Rapid microbiological diagnosis for VAP and sepsis

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Ivor S. Douglas, MD
Denver Health Medical Center &
U Colorado, Denver
idouglas@dhha.org
ISD Disclosures

• Grant support to Denver Health Medical Center
• Dept. of Defense (CDMRP) Award 2012-2015 (PI)
  • Accelerate Diagnostics (development partner)
• NIH-NIAID R01 2015-2020
• No Tobacco industry funding
• Accelerate Diagnostics Ad Board – payment to DHMC
The Sepsis Gap: Unfulfilled clinical need for accurate, prompt diagnosis

- Inappropriate/Delayed Abx correlate directly with increased mortality
  
  *KT Huang Chang Gung Med J. 2010*

- Indiscriminate Abx leads to multi-resistant strains
  
  *CM Luna, ERJ 2006*

- Empiric antibiotic therapy predisposes to infection with more virulent strains
  
  - Increased Pseudomonas and Acinetobacter from 19% to 65% when antibiotics given before the development of an actual pneumonia
  
  - Increased mortality in previously-treated group (48% vs. 83%)
  
  *Fagon et al, Am Rev Respir Dis, 1989*
Ventilator Associated Pneumonia (VAP) – High sepsis-associated mortality

- 2nd most common HAI
  - 15% of all hospital acquired infections

- Incidence = 9% to 27% of MV
  - Increases ICU LOS by several days
  - Increases hospital LOS 1 to 3 weeks
  - Mortality = 13% to 55%

- Added costs of $40,000 - $50,000
  - MRSA vs MSSA: (€60,684 vs €38,731; P=0.01) – Ott 2010
  - MRSA vs MSS Excess cost US$7731 (P = 0.35) – Shorr 2006

- Remains difficult to diagnose conclusively

M Bekaert AJRCCM 2011; 184(10):1133-9
J. Hunter, BMJ 2012;344:e3325
“Know (and rapidly diagnose) thy enemy”

- Difficult in ventilated patient
  - Recent antimicrobials
  - Structural lung disease
- No consistently reliable combination of clinical diagnostic criteria
  - Typical criteria
    - Clinical
    - Radiographic
    - Laboratory
- Evolving surveillance/diagnostic criteria
  - CDC: 2-1-2
  - Modified iVAC → VAP

### Severe HCAP/HAP/VAP – mainly MDRO

<table>
<thead>
<tr>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>Acinetabacter spp.</td>
</tr>
<tr>
<td>Enterobacteriaceae (Klebs, E. Coli, Enterobacter spp.)</td>
</tr>
<tr>
<td>S. aureus</td>
</tr>
</tbody>
</table>

VAPs, VAEs, IVACs, VATs, HCAPs?!

M. Klompas, Crit Care Med. 2012 40:3154-3161
OUTCOMERA: VAC, IVAC, VAP (1996-2012)

At risk
- 13,702 MV patients
- 3,028 MV > 5 days
- Baseline stability → worsening oxygenation

VAC
- 2,331 (77%) at least one VAC episode

iVAC
- 869 (29%) at least one iVAC with ABX >4 days (-2d through +2d VAC)

VAP
- Possible/Probable VAP 240 (ONLY 27.6%); Microbiological confirmation
- Blood or CLABSI 134 (16%)

L. Bouadma, Critical Care Medicine 2015, 43(9)
## Performance Characteristics of VAC and IVAC for Probable VAP

<table>
<thead>
<tr>
<th></th>
<th>VAC</th>
<th>IVAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.92 (0.90–0.93)</td>
<td>0.67 (0.64–0.70)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.28 (0.27–0.30)</td>
<td>0.75 (0.73–0.77)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.32 (0.30–0.34)</td>
<td>0.50 (0.47–0.53)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.90 (0.88–0.92)</td>
<td>0.86 (0.84–0.87)</td>
</tr>
</tbody>
</table>

