commercially available tests include GenoType MTBDRplus VER 1 and 2 (Hain Lifescience, Germany), Nipro NTM+MDRTB detection kit 2 (Nipro, Japan). Suitable for use on smear-positive specimens or culture isolates.
- Second-line line probe assay (SL-LPA) as a rapid diagnostic test in patients with confirmed rifampicin-resistant TB or MDR-TB to detect resistance to fluoroquinolones and the second-line injectable drugs.

Advantages of Molecular Diagnosis: TB

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Molecular Diagnosis of TB in Resource Limited Areas

- Advantages:
  - Rapidity of result delivery
  - Standardization of assay techniques
  - Potential for high throughput
  - Reduced requirements for biosafety
  - Sensitivity as high as 95% for some platforms

- Disadvantages:
  - Calibration
  - Instrumentation
  - Constant electric supply

Real-Time PCR Assay Technologies: RECAP

**Advantages**
- Minimizes cross-contamination
- Automated, low-complexity
- Quantitative
- High sensitivity

**Disadvantages**
- Known pathogens only
- Only 2-6plex
- Primer design: challenging for melt-curve
- Fluorescent-labeled primers: expensive
Syndromic Approach to Diagnostics

Fournier et al. Modern Clinical Microbiology Nature Reviews Microbiology 2013. 11:574.

The GI Panels

<table>
<thead>
<tr>
<th>Target</th>
<th>Verigene FF</th>
<th>FilmG巨 Rif</th>
<th>xTIG-GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. difficile (tox/AF)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhoid spp.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>EPEC</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>EHEC</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>S. suis (G1 and G2)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Z. pyogenes</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. jejuni</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>H. pylori</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>F. tularensis</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>


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The Problem with Diarrhea

- **Issues with lab diagnosis**
  - Wide range of pathogens causing different types of diarrhea
  - Childhood diarrhea different from adult
  - A multitude of techniques needed for diagnosis
    - ELISA for toxins
    - Fluorescent microscopy for parasites
  - Global problem with varying etiology

- **Solution?**
  - Antimicrobial susceptibility and public health impact?
  - False positives with Vibrio and Entamoeba
  - Reimbursement: Do all patients need to get all 17 targets?
  - What will we miss if we move solely to panel based testing? (Aerocnenas)

The Problem with Gold Standards: STI

- PCR found to be 20-50% more sensitive for diagnosis of chlamydial infections than traditional culture methods
- Comparing results to an imperfect method produces biased sensitivity and specificity
- Prevalence being constant, sensitivity of older assays will be overestimated if standard tests are suboptimal
- Comparison with comparable technology
- Caveats?
Impact of Commensal Flora

- Extra-genital Chlamydia and Neisseria: Impact the detection in pharyngeal rectal specimens impacts MSM, minors where detection in extra genital sites of importance

Respiratory Viral Panels

<table>
<thead>
<tr>
<th>Assay Name</th>
<th>Assay Format/Method</th>
<th>Specimen</th>
<th>Extraction of Nucleic acids required</th>
<th>Sensitivity/Specificity</th>
<th>Pathogens detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProFAST90™ (Promega, Gen-Probe, Affymetrix)</td>
<td>Multiplex real-time reverse transcription PCR</td>
<td>NP pellets</td>
<td>No</td>
<td>50-80%</td>
<td>Influenza A, B, RSV, RS V (including variants)</td>
</tr>
<tr>
<td>FlexArray (Biomerieux)</td>
<td>Real-time PCR with melt curve analysis</td>
<td>NP pellets, respiratory secretions</td>
<td>No</td>
<td>85-100%</td>
<td>Influenza A, B, RSV</td>
</tr>
<tr>
<td>Xpert Flu (Cepheid)</td>
<td>Multiplex real-time reverse transcription PCR assay</td>
<td>NP pellets</td>
<td>No</td>
<td>80-90%</td>
<td>Influenza A, B, RSV</td>
</tr>
<tr>
<td>Biofire Flu RAPID AB Test (BioFire Diagnostics)</td>
<td>PCR, hybridization and detection, lid-based</td>
<td>NP pellets, respiratory secretions</td>
<td>Yes</td>
<td>97-100%</td>
<td>Influenza A, B, RSV, RS V (including variants)</td>
</tr>
<tr>
<td>xMark™ Respiratory Virus Panel (GenMark)</td>
<td>PCR followed by hybridization and detection</td>
<td>NP pellets, respiratory secretions</td>
<td>Yes</td>
<td>90-95%</td>
<td>Influenza A, B, RSV, RS V, adenovirus, parainfluenza, rhinovirus</td>
</tr>
<tr>
<td>Verigene RV (Bionsearch)</td>
<td>Multiplex reverse transcription PCR followed by hybridization and detection</td>
<td>NP pellets</td>
<td>No</td>
<td>46-60%</td>
<td>Influenza A</td>
</tr>
<tr>
<td>Verigene RV (Bionsearch)</td>
<td>Multiplex reverse transcription PCR followed by hybridization and detection</td>
<td>NP pellets</td>
<td>No</td>
<td>46-60%</td>
<td>Influenza B</td>
</tr>
<tr>
<td>Access (Hologic)</td>
<td>Isothermal PCR assay with real-time analysis</td>
<td>NP pellets, respiratory secretions</td>
<td>No</td>
<td>95-100%</td>
<td>Influenza A, B</td>
</tr>
<tr>
<td>LRT (Roche)</td>
<td>Multiplex real-time reverse transcription PCR assay</td>
<td>NP pellets</td>
<td>No</td>
<td>68-70%</td>
<td>Influenza A and B</td>
</tr>
</tbody>
</table>

