Robert A. Bonomo, MD
Barry Kreiswirth
Scott Evans, PhD

RAPID MOLECULAR DIAGNOSTICS
INFORMING ANTIBIOTIC TREATMENT DECISIONS
Acknowledgements and Disclosures

- ECCMID Program Committee; special thanks to Professors Orjan Samuelson and Carolina Marquez.
- Dr. Maria Virginia Villegas, ECCMID Fellow
- VA Merit Review, NIH, ARLG, Harrington Foundation
- Research Grants (beta-lactamase inhibitors)
  - AstraZeneca
  - Allergan
  - Merck
  - Wockhardt
  - GSK
  - Roche
Case Study (Adapted)

• A 41-year-old man w/ type 1 DM underwent kidney transpltx in Bogota, Colombia, after being maintained on HD x 4 years.

• Induction immunosuppression included ATG, mycophenolate mofetil, tacrolimus, and methylprednisolone.

• Pre-transplantation evaluation had revealed that the patient was CMV-seronegative; he received valganciclovir, as well as TMP-SMX and fluconazole prophylaxis.

Patel et al, Transplant Infectious Diseases, 2015
Case Study

- On POD 4, VS = 34.9°C, 115 bpm, BP = 90/60 mmHg, and WBC = 1.7 cells × 10^3/mm).
- Upon PE, the abd- distended. Blood and urine cultures were obtained; vancomycin and pip/tazo were started empirically, and fluconazole continued.
- An EX LAP was performed on the evening of POD 5 because of concern for an intra-abdominal source of sepsis.
- 2 L of serosanguinous peritoneal fluid were drained; renal allografts was deemed viable, and there was no evidence of necrosis.
- Gram stain of a sample of peritoneal fluid revealed WBC 4+ and GNRS
Case Study

- A repeat EX LAP was performed on the morning of POD 6. The nonviable renal allograft was removed.
- Shock, CP arrest
- On the morning of POD 6, cultures obtained from peritoneal fluid yielded 4+ growth of gram-negative bacilli.
  - *K. pneumoniae* resistant to pip/tazo, all carbapenems, all cephalosporins, fluoroquinolones, and aminoglycosides.
  - In contrast, the isolate was susceptible to colistin, polymyxin B, and tigecycline.
Would a RMD have saved the allograft and this patient’s life?
By 2050, increases in antimicrobial resistance (AMR) will be responsible for 300 million deaths

By setting out the full magnitude of the potential human and economic costs of rising drug resistance, this paper demonstrates that there is a clear global imperative to take this threat seriously and start finding solutions, not least as action taken now could dramatically reduce both the enormous financial and human impact of resistant infections in the future.

Total GDP Loss
100.2 Trillion USD

The Promise of Rapid Molecular Diagnostics (RMDs)
Better Tests, Better Care: Improved Diagnostics for Infectious Diseases

Angela M. Caliendo,1 David N. Gilbert,2,3 Christine C. Ginocchio,4,5,6 Kimberly E. Hanson,7,8 Larissa May,9 Thomas C. Quinn,10,11 Fred C. Tenover,12 David Alland,13 Anne J. Blaschke,14 Robert A. Bonomo,15,16,17,18 Karen C. Carroll,19,20 Mary Jane Ferraro,21,22 Lisa R. Hirschhorn,23,24 W. Patrick Joseph,25,26,27,28 Tobi Karchmer,29 Ann T. MacIntyre,30,31
L. Barth Reller,32,33 and Audrey F. Jackson,34 for the Infectious Diseases Society of America (IDSA)

How do we make this happen?? Can we make it relevant?
Value of RMDs
- POC and direct patient mgmt, novel agents, stewardship

Unmet diagnostic needs in the clinical setting
- OPD, ICU, CSF infx, sepsis, immunocompromised hosts

New/developing technologies: impact on unmet clinical needs
- MALDI, T2, etc.

Challenges to diagnostics R&D

Challenges to the adoption of diagnostic tests
- Regulatory hurdles, understanding of assay results
“Criteria used in evaluating a diagnostic test”

- Sensitivity, specificity, positive/negative predictive values and likelihood ratios, and accuracy (the overall percentage that is correctly classified)
- “Very major error rates” diagnostic test indicates susceptibility when the reference test indicates resistance
- “Major error rates” diagnostic test indicates resistance when the reference test indicates susceptibility
Which diagnostic should be selected to optimize clinical outcomes???
Micro Lab in Transition
Crawl, Walk, Run, Fly....

Dr. Vance Fowler
PRIMERS (Platforms for Rapid Identification of MDR-gram negative bacteria and Evaluation of Resistance Studies)

- PRIMERS-I and –II; introduction, defining an approach to the challenge of RMD and bacterial resistance (*E. coli* and *K. pneumoniae*)

- PRIMERS-III; applying what was learned to an MDRO (*Acinetobacter* spp.)