Good correlation between VAC and VAP: \( R^2 = 0.69 \)
IVAC and VAP: \( R^2 = 0.82 \) (\( p < 0.0001 \))
Temporal VAP risk

- Before day 5 40.1%
- Days 6 – 10 41.2%
- Days 11-15 11.3%
- Days 16 - 20 2.8%
- After day 21 4.5%

D. Koulenti, CCM 2009
D. Park, Resp Care 2005
Dilemma with Conventional Cultures

- Gram stain provides limited information. Cannot tell the species or susceptibility
- Culture is too slow
- Clinicians can’t wait an additional 1-3 days to treat the infection
  - Leads to inappropriate and ineffective therapy for some patients and unnecessarily broad therapy for others
Innovations in Micro Testing May Not Speed Assay Turn Around Time

- Nucleic acid testing
  - qPCR
- Molecular finger printing
- MALDI-TOF MS
- Nextgen 16S rDNA
- Volatile organic compounds (VOCs)
- Multiplexed Microscopy/FISH

A. Van Belkum, Ann Lab Med. 2013
# FDA 510K Cleared/ or CE Molecular Diagnostics

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Molecular (Genotypic)</th>
<th>Proteomics</th>
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<tbody>
<tr>
<td></td>
<td>BioFire Dx</td>
<td>Nanosphere</td>
</tr>
<tr>
<td>Technology</td>
<td>Multiplex PCR</td>
<td>Direct DNA</td>
</tr>
<tr>
<td>Identification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targets</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Markers</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Antibiotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptibility</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**FilmArray Blood Culture Identification (BCID) Panel - Multiplex PCR**

- **Gram-positive bacteria**
  - Enterococcus
  - Listeria monocytogenes
- **Staphylococcus**
  - Staphylococcus aureus
- **Streptococcus**
  - Streptococcus agalactiae
  - Streptococcus pneumoniae
  - Streptococcus pyogenes

- **Gram-negative bacteria**
  - Acinetobacter baumannii
  - Haemophilus influenzae
  - Neisseria meningitidis
  - Pseudomonas aeruginosa
- **Enterobacteriaceae**
  - Enterobacter cloacae complex
  - Escherichia coli
  - Klebsiella oxytoca
  - Klebsiella pneumoniae
  - Proteus
  - Serratia marcescens

- **Yeast**
  - Candida albicans
  - Candida glabrata
  - Candida krusei
  - Candida parapsilosis
  - Candida tropicalis

- **Antimicrobial resistance genes**
  - meca - methicillin resistance
  - vanA/B - vancomycin resistance
  - KPC - carbapenem resistance
VITEK® MS – MALDI-TOF Mass Spec

• Clinically relevant species with more than 25,000 spectra.
• Robust validation using Advanced Spectra Classifier.
Abbott Iridica PCR/ESI-MS

- RADICAL study

<table>
<thead>
<tr>
<th>Culture</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower respiratory tract</td>
<td>84% (95% CI, 74–91%)</td>
<td>53% (95% CI, 43–63%)</td>
<td>58% (95% CI, 40–67%)</td>
<td>81% (95% CI, 70–89%)</td>
</tr>
<tr>
<td>PCR/ESI-MS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>68</td>
<td>49</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>13</td>
<td>55</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>104</td>
<td>185</td>
<td></td>
</tr>
<tr>
<td>Sterile fluid and tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR/ESI-MS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>45</td>
<td>33</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>8</td>
<td>24</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>57</td>
<td>110</td>
<td></td>
</tr>
</tbody>
</table>

PPV = positive predictive value, NPV = negative predictive value.