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Elderly Gentleman with Pneumonia?

- 85YO male in clinic on 13th January with moderate productive cough for 1 day, fever up to a 100°F
- Chest X-ray ordered, patient prescribed cefpodoxime and advised to come back if worse
- Admitted on 14th of January
  - Fever of 101.5, congestion, generalized weakness
  - Concern for pneumonia
  - Antibiotics: vancomycin and zosyn continued
  - Blood culture and NP swab for respiratory virus were sent

Acute Exacerbation of Asthma?

- 65YO female with asthmatic bronchitis presents to ED on 25th January with complaints of 5 days of cough and shortness of breath
- Associated wheezing, nausea, vomiting, diarrhea and body ache
- Started on nebulizer and IV steroids-- minimal improvement; transferred to ICU for further management
- NP swab sent for respiratory viruses
Fever in an Infant

- Otherwise healthy 12 month old female presents to Urgent Care Center on February 6th with fever since waking this morning
- No other symptoms
- Does not attend day care, no sick contacts at home
- Vaccinations up-to-date
- NP swab sent for respiratory viruses

Laboratory Testing Viruses: The Past

- Specimen
- Chorioallantoic Membrane & Allantoic cavity
- Harvest from infected egg (~1 week)
- Hemagglutination/Hemagglutination inhibition
- Viral Cell Culture Flask
- Uninfected Cell Line
- Viral Cytopathic Effect (~1 week)
- Hemadsorption to RBC for identification

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Direct Fluorescent Antibody

- Fluorescein-Labeled Specific Antibody
- Fluorescein-Labeled Anti-IgG
- Unlabeled Specific Antibody

A. Direct  B. Indirect
Fixed Specimen on Glass Slide

Antibody

POSITIVE

Differential identification of viruses, HSV-1 vs. HSV-2

Rapid Antigen Immunoassay

- Influenza A and RSV
- Performance inferior to PCR

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Laboratory Testing Viruses: Present

Turn around time

Complex

Simple

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### Our Patients’ Lab Results

#### Case 2
- Elderly inpatient with CAP
- **Turn around time up to ~6h**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>Positive</td>
</tr>
<tr>
<td>Influenza A(H1)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Influenza A(H3)</td>
<td>Positive</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Respiratory Syncytial virus (RSV) A</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Respiratory Syncytial virus (RSV) B</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 1</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 2</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 3</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 4</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Human Metapneumovirus (HMPV)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Human Rhinovirus (HRV)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

#### Case 3
- Asthmatic in ICU
- **Turn around time ~3-4h**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>Positive</td>
</tr>
<tr>
<td>Influenza A(H1)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Influenza A(H3)</td>
<td>Positive</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Respiratory Syncytial virus (RSV) A</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Respiratory Syncytial virus (RSV) B</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 1</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 2</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 3</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 4</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Human Metapneumovirus (HMPV)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Human Rhinovirus (HRV)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