- PRIMERS-IV; can we mimic clinical and registration trials (*P. aeruginosa*)

- BED-FRAME: why PRIMERS worked
Addressing the challenge of RMD in antimicrobial resistance: needed a new/better approach

Rapid Molecular Diagnostics, Antibiotic Treatment Decisions, and Developing Approaches to Inform Empiric Therapy: PRIMERS I and II

Scott R. Evans, Andrea M. Hujer, Hongyu Jiang, Kristine M. Hujer, Thomas Hall, Christine Marzan, Michael R. Jacobs, Rangarajan Sampath, David J. Ecker, Claudia Manca, Kalyan Chavda, Pan Zhang, Helen Fernandez, Liang Chen, Jose R. Mediavilla, Carol B. Hill, Federico Perez, Angela M. Caliendo, Vance G. Fowler Jr, Henry F. Chambers, Barry N. Kreiswirth, and Robert A. Bonomo, for the Antibacterial Resistance Leadership Group
A New Analytical Approach-I
New language

- **Susceptibility sensitivity**
  - the probability that the test result is susceptible when the reference standard is susceptible

- **Resistance sensitivity**
  - the probability that the test result is resistant when the reference standard is resistant
Novel Analytical Approach

Sensitivity

Resistance Sensitivity

Specificity

Susceptibility Sensitivity
**Novel Analytical Approach-II; SPV, RPV, Dim Sum….**

- The **SPV** (susceptibility predictive value) and **RPV** (resistance predictive value) are functions of the prevalence of susceptibility.
- Since there are temporal and geographic variations in the prevalence of susceptibility, the SPV and RPV can be assessed as a function of the prevalence of susceptibility (with 95% confidence bands) to allow for interpretation across the spectrum of prevalence.
- **Discrimination summary plots** are used to display the 95% confidence interval (CI) estimates of susceptibility sensitivity and resistance sensitivity.
PRIMERS-I and II

- PRIMERS-I: Four RMDs for identifying β-lactam resistance (\textit{bla}) in isolates belonging to \textit{Enterobacteriaceae}
  - 72 strains of highly drug-resistant \textit{E. coli} and \textit{K. pneumoniae}
- MB
- PCR/ESI-MS
- NA Microarray (Checkpoints)
- WGS
**bla genes selected; genotype responsible for phenotype**

The complexity of this search was challenging

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>bla Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin/Sulbactam</td>
<td>SHV-WT (SHV-1), KPC, NDM, VIM, IMP, OXA-48, CMY-1/MOX, CMY-2/FOX</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic</td>
<td>KPC, NDM, VIM, IMP, OXA-48, CMY-1/MOX, CMY-2/FOX</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>KPC, NDM, VIM, IMP, OXA-48, CMY-1/MOX, CMY-2/FOX</td>
</tr>
<tr>
<td>Cefoxitin (II)</td>
<td>KPC, NDM, VIM, IMP, OXA-48, CMY-1/MOX, CMY-2/FOX</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>KPC, NDM, VIM, IMP, OXA-48</td>
</tr>
<tr>
<td>Imipenem</td>
<td>KPC, NDM, VIM, IMP, OXA-48</td>
</tr>
<tr>
<td>Meropenem</td>
<td>KPC, NDM, VIM, IMP, OXA-48</td>
</tr>
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</table>

\(^a\) Indicates CTX-M group.
PRIMERS-I

<table>
<thead>
<tr>
<th>Isolate Count</th>
<th>Amoxicillin Clavulanic acid</th>
<th>Ampicillin</th>
<th>Aztreonam</th>
<th>Cefazolin</th>
<th>Cefepime</th>
<th>Cefotaxime</th>
<th>Cefozitin</th>
<th>Ceftriaxone</th>
<th>Ertapenem</th>
<th>Imipenem</th>
<th>Meropenem</th>
<th>Piperacillin Tazobactam</th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>6</td>
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72 isolates
37 R to all cephs and carbs

PRIMERS-II

<table>
<thead>
<tr>
<th>Isolate Count</th>
<th>Amoxicillin Clavulanic acid</th>
<th>Ampicillin</th>
<th>Aztreonam</th>
<th>Cefazolin</th>
<th>Cefepime</th>
<th>Cefotaxime</th>
<th>Cefozitin</th>
<th>Ceftriaxone</th>
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<th>Meropenem</th>
<th>Piperacillin Tazobactam</th>
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<td>R</td>
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<td>R</td>
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<td>S</td>
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</tr>
</tbody>
</table>
PRIMERS-I  Resistance Sensitivity
Similar performance of all 4 RMDs vs. 14 β-lactams (95% CIs).