*JL Vincent CCM. 2015;43(11):2283-91*
# Limitations of Rapid Diagnostic Technologies

<table>
<thead>
<tr>
<th>Objective</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed, preferably ≤2 hours</td>
<td>Gene Marker</td>
</tr>
<tr>
<td></td>
<td>Immuno-detection</td>
</tr>
<tr>
<td></td>
<td>Bacteriophage</td>
</tr>
<tr>
<td>Directly specimen analysis without colony isolation</td>
<td>x</td>
</tr>
<tr>
<td>Polymicrobial samples (multi-species and multi-strain)</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>x</td>
</tr>
<tr>
<td>High diagnostic accuracy</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Organism panel most prevalent, MDR prone species</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Abx resistance for multiple antibiotic classes</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiate live from dead organisms</td>
<td></td>
</tr>
<tr>
<td>Quantitative analysis for each target CFU/ml</td>
<td></td>
</tr>
<tr>
<td>“Random access” specimen analysis (stat capability)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Rapid Microbiological Diagnostics in Sepsis

Method

- Culture
- Biofire
- MS
- PNA-Fish
- Septifast
- liridica
- T2-bio
- RT-PCR
- AXDX ID-AST

Techniques
- PCR
- MALDI-TOF
- FISH
- RT-PCR
- ESI-MS
- PCR-NMR
- FISH-rapid microscopy

Time (h)
Rapid Dx: Live Cells, Multiplex Automated Digital Microscopy (MADM)

- Quantitative ID: 2 hours from specimen
  - Viable CFU quantitation, FISH confirmation
    - INFECTION VS COLONIZATION
- Major resistance typing: 6 hours
- Direct from specimen
  - Immobilize intact live bacteria
- Automated microscopy and image analysis
- >95% coverage of multi-resistance species and types
  - By disease panel: pneumonia, wound, etc.
Accelerate Diagnostics ID/AST Instrument

Overview

• Phenotypic MIC susceptibilities
• Direct from positive blood culture
• Future development direct from specimen such as BAL, wound, urine
• 1hr ID & 5hr MIC/susceptibilities
• Polymicrobial ID & MICs

+BC Sample → Sample prep Gel Electrofiltration → Immobilize live microbial cells → FISH based Identification → MICs via Imaging of cells
Analytical Process

Bacteria enter the flowcell (cutaway side view)

Electrical field forces cells to surface, remaining adhered to coating

Bacteria grow on surface (microscope view)

Subsequent susceptibility testing in multichannel flow cell

Change in growth rate over time reveals drug effect, clone by clone
Time-lapse Imaging of Live Cells

- Microscopy images are computed into individual cell (grey) growth curves
- Test strain growth kinetics are matched to a proprietary BMD databank to calculate MIC (solid red line)
- Features such as change in mass, morphology, division rate are analyzed

Example A - *E. coli* vs. 4 μg/mL Pip/Taz. MIC=8 (S)

Example B - *E. coli* vs. 4 μg/mL Pip/Taz. MIC=128 (R)
Polymicrobial Infections & Susceptibilities

Morphology, division rates, growth patterns, and signal intensity distinguish bacteria

vs. Erythromycin & Clindamycin

vs. Cefoxitin
AST: MIC from Single Concentration

Sample Prep

Concentrate, Immobilize, and Map

Identification

Phenotyping

Growth is centered to the S-I-R MIC range by adjusting challenge concentration.

Population Growth Range

Susceptibility score

0.25 0.5 1 2 4 8 16 32 64 128 256

S I R
AST: MIC from Single Concentration

P. aeruginosa isolates challenged with a single CAZ dilution

Sample Prep

Concentrate, Immobilize, and Map

Identification

Phenotyping

MIC = 32

(R)

MIC = 16

(I)

MIC = 8

(S)
**Ultrarapid MADM Detection and AST: A. baumannii in Blood Cultures**

**Bacterial cell population response profiles for S (8), R (64)**

**Automated Microscopy**

<table>
<thead>
<tr>
<th>Broth Micro-Dilution</th>
<th>≤4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>≥64</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**CLSI Breakpoints (n=47)**