#### Case 4
- Infant in ACC
- **Turn around time <1h**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>Positive</td>
</tr>
<tr>
<td>Influenza A(H1)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Influenza A(H3)</td>
<td>Positive</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Respiratory Syncytial virus (RSV) A</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Respiratory Syncytial virus (RSV) B</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 1</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 2</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 3</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Parainfluenza (PIV) 4</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Human Metapneumovirus (HMPV)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Human Rhinovirus (HRV)</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

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Impact on Clinical Decision

### Table 1

<table>
<thead>
<tr>
<th>Clinical Indicator</th>
<th>% Immediate Influence Change</th>
<th>% Immediate Influence Reduction</th>
<th>% Immediate Influence Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics prescribing failure</td>
<td>20% (10/50)</td>
<td>10% (5/50)</td>
<td>10% (5/50)</td>
</tr>
<tr>
<td>Antibiotics prescribing success</td>
<td>30% (15/50)</td>
<td>10% (5/50)</td>
<td>10% (5/50)</td>
</tr>
<tr>
<td>Antiviral prescribing failure</td>
<td>20% (10/50)</td>
<td>10% (5/50)</td>
<td>10% (5/50)</td>
</tr>
<tr>
<td>Antiviral prescribing success</td>
<td>30% (15/50)</td>
<td>10% (5/50)</td>
<td>10% (5/50)</td>
</tr>
<tr>
<td>Influenza diagnosis</td>
<td>10% (1/10)</td>
<td>10% (5/50)</td>
<td>10% (5/50)</td>
</tr>
</tbody>
</table>

*Based on total number of 50 patients receiving antivirals, or twice 50.*

**p < 0.05, significant difference in antiviral prescribing when positive and negative.


Impact on Clinical Decision: Outpatient Study

<table>
<thead>
<tr>
<th>Test Result</th>
<th>Number</th>
<th>Prescribed Medications</th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Antiviral*</td>
<td>Antibiotic**</td>
</tr>
<tr>
<td><strong>LIAT (POC-PCR) – Site A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>125</td>
<td>3</td>
<td>57</td>
</tr>
<tr>
<td>Positive (FluA)</td>
<td>63</td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td>Positive (FluB)</td>
<td>51</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td><strong>RADT (POC-Antigen) – Site B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>108</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>Positive (FluA)</td>
<td>82</td>
<td>69</td>
<td>3</td>
</tr>
<tr>
<td>Positive (FluB)</td>
<td>51</td>
<td>33</td>
<td>6</td>
</tr>
</tbody>
</table>

Significant difference in antiviral prescription for patients with negative LIAT

Brenninkmeijer et al., Abstract Clinical Virology Symposium 2018

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Multiplexed PCR Assays: Syndromic approach

- Concept of one test for one diagnosis:
  - Pneumonia panel
  - Sepsis panel
  - Diarrhea panel
  - Meningitis panel

- Advanced technology:
  - Appeal of complete automated sample handling
  - Easy read-out and software for analysis

- Strategic combination of targets:
  - Short time for broad range of pathogens
  - Provides some anti-infective information

Antimicrobial Stewardship: A lost Cause?

“There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react in some way acquiring ‘fastness’ [resistance]” — Alexander Fleming, 1946

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Elderly Gentleman with Sepsis

- 76 year old male from nursing home, Parkinson’s disease
- On chronic Foley catheter, and failure to thrive
- Brought to ED for fever, low blood pressure
- Alert and oriented but hypotensive
- Laboratory Results
  - Serum lactate level: 6.3
  - WBC count: 30,000/ul
- Working Diagnosis: Sepsis, blood cultures sent and started on 3rd generation cephalosporin

Use of Molecular Assays on Blood Culture

<table>
<thead>
<tr>
<th>Cultures collected</th>
<th>Blood culture positive</th>
<th>Colonies available for ID</th>
<th>Susceptibility Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>0hr</td>
<td>~12hr</td>
<td>~24-36hr</td>
<td>~36-48hr</td>
</tr>
</tbody>
</table>

Molecular Assay (prelim Susceptibility result) | 0hr | ~12hr | ~14-15h
Molecular Assays for Antimicrobial Stewardship

• Elderly sepsis patient
  – E. coli by molecular assay (14h after blood culture collection)
  – Prelim result CTXM positive
  – 3rd generation cephalosporin changed to carbapenem
    » De-escalated to beta-lactamase inhibitor combination after full susceptibility

Clinical Decision Support

Molecular Assay vs. Conventional Susceptibility:
Negative Predictive Values for ceftriaxone susceptibility in *Escherichia coli* and *Klebsiella pneumoniae* in the absence of either CTX-M or a carbapenemase gene were 98% and 93 to 94%, respectively.

