Symposium: The future of hospital antibiotic stewardship - what should we achieve by 2020?

Rapid DNA-based tests

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Disclosures
Research funding form Pfizer and Abbott
Speaker’s honorarium from Becton-Dickinson
Rapid DNA Tests in Infectious Diseases

• **Screening Assays**
  – MRSA
  – MDR Enterobacteriaceae

• **Infection Detection Assays**
  – Sepsis

• **Envisioning 2020**
Classical Methods in Bacterial Identification

- Gram’s stain
- Culture / antibiotic susceptibility
- Biochemical identification
Two Sides of Rapid DNA Testing

ADVANTAGES

• High sensitivity
  – Theoretically detects a single organism

• High specificity
  – Specific genotypes
  – Drug resistance
  – Predict virulence

• Speed
  – Quicker than culture

• Simplicity
  – Some assays are now automated

DISADVANTAGES

• Too sensitive? clinical relevance of results?
  – Especially in antibiotic-treated patients

• So specific that clinical data of aetiology needed before testing

• New organisms missed unless molecular unknowns are sequenced

• Expensive
Rapid DNA Tests in Infectious Diseases

- **Screening Assays**
  - MRSA
  - MDR Enterobacteriaceae

- **Infection Detection Assays**
  - Sepsis

- **Envisioning 2020**
Rapid Screening Tests for MRSA

BD GeneOhm MRSA and Cepheid GeneXpert MRSA assays targeting the SCCmec-orfX junction

- Real-time PCR
- Definitive identification of MRSA
- Target both HA- and CA-MRSA
- Time to result is $\approx 1.30$ hrs
Rapid Screening Tests for MRSA

BD GeneOhm MRSA and Cepheid GeneXpert are comparable and useful

- GeneOhm: Batched and may need technical expertise, € 25/test
- Xpert: Modular/batched, user-friendly, € 35/test

<table>
<thead>
<tr>
<th>Assay</th>
<th>Samples</th>
<th>Sensitivity (95%CI)</th>
<th>Direct-culture</th>
<th>Preenriched-culture</th>
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<tbody>
<tr>
<td>GeneOhm</td>
<td>Nasal</td>
<td>90.9%</td>
<td>71.4%</td>
<td>(82.5–99.4)</td>
</tr>
<tr>
<td></td>
<td>Groin</td>
<td>100%</td>
<td>90.5%</td>
<td>(81.6–100)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>96.4%</td>
<td>81.0%</td>
<td>(82.3–99.4)</td>
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<tr>
<td>Xpert</td>
<td>Nasal</td>
<td>100%</td>
<td>57.1%</td>
<td>(74.1–100)</td>
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<tr>
<td></td>
<td>Groin</td>
<td>88.2%</td>
<td>76.1%</td>
<td>(65.7–96.7)</td>
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<tr>
<td></td>
<td>All</td>
<td>92.9%</td>
<td>66.7%</td>
<td>(77.4–98.0)</td>
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</table>

Malhotra-Kumar et al., JCM, 2010
Rapid DNA tests

Disadvantages of targeting the SCCmec-orfX region

- **OrfX** is also a preferred site for insertion of SCCmec in other staphylococci
- False-positive reactions with MRCoNS on both assays
  - Upto 99% sequence homology with *S. aureus* orfX
  - *MecA* excised ‘former’ MRSA
Impact of Rapid MRSA Tests

- On MRSA acquisition rates/1000 patient-days
  - No effect on MRSA acquisition (vs. culture screening)

- On incidence of MRSA bloodstream infections/1000 patient days
  - Significant decrease (46%) in MRSA bloodstream infections (vs no screening)

- On MRSA surgical site infections/100 surgical procedures
  - Non-significant trend to reduction in surgical-site infections (vs no screening)

Tacconelli et al, LID, 2010
Current MRSA Screening Methods in Europe

Results from questionnaires submitted by 23 hospitals participating in MOSAR

<table>
<thead>
<tr>
<th>In-house methods</th>
<th>Hospital laboratories (n=23)</th>
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</thead>
<tbody>
<tr>
<td>Conventional media</td>
<td>7 (30%)</td>
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<tr>
<td>Chromogenic media</td>
<td>9 (39%)</td>
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<tr>
<td>ChromID (BioMerieux)</td>
<td>5</td>
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<tr>
<td>CHROMagar MRSA (BD Diagnostics)</td>
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<tr>
<td>MRSA Select (Bio-Rad Laboratories)</td>
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<tr>
<td>Brilliance MRSA/Remel Spectra (Oxoid)</td>
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</tr>
<tr>
<td>Conventional and chromogenic media</td>
<td>7 (30%)</td>
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</table>

Gazin et al, EQA of culture-based detection of MRSA by a network of European laboratories
P1038, May 8, 12.30-13.30, Detection of resistance in Gram-positive bacteria
Decreasing MRSA Trends in Europe