Discrimination summary plots used to display estimates of S and R sensitivity for certain carbapenems and β-lactam/β-lactamase combinations (A/C, P/T) was “suboptimal”

“the probability that the test result is resistant when the reference standard is resistant”

Platform did not matter
• PRIMERS-II, 2 platforms (PCR/ESI-MS and MB) were selected and tested in a blinded fashion against a heterogeneous collection of 196 susceptible and drug-resistant isolates of *E. coli* and *K. pneumoniae*

- Can an RMD discriminate S vs. R?
- Can an RMD improve clinical decision-making regarding the selection of empiric antimicrobial therapy.
Results--PRIMERS-II PCR/ESI-MS (E. coli and K. pneumoniae); did we do as well???

Discrimination summary plots used to display estimates of S and R sensitivity.
PRIMERS-II MB (E. coli and K. pneumoniae)
PRIMERS-II Ion Torrent and NA Microarray (E. coli and K. pneumoniae) Resistance Sensitivity

Discrimination
Summary plots
How does one apply these findings to our patient??

What is the prevalence of carbapenem resistant K. pneumoniae in Bogota, Colombia?
If 50% prevalence of resistance, RMDs perform differently.

- Imipenem\textsuperscript{R} \textit{K. pneumoniae} prevalence = 5%, the SPVs of PCR/ESI-MS and MB are 100% and 99% for Imi, respectively.

- In contrast, for that same situation, RPVs of PCR/ESI-MS are 41%.
200 *Acinetobacter* spp. isolates

Carb R vs. S and decision to use more complex rx

*bla* \(\text{NDM, } -\text{VIM, } -\text{IMP, } \text{KPC;}\)

*bla* \(\text{OXA-23, } \text{bla}_{\text{OXA-24/40, -58}}\)

MICs

Chose isolates that reflect current crisis in resistance
Differences in the actual number that are S or R may be due to (i) the ability of different carbapenems to penetrate the outer membrane of *Acinetobacter* spp. and (ii) the activities/potencies of each carbapenem versus the carbapenemase harbored by the strain.

**TABLE 2** Carbapenem phenotypic profile of 200 isolates studied

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>Susceptibility to(^a):</th>
<th>Doripenem</th>
<th>Imipenem</th>
<th>Meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>99 S, 101 R</td>
<td>102 S, 98 R</td>
<td>100 S, 100 R</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)S, susceptible; R, resistant.

**TABLE 3** Genotypic profile of 200 isolates studied via PCR ESI-MS/MB

<table>
<thead>
<tr>
<th>No. of isolates(^a)</th>
<th><em>bla</em>(^{OXA-23})</th>
<th><em>bla</em>(^{OXA-24/40})</th>
<th><em>bla</em>(^{OXA-58})</th>
</tr>
</thead>
<tbody>
<tr>
<td>89/106</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>79/82</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24/9</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3/1</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5/2</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)Isolate count for PCR ESI-MS/MB.
## Results

<table>
<thead>
<tr>
<th></th>
<th>PCR/ESI-MS</th>
<th>MB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resistance</strong></td>
<td><strong>Susceptibility</strong></td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td>CI (95%)</td>
<td>CI (95%)</td>
</tr>
<tr>
<td>96%</td>
<td>(91-99%)</td>
<td>89%</td>
</tr>
<tr>
<td>(74-89%)</td>
<td>(81-94%)</td>
<td>92%</td>
</tr>
<tr>
<td>(85-97%)</td>
<td>(85-97%)</td>
<td></td>
</tr>
</tbody>
</table>
Distribution of *Acinetobacter* spp. carbapenem MICs versus target (gene) identification for PCR/ESI-MS and MB platforms. All gene targets were examined; only identified targets are presented.

- + gene targets in the S range
- Interestingly, very few isolates that demonstrate MICs near the breakpoint possessed *bla* carbapenemase genes.
If the RMD detects resistance gene, you are pretty sure it is resistant.

However, if the platform does NOT detect resistance, may miss!
In a setting where the level of CR Acinetobacter spp. is low, there is an increased likelihood for the RMD to inaccurately identify resistance.