Similar results were seen with other target bug-drug scenarios, with NPVs of 94 to 100% with the exception of *P. aeruginosa*, for which NPVs were poor, likely due to the more complex nature of resistance in this pathogen.

Infection Control Efforts and Surveillance

MRSA: The Success Story

Impact of active screening and decolonization in ICU
- MRSA infection decreased from 3.58 to 0.42% ($p<0.05$)
- Interruption of active surveillance and decolonization led to resurgence of infection rates up to 2.21%
- Reintroduction of intervention reduced in-hospital MRSA infection rates to 0.16%
- Decolonization and active surveillance saved $22 for each $1 spent on the intervention

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Molecular Diagnostics and it’s Role in Infection Prevention
Dr. Sanchita Das, University of Chicago
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MRSA: The Success Story

- What qualities ensure success of MRSA screening test?
  - PCR test with quick result makes intervention simple and impactful
  - Nasal swabs simple to collect and easy to test (not messy)
  - Site specific insertion of resistance gene, easy PCR design
  - Buy in from hospital and public health authorities

C. difficile: The failure

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The difference is in the disease!

![Diagram showing the difference between unaltered and antibiotic-disrupted microbiota](image)


The Art of Assay Design: *Unlimited?*

- Ideal Molecular Assay: Key Challenges
  - Rapid with relatively low technical complexity
  - **Minimal handling of samples**
  - **High throughput low turnaround time**
  - **A strategic combination of molecular targets based on specimen type**
  - Multiplexing: a panel for a syndrome
  - Guide to anti-infective therapy
  - Cost effective
  - Easy readout, software for analysis
The Art of Assay Design: Unlimited?

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Fever Spikes....Again?!!!

Case

Present Illness
48y/o female presents with pyelonephritis
Nephrostomy tubes placed
Drained fluid sent for culture
Cultures negative
Sent home on p/o antibiotics

Disease Course
Comes back to ED in a few hours for fever and pain
High WBC count 16000
Fever up to a 103
Blood, urine and drain fluid sent for culture
Rocephin and Vanco started
All cultures negative

Management
Continues to be febrile to 102.5
Pain at site of nephrostomy and back
Nephrostomy tube draining well
WBC 15.2
CRP 256

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Gram Stain and Culture of Nephrostomy Drainage

Culture Results:
Aerobic and anaerobic culture
No organism isolated

4+ WBCs (PMNs)
No organisms seen

The Panbacterial Target: 16SrRNA Gene

Culture Results:
Aerobic and anaerobic culture
No organism isolated
Ureaplasma species. Isolate Comment: Detected by 16S rRNA gene sequencing. Results discussed with ID physician

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Idnt</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ureaplasma urealyticum strain B, strain ATCC 27961 16S ribosomal RNA gene, partial sequence</td>
<td>983</td>
<td>863</td>
<td>98%</td>
<td>0.0</td>
<td>98%</td>
<td>NR_34716.1</td>
</tr>
<tr>
<td>Ureaplasma parvum strain ATCC 27915 16S ribosomal RNA, complete sequence</td>
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<td>863</td>
<td>98%</td>
<td>0.0</td>
<td>98%</td>
<td>NR_374761.2</td>
</tr>
</tbody>
</table>
The Future: New Technology?

Next Generation Sequencing: The Future?
- Ribosomal RNA gene sequence is used for ID
- Sequence information of whole genome
- Known pathogens and novel organisms
- Identify mutations for virulence and resistance
- Trace spread of pathogens

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Next Generation Sequencing: The Future?

- Ribosomal RNA gene sequence is used for ID
- Sequence information of whole genome
- Known pathogens and novel organisms
- Identify mutations for virulence and resistance
- Trace spread of pathogens

- Reduction in running cost
- Clinical interpretation of data
- Streamlining bioinformatics and data analysis

Targeted PCR vs. Sanger Sequencing

Identification in few hours
Next Generation Sequencing: Workflow

- Template Preparation
  - Randomly break into fragments
  - Template needs to be amplified

- Sequencing and Imaging
  - Reversible termination with fluorescent dyes
  - Ligation with fluorescent dyes
  - Semiconductor that measures pH when base added
  - Nanopore measures changes in electrical impulse

- Data Analysis
  - Align sequence to a reference database OR assemble de novo

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Next Generation Sequencing: WGS

Genotype: Phenotype Correlation Simplified?