Invasive MRSA trends, EARS-Net, 2009
Courtesy D. Monnet

Wolk et al, JCM, 2009
Rapid DNA tests

MDR Enterobacteriaceae: Emerging Superbugs

- Optimum control strategy unclear
- Screening for MDR Enterobacteriaceae limited

Invasive *K. pneumoniae* resistant to 3rd gen cephalosporins EARS-Net, 2009
Courtesy D. Monnet
Screening for MDR Enterobacteriaceae

- **Check-Points arrays**
  - Check-ESBL: CTX-M, TEM, SHV
  - Check-KPC ESBL: KPC, CTX-M, TEM, SHV
  - Check-MDR CT101: NDM-1, KPC, CTX-M, TEM, SHV, and several AmpCs
  - Check-MDR CT102: NDM-1, VIM, IMP, OXA-48, KPC, and CTX-M, TEM, SHV

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>SHV</td>
<td>98.8%</td>
<td>100%</td>
</tr>
<tr>
<td>TEM</td>
<td>100%</td>
<td>96.4%</td>
</tr>
<tr>
<td>KPC</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

PCR-microarray based commercial assays

Endimiani et al., JCM, 2010
Screening for MDR Enterobacteriaceae

- **Identibac AMR-ve genotyping array**
  - vs. in-house PCR-sequencing

<table>
<thead>
<tr>
<th>Species</th>
<th>CTX-M</th>
<th>SHV</th>
<th>TEM</th>
<th>Agreements</th>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>PCR</td>
<td>Array</td>
<td>Sensitivity %</td>
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<tr>
<td>E. coli</td>
<td>15</td>
<td>13</td>
<td>8</td>
<td>62</td>
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<tr>
<td>K. pneumoniae</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>P. mirabilis</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>S. marcescens</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>E. cloacae</td>
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<td>2</td>
<td>1</td>
<td>50</td>
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<tr>
<td>C. freundii</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>18</td>
<td>12</td>
<td>67</td>
</tr>
</tbody>
</table>

Gazin et al, Evaluation of a commercial miniaturised DNA microarray for detection of beta-lactamase genes in Gram-negative bacteria, **P640, May 7, 15.30-16.30**
Screening for MDR Enterobacteriaceae

- Direct detection from clinical samples is still culture-based
  - Diagnostic delay of at least 24 hours
- More complex than MRSA detection
  - Fecal samples, numerous targets
- Molecular diagnostic solutions with off-board sample preparation

(Resistance in Gram-Negative Organisms: Studying Intervention Strategies)
Rapid DNA Tests in Infectious Diseases

- Screening Assays
  - MRSA
  - Multi-resistant Enterobacteriaceae

- Infection Detection Assays
  - Sepsis

- Envisioning 2020
Rapid DNA tests

- Hybridization
  - Oligonucleotide–FISH, PNA-FISH

- Prove-it™ Sepsis (Mobidiag)
  - Broad-range PCR + mecA detection
  - 86% pathogen coverage
  - Sens 95% Spec 99%
  - Fast method

- LightCycler SeptiFast® PCR (Roche)
  - TAT ≈ 6hrs for 8 samples
  - Sens 76–90% Spec 85–98% (vs. culture)
  - 82% pathogen coverage
  - Limited sens/spec vs. culture

Tissari et al., Lancet, 2010

Jaton-Ogay et al., ECCMID 2008
Regueiro BJ et al, ECCMID 2008
Diagnostic Tests for Sepsis

ADVANTAGES

• Relatively rapid

• Increase detection sensitivity especially useful in particular clinical settings
  – In patients who have been treated with antibiotics
  – Difficult to grow bacteria e.g. Bartonella spp,
  – Neonatal sepsis, …

DISADVANTAGES

• Total TAT should still be shorter to directly impact therapeutic management

• Select clinically relevant pathogens not in the panel

• Sample preparation laborious

• Careful validation in real life is necessary

• Cost
Rapid DNA tests in Infectious Diseases

- **Screening Assays**
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- **Envisioning 2020**
Rapid DNA tests

- Omics
- Technology
- Automation
- Software
Paradigm Shift in Diagnostics

Integrated point of care test/personalized medicine

Rapid DNA tests
Why Require Better, Rapid Point-of-Care Assays?

- There has been under-investment in rapid diagnostics for improving the quality of care for patients with suspected infections
  - Diagnostics influence 60-70% of health care decision making but account for less than 5% of hospital costs (Lewin report 2006)

- POCTs improve antibiotic targeting to only those who will benefit, thus reducing overuse
  - The commonest reason for prescribing antibiotics in the community is acute cough, and these prescriptions virtually never benefit patients (Butler et al, BMJ 2009)
Why Require Better, Rapid Point-of-Care Assays?