- Essential Agreement = 98%
- Essential Agreement = 98%

**MIC Breakpoints**

- MIC = 8 (S)
- MIC = 64 (R)
# MADM Detection Validation - MDRO: Spiked Respiratory Specimens

<table>
<thead>
<tr>
<th>Description of Test</th>
<th>Number</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>250</td>
<td>84%</td>
<td>88%</td>
</tr>
<tr>
<td><em>P. aeruginosa</em>/amikacin</td>
<td>72</td>
<td>89%</td>
<td>94%</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>274</td>
<td>85%</td>
<td>95%</td>
</tr>
<tr>
<td><em>A. baumannii</em>/imipenem</td>
<td>92</td>
<td>92%</td>
<td>98%</td>
</tr>
<tr>
<td><em>A. baumannii</em>/ceftazidime</td>
<td>76</td>
<td>98%</td>
<td>82%</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>103</td>
<td>87%</td>
<td>83%</td>
</tr>
<tr>
<td>MRSA phenotype</td>
<td>307</td>
<td>99%</td>
<td>99%</td>
</tr>
<tr>
<td>MLSb phenotype</td>
<td>134</td>
<td>97%</td>
<td>99%</td>
</tr>
<tr>
<td>SA-hVISA</td>
<td>23</td>
<td>93%</td>
<td>100%</td>
</tr>
<tr>
<td>ESBL phenotype</td>
<td>126</td>
<td>94%</td>
<td>95%</td>
</tr>
<tr>
<td>KPC phenotype</td>
<td>20</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Bronchoaveolar Lavage (BAL)

- Fibreoptic bronchoscopy
- BAL samples larger portion of lung than TA
  - Sens 72-100%, spec 69-100%
  - Increased with protected catheter
  - Sens 92%, Spec 97%
  - Threshold of 104 CFU/ ml
- Blind mini-BAL – telescoping catheter
  - Performed by resp technicians
  - Protected catheter-approach
  - Compares favorably to bronchoscopic BAL
  - Threshold of 104 CFU/ ml

- However, regardless of technique, laboratory processing is
  - Cumbersome
  - Slow
  - Labor intensive
  - Associated Costs
Proactive Mini-BAL Surveillance

CHALLENGE: DIFFERENTIATING COLONIZATION FROM INFECTION

And DEPENDS OF ROUTINE Cx – 48hr Turn around time

- Surveillance Cx (mini-BAL 3x/week) within 12 h of MV then 3x/wk. (58 VAP in 50 patients)
  - Argyle (De Lee) suction catheter; 30mL NS; 10% return
  - Gram stain; Quantitatively cultured

- Concordant pathogens: 85% from mini-BAL & ETA 2 days prior to VAP – (2652 mini-BALs)
  - Mini BAL useful for early sampling – minimal EA.

- $>10^4$ cfu/mL colonies for predicted VAP:
  - Sens 84%, Spec 50%, PPV 31%; NPV 93%

R. Boots Respirology. 2008
Mini-BAL Surveillance

- No CAP/VAP: 37% colonized with potential pathogens
  - 27% $\geq 10^4$ cfu/mL
- Stable abiogram from d -4
- VAP causative organism may not be present even 24 h prior diagnostic specimens must continue to be collected

R. Boots Respirology. 2008
BAL Specimens

The Good

The Bad

The Ugly
BAL Sample Clean up

1. Tube shearing

Prolonged Vortexing Histidine. SDS .03%
Mucus Clean-up with S. griseus Pronase ATCC Spiked BAL Samples

<table>
<thead>
<tr>
<th>Pronase mg/mL</th>
<th>Mean Clone Number/FOV</th>
<th>Mean Division/Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>28.0</td>
<td>2.0</td>
</tr>
<tr>
<td>0.25</td>
<td>30.3</td>
<td>2.0</td>
</tr>
<tr>
<td>0.5</td>
<td>59.7</td>
<td>1.9</td>
</tr>
<tr>
<td>1</td>
<td>29.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