- How often does genotype predict resistance clinically?
- Can population genome graphs be used for identification and susceptibility?
- Is it better or rapid compared to existing methods?
- Algorithmic approach could work for some pathogens?
M. tuberculosis AST: Game changer?

- AST is performed by proportion method (subjective and slow)
- Slow growing pathogen, time to result can be weeks
- XDR and MDR TB is on the rise and rapid identification is key to control

- All drug resistance is mediated through chromosomal mutations
- Mutations known and annotated
- Mutation database publicly available

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The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee

M.J. Ellington 1,2,3, O. Ekelund 1,2,3, J.M. Aarestrup 2, R. Cantor 4, M. Doumith 4, C. Giske 5, H. Grundman 1, H. Hasman 1, M.T.C. Holden 6, K.L. Hopkins 1, J. Iredell 7, G. Kahlmeter 8, C.U. Köser 1, A. MacGowan 9, D. Mevius 10,11, M. Mulvey 1, T. Haas 10, T. Petro 10, J.-M. Robin 1, O. Samuelson 10, N. Woodford 3.
Single nucleotide changes do not reflect plasmid mediated resistance


Single molecule sequencing to track plasmid diversity of hospital-associated carbapenemase-producing *Enterobacteriaceae*

Sean Conlon¹, Pamela J. Thomas², Clayton Deming¹, Morgan Park², Anna F. Leu², John P. Dekker², Evan S. Smitkin¹, Tyson A. Clark³, Khai Luong³, Yi Song³, Yu-Chih Tsai¹, Matthew Bottino⁴, Jyoti Gupta¹, Shetlise Y. Brooks¹, Brian Schmidt¹, Alice C. Young², James W. Thomas₂, Gerard G. Bouffard², Robert W. Bakesley². NICE Comparative Sequencing Program², James C. Mulliken², James Korfhage¹, David K. Henderson³, Karen M. Frank³, Tara N. Palmere⁵, and Julia A. Segre¹,²

¹National Human Genome Research Institute, Bethesda, MD

---

Tracking a plasmid during outbreak of KPC+ isolates at NIH

- Wide array of plasmids with carbapenem resistance genes found in several *Enterobacteriaceae* spp.
- Horizontal transfer of plasmids in the hospital environment
- Difficult to pin down person to person transmission

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Roadblocks

- Cost and Time
- Bioinformatics consideration: making sense of the data
- Genotype: phenotype correlation (expression)
- Significance of a genome within a specimen: clinical correlation
- Quality Assurance: Curating and maintaining reliable database
- Regulatory considerations
- Patient outcomes

Molecular Assays: Making the Difference?

- Summary
  - New technology can provide accurate diagnosis in a shorter time
  - Consultative microbiology could enhance understanding of the “lab report”
  - Consultation with clinical pathologist help in choice of platform
  - With novel pathogens, antimicrobial resistance and technology laboratory stewardship becomes an integral part of patient care
  - Applications of NGS in clinical microbiology is promising but need careful cost benefit evaluation depending on the application

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Thank You!

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THE SILENT TSUNAMI OF AZOLE-RESISTANCE IN THE OPPORTUNISTIC
FUNGUS ASPERGILLUS FUMIGATUS
Speaker: Prof. Paul E. Verweij, Radboud University Center of Expertise in
Mycology, The Netherlands

CHLORHEXIDINE USE AND BACTERIAL RESISTANCE
Speaker: Prof. Jean Yves Maillard, Cardiff University, Wales

(FREE European Teleclass - Broadcast live from the 2018 IPS conference)
Cottrell Lecture ... SURVEILLANCE BY OBJECTIVES: USING MEASUREMENT
IN THE PREVENTION OF HEALTHCARE ASSOCIATED INFECTIONS
Speaker: Prof. Jennie Wilson, University of West London

(FREE European Teleclass - Broadcast live from the 2018 IPS conference)
Ayliffe Lecture ... (TO BE POSTED)
Speaker: Prof. Shaheen Mehtar, Stellenbosch University, Cape Town, South Africa

(IN FREE CBIC Teleclass)
INFECTION CONTROL: CHAMPIONS ARE MADE, NOT BORN

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