- POCTs **enhance surveillance** of pathogens and infectious diseases
  - e.g. H1N1 flu pandemic

- POCTs support **rapid initiation and cessation of treatment**
  - Sepsis is associated with 7% increased mortality for every hour delay in the administration of appropriate antibiotics (Kumar et al, CCM 2006)

- POCTs **decrease the size and cost of antibacterial clinical trials**
  - We URGENTLY need new antibiotics (ECDC/EMA report 2009)
Can you imagine the challenges of shrinking a huge laboratory filled with people and equipment onto a single chip the size of a matchbox?
Huge Challenges and Synergies

**Biotechnologies**
- Integrated sample prep solutions
- Targeting NA + host/pathogen biomarkers
- Novel surface chemistries

**Clinical practice**
- Selection of relevant targets/applications
- Validation of analytical, clinical performance

(Micro)technologies
- Lab-on-a-chip/microfluidics
- Photonics
- Biosensors

Rapid DNA tests
A Big Bottleneck in Developing POCTs: Sample Preparation

- Off-chip (macroscale) sample prep
  - Laborious
  - Refrigerated/frozen reagents
  - Large sample volumes
  - Requires centrifuges, bead beaters, several machines
  - Few hours

- On-chip sample prep
  - Room temperature stable reagents (disposable chips with on-chip storage)
  - Microliter volumes
  - Few minutes!!
Disease-specific challenges... and some solutions

Sepsis
- Blood

LRTI (VAP & CAP)
- Broncho-alveolar lavage (BAL), endotracheal aspirate, sputum, nasopharyngeal aspirates, throat, nasal and nasopharyngeal swab samples in buffer

TB
- Sputum

Bacteria, fungi
- Size based separation and/or selective lysis
- NA extraction

Bacteria, fungi, virus, host protein
- Immunoassay based separation

Sample concentration & extraction

Amplification & labeling

Sample detection

Solution based fluorescent detection

Surface array based
- Flourescent DNA/Immuno array
- DNA/immuno array

(Dual colour) fluorescent detection

Labeled
- Label free

Micro-PCR

No amplification (VAP bacteria)
- No labeling

No amplification
- No labeling
- No amplification
On-chip Bacterial Lysis and DNA Purification

Development of a proprietary bacterial lysis and DNA purification protocol and its successful application on a prototypal microfluidic chip for a CA-LRTI assay

Van Heirstraeten et al., Integrated DNA extraction and purification on an automated microfluidic LOC from bacterial pathogens causing CA-LRTI

P1909, May 9, 13.30-14.30, Molecular Diagnostics

In collaboration with Institut für Mikrotechnik, Mainz, Germany
On-chip Bacterial Lysis, DNA Purification/Amplification

Development of a sample prep solution and on-chip micro-PCR for a rapid patient bed-side sepsis assay

In collaboration with KTH Royal Institute for Technology, Stockholm, Sweden
Developing an efficient, rapid and accurate POCT

- The joint efforts of academia and industry can bring this to reality
- IMI supports collaborative research projects and builds networks of industrial and academic experts in order to boost pharmaceutical innovation in Europe

Microfluidic ChipShop GmbH, Germany
Primary care
Hospital care

Rapid DNA tests
### Collaborators

<table>
<thead>
<tr>
<th>EFPIA MEMBER COMPANIES</th>
<th>UNIVERSITIES, RESEARCH ORGANISATIONS, PUBLIC BODIES &amp; NON-PROFIT</th>
<th>SMEs</th>
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<tbody>
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<td>- GlaxoSmithKline</td>
<td>- Cardiff University, UK</td>
<td>- LIONEX, Germany</td>
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<td>- Virco-Janssen</td>
<td>- Catholic University of Leuven, Belgium</td>
<td>- Microfluidic ChipShop, Germany</td>
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<td>- Merck</td>
<td>- IMEC, Belgium</td>
<td>- Mobidiag, Finland</td>
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<td>- Novartis</td>
<td>- University of Cambridge, UK</td>
<td>- Q-linea, Sweden</td>
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<td>- Sanofi-Aventis</td>
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<td>- Royal Institute of Technology, Sweden</td>
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<td>- University of Antwerp, Belgium</td>
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<td>- University of Twente, Netherlands</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Uppsala University, Sweden</td>
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</table>
Rapid DNA tests

Overall Objective of RAPP-ID

RAPP-ID will develop a Point-of-Care Test (POCT) for rapid (hospital <2h, primary care <30min) detection of bacteria, mycobacteria, fungi, as well as viruses and host biomarkers by combining novel specific probes, novel methods of sample preparation, and demonstrated ultra-high sensitive detection methods. The platforms will also determine resistance to antimicrobial drugs.
Will We Succeed in Developing a POCT?

• Develop scenarios for the targeted diseases (from sample collection to readout)

• Familiarize with technologies and amalgamate with (current) diagnostic and clinical algorithms
  – Potential clinical benefit of optimized disease diagnosis
  – Requirements and profiles of POCT
  – Quantitated information on micro-organisms and host and pathogen biomarkers in different clinical samples

Not just personalized but also microbialized medicine!
2020: An Integrated POCT in Primary Care

1. The patient visits the GP practice with a suspect LRTI. The visit includes testing a swab sample with the TheraTEST assay