- Negative BAL sample “spiked” with ATCC S. Aureus
- Clone numbers and Division Rates
- 0.5mg/mL Pronase optimal
### Antimicrobial Susceptibility Results

<table>
<thead>
<tr>
<th>Species</th>
<th>Antibiotic</th>
<th>Essential Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. baumannii</em></td>
<td>Amikacin</td>
<td>94</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>Ciprofloxacin</td>
<td>92</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>Imipenem</td>
<td>91</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>Meropenem</td>
<td>96</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Daptomycin</td>
<td>100</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Doxycycline</td>
<td>93</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Gentamicin 500</td>
<td>100</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Linezolid</td>
<td>93</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Streptomycin 1000</td>
<td>100</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Vancomycin</td>
<td>93</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>Amikacin</td>
<td>93</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>Cefepime</td>
<td>93</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>Ceftazidime</td>
<td>92</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>Colistin</td>
<td>94</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>Meropenem</td>
<td>93</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Ciprofloxacin</td>
<td>92</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Colistin</td>
<td>96</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Imipenem</td>
<td>97</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Tobramycin</td>
<td>93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Isolates</th>
<th>Number of Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. baumannii</em></td>
<td>308</td>
<td>783</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>918</td>
<td>2263</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>117</td>
<td>334</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>250</td>
<td>598</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1593</strong></td>
<td><strong>3978</strong></td>
</tr>
</tbody>
</table>

![Essential Agreement Graph](image-url)

*Accelerate Diagnostics internal development data on system prototype*
Cell death in Flow cells 2 and 3 indicate *Staph aureus* infection will be susceptible to Cefoxitin and Clindamycin.

Traditional disc diffusion methods indicate that the isolate of *Staph aureus* is susceptible to both antibiotics.

MADM result reported within hours instead of 2-3 days.
Time lapse images
*S. aureus* in growth well vs cefoxitin well

Flow cell 1
(growth well)

Flow cell 4
(cefoxitin)
S. aureus Clone Report with and without antibiotic exposure in growth media

Flow cells 1-2 “Growth wells” with no antibiotic; Flow cell 3 contains 1µg/mL Clindamycin; Flow cell 4 contains 6µg/mL Cefoxitin
Divisions/Hr for *S. aureus* with and without antibiotic exposure in growth media

Flow cells 1-2 “Growth wells” with no antibiotic; Flow cell 3 contains 1μg/mL Clindamycin; Flow cell 4 contains 6μg/mL Cefoxitin
CO-PILOT Rapid MDRO VAP study

Surveillance sampling (prolonged ventilation, VAP risk)

- 6-8 hr rapid flowcell quantitative ID & sens
- Comparison with conventional methods

Delayed clinical VAP diagnosis; empiric Abx

- 72 hr conventional micro ID & sensitivities
- Potential Abx failure; Abx resistance
- 72 hr conventional micro ID & sensitivities
- ↑ morbidity/mortality

- Reduced Abx failure; controlled Abx resistance
- ? ↓ morbidity/mortality

I Douglas AJRCCM. 2015 Mar 1;191:566-73.

NCT00938002
• Adult MICU patients
  • Within 72h of intubation anticipated to require MV >48h
• Mini-BAL on Day 1, 3, 5, 7 and 10
• Samples processed
  • Routine quantitative culture/susceptibility and
  • ID/AST – rapid micro diagnostic
• Viable bacteria identified using growth analysis and response to key antibiotics
  • Focused VAP antibody panel (S. aureus, P. aeruginosa, A. baumannii) for specific ID
## Target Species and Resistance Phenotypes Tested

<table>
<thead>
<tr>
<th>Species</th>
<th>Resistance phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>MRSA phenotype&lt;br&gt;clindamycin resistance&lt;br&gt;(constitutive or inducible)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>amikacin resistance&lt;br&gt;piperacillin/tazobactam resistance</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>imipenem resistance&lt;br&gt;ceftazidime resistance</td>
</tr>
</tbody>
</table>
CO-PILOT Rapid MDRO VAP study