Greatest impact, high prevalence of CR Acinetobacter spp. (24/7 lab)
PRIMERS-IV: mimic a clinical registration trial

- 197 isolates of *P. aeruginosa*
- R or S to ceftazidime/avibactam and ceftolozane/tazobactam (CZA and TOL/TAZO).
- RMDs
  - Verigene BC-GN
  - Acuitas Resistome Test
**Susceptibility Sensitivity**

<table>
<thead>
<tr>
<th>Antimicrobial Combination</th>
<th>Sensitivity</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftolozane/Tazobactam (n=144)</td>
<td>1.00</td>
<td>(0.97, 1.00)</td>
</tr>
<tr>
<td>VERIGENE BC-GN</td>
<td>1.00</td>
<td>(0.97, 1.00)</td>
</tr>
<tr>
<td>Acuitas Resistome Test</td>
<td>1.00</td>
<td>(0.97, 1.00)</td>
</tr>
<tr>
<td>Ceftazidime/Avibactam (n=152)</td>
<td>1.00</td>
<td>(0.98, 1.00)</td>
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<tr>
<td>VERIGENE BC-GN</td>
<td>1.00</td>
<td>(0.98, 1.00)</td>
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<tr>
<td>Acuitas Resistome Test</td>
<td>1.00</td>
<td>(0.98, 1.00)</td>
</tr>
</tbody>
</table>

**Resistance Sensitivity**

<table>
<thead>
<tr>
<th>Antimicrobial Combination</th>
<th>Sensitivity</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftolozane/Tazobactam (n=53)</td>
<td>0.66</td>
<td>(0.52, 0.78)</td>
</tr>
<tr>
<td>VERIGENE BC-GN</td>
<td>0.66</td>
<td>(0.52, 0.78)</td>
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<tr>
<td>Acuitas Resistome Test</td>
<td>0.66</td>
<td>(0.52, 0.78)</td>
</tr>
<tr>
<td>Ceftazidime/Avibactam (n=45)</td>
<td>0.33</td>
<td>(0.20, 0.49)</td>
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<tr>
<td>VERIGENE BC-GN</td>
<td>0.33</td>
<td>(0.20, 0.49)</td>
</tr>
<tr>
<td>Acuitas Resistome Test</td>
<td>0.33</td>
<td>(0.20, 0.49)</td>
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BED-FRAME: the basis for analysis

Clinical Infectious Diseases
INVITED ARTICLE
HEALTHCARE EPIDEMIOLOGY: Robert A. Weinstein, Section Editor

Benefit-risk Evaluation for Diagnostics: A Framework (BED-FRAME)

Scott R. Evans,1,2 Gene Pennello,3 Norberto Pantoja-Galicia,3 Hongyu Jiang,2 Andrea M. Hujer,4 Kristine M. Hujer,4 Claudia Manca,5 Carol Hill,6 Michael R. Jacobs,4 Liang Chen,5 Robin Patel,7 Barry N. Kreiswirth,5 and Robert A. Bonomo4, for the Antibacterial Resistance Leadership Group

• Diagnostic yield depends on prevalence

• Different diagnostic errors (false positive and false negative) carry different clinical consequences

Why PRIMERS worked
Using PRIMERS-III data....

12,000 cases of multidrug-resistant (MDR) *Acinetobacter* spp. infections occur and are associated with approximately 500–750 deaths per year. Approximately 63% of infections are MDR.

The benefit risk tradeoff
Slide-rule profile plots indicate the expected diagnostic yield (the expected distribution of true susceptible [TS], true resistance [TR], false susceptible [FS], and false resistance [FR] results) as a function of the imipenem susceptibility rate.
Can a clinician pick better empiric rx in the case of Ec and Kp BSI using an RMD?

**What we are doing now...PRIMERS I-II, -III, -IV**

- Blood culture identifies GNR
- VINCI Data Base
  - E. coli
  - K. pneumoniae
  - BSI, 10 years

**RMD**

**Choices**
- GNRx1
- GNRx1+ GNRx2
- GN Rx3

**D/C GNRx**

**ID and AST**

**“Best” GNrx**
Conclusions-I

1) It really matters what the population is that the RMD is applied to (ICU, LTAC, hospital >> community, doctor office) - prevalence of resistance is paramount; this is addressed in the BED FRAME paper.

2) It is very difficult to find a platform that defines (or will define) every possible mechanism of resistance; we have "experience with Gram negatives", what about Gram-positives???

3) Even the best approach (WGS ??) will have limitations (expression levels not really assessed well).

4) We need to accept that RMDs need to be defined with regard to outcomes.
Conclusions-II

5) We still have to make decisions regarding risks (especially if applied to stewardship); BED FRAME again...

6) Novel mechanisms of resistance will be missed;

7) For some antibiotics, we may never reach the level of confidence we need (beta-lactam beta-lactamase inhibitor combinations);

8) We still need to know how well RMDs do compared to the "best guess" that clinicians make. In other words, how bad is empiric therapy now? How much better would a RMD improve this (searching the VA data base for this)?

9) Although we did not address this, many RMDs will yield a DNA “answer” and cultures will be negative. How do we interpret this??
Thank you!