- 33 MV patients
  - Age 55 (27-84) years
  - Female 38%; Active smokers 50%
  - APACHEII: 21 (16-24); 33% ICU mortality
  - CXR infiltrates: 11 (32%) focal; 20 (60.6%) patchy
  - MV days: 4 (6-10)
  - ICU days: 10.5 (6.5-18.2)
- 77 miniBALs (median 2; Range 1-7 per patient)
  - 73 miniBALs subjected to ID/AST.
- 15 BAL samples (11 pt) colonies at >10⁴ CFU/mL by QCx.
- 21 unique isolates

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4 S. aureus, (2 MRSA)</td>
<td>2 K. pneumonia;</td>
</tr>
<tr>
<td>2 S. maltophilia;</td>
<td>1 NLF GNB</td>
</tr>
<tr>
<td>1 H. Influenza;</td>
<td>11 mixed respiratory flora.</td>
</tr>
</tbody>
</table>

ID/AST prototype analysis

• 10 samples ($>10^4$ CFU/mL) pathogens by QCx.
  • ID/AST identified 5 of 6 target panel organisms accurately
    • matched antimicrobial sensitivity patterns in 4 samples
      • (2 S. maltophilia, 2 MRSA).
  • Off-panel organisms detected by QCx but not ID/AST
    • 2 NLF GNB, 1 LF-GNB, 1 H. inf. None developed clinical VAP.
  • ID/AST : 100% specific for samples negative by QCx
    • (28 of 28).
  • 1 VAP was diagnosed by CPIS and clinical criteria
    • (QCx negative; ID/AST detected untypable enteric organisms ($>10^5$CFU/mL) on day.

I Douglas AJRCCM. 2015 Mar 1;191:566-73.
## Microbiologically-Positive Identifications

### Clinical Culture vs Automated Microscopy

<table>
<thead>
<tr>
<th>(Pt.-Day)</th>
<th>CPIS</th>
<th>Discharge Status</th>
<th>Clinical Culture:</th>
<th>Automated Microscopy:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Identification</td>
<td>Identification</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Concentration (CFU/ml)</td>
<td>Concentration (CFU/ml)</td>
</tr>
<tr>
<td>3-D1</td>
<td>4</td>
<td>SNF</td>
<td>S. aureus*,†</td>
<td>fastidious organism‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$-$10^5$</td>
<td>$1.07\times10^4$</td>
</tr>
<tr>
<td>5-D7</td>
<td>3</td>
<td>Died</td>
<td>no isolate§</td>
<td>enteric‖</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>$1.28\times10^5$</td>
</tr>
<tr>
<td>6-D1</td>
<td>6</td>
<td>Home</td>
<td>S. maltophilia</td>
<td>S. maltophilia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$&gt;10^5$</td>
<td>$7.68\times10^5$</td>
</tr>
<tr>
<td>6-D3</td>
<td>9</td>
<td>Home</td>
<td>S. maltophilia</td>
<td>S. maltophilia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$-$10^5$</td>
<td>$1.60\times10^4$</td>
</tr>
<tr>
<td>8-D7</td>
<td>9</td>
<td>Died</td>
<td>S. aureus*,**</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$&gt;10^5$</td>
<td>$1.11\times10^6$</td>
</tr>
<tr>
<td>8-D10</td>
<td>9</td>
<td>Died</td>
<td>S. aureus</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$-$10^5$</td>
<td>$1.42\times10^5$</td>
</tr>
<tr>
<td>17-D1</td>
<td>7</td>
<td>SNF</td>
<td>K. pneumoniae*</td>
<td>unknown/ enteric</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$-$10^5$</td>
<td>$1.87\times10^4$</td>
</tr>
<tr>
<td>22-D3</td>
<td>8</td>
<td>Home</td>
<td>S. aureus*</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$-$10^5$</td>
<td>$4.00\times10^4$</td>
</tr>
<tr>
<td>33-D7</td>
<td>6</td>
<td>Home</td>
<td>Candida*††</td>
<td>S. aureus‡‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^4$-$10^5$</td>
<td>$6.64\times10^4$</td>
</tr>
</tbody>
</table>
# ID:AST Performance – MDRO VAP

<table>
<thead>
<tr>
<th></th>
<th>Clinical Micro $\geq 10^4$ CFU/mL</th>
<th>Present/Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Automated Microscopy</strong></td>
<td>Positive</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>64</td>
</tr>
</tbody>
</table>

- **Sensitivity**: 100%
- **Specificity**: 97%

I Douglas AJRCCM. 2015 Mar 1;191:566-73.
Simulated Impact of Microscopy ID/AST on Antimicrobial Prescribing

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample</th>
<th>Discontinued</th>
<th>Started</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Day 1</td>
<td></td>
<td></td>
<td>no change</td>
</tr>
<tr>
<td>5</td>
<td>Day 7</td>
<td>piperacillin-tazobactam, vancomycin</td>
<td>amikacin, imipenem</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Day 1</td>
<td>imipenem, levofloxacin, vancomycin</td>
<td>trimethoprim-sulfamethoxazole</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Day 3</td>
<td></td>
<td></td>
<td>no change</td>
</tr>
<tr>
<td>8</td>
<td>Day 7</td>
<td>metronidazole, piperacillin-tazobactam</td>
<td>vancomycin</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Day 10</td>
<td></td>
<td></td>
<td>no change</td>
</tr>
<tr>
<td>17</td>
<td>Day 1</td>
<td></td>
<td></td>
<td>no change</td>
</tr>
<tr>
<td>22</td>
<td>Day 3</td>
<td></td>
<td></td>
<td>no change</td>
</tr>
<tr>
<td>33</td>
<td>Day 7</td>
<td></td>
<td></td>
<td>no change</td>
</tr>
</tbody>
</table>

I Douglas AJRCCM. 2015 Mar 1;191:566-73.
The Numbers Challenge: What is the system’s lower limit of detection?

- Positive Blood culture bottle from instrument contains very high numbers of bacteria ($10^7$ to $10^9$ bacteria/mL).
- Diagnostic threshold for a BAL is $10^4$ bacteria/mL.
- Strategies for recovering low numbers in BAL:
  - Pre-growth in nutrient broth before or during process
  - Higher concentrations of reagent requiring lower dilutions of specimen
  - Optimal recovery from GEF well
  - Initial volume concentration of sample
Biomarkers

- Risk Prediction
- Early detection
- Diagnosis
  - Microbial
  - Host immune response
- Severity assessment
- Treatment response monitoring
- Proof of cure/progression

- Plasma
  - PCT
  - CRP

- BALF
  - IL-1b
  - IL-8
  - sTREM-1
  - Cathelecidin

TP Hellyer Thorax. 2015 Jan;70(1):41-7
Volatile Organic Compounds (VOC)

- E-nose:

S. aureus and C. albicans
E. Coli

- 2-pentanone
- acetonitrile
- dimethylsulfide
- propane
- 4-heptanone

Rapid Diagnostics – Future Challenges

1. Direct from (difficult) specimens
   - Skin, soft-tissue, bone, devices
2. Clinically relevant rapid TAT
3. Broad, clinically relevant drug-bug combos
4. Point-of-care platforms
5. Non bacterial assays
6. Integration with relevant serological/other biomarkers for risk prediction, treatment responsiveness prediction
7. Cost, cost, cost
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• Ellen Tuttle
• Meghan Mensack
• Lawrence Mehren

CDMRP
• Dwayne Taliaferro
• Mirlene Desir
• Amber Linde
Now I Know Why......

QUESTIONS?

idouglas@dhha.org
for lecture hand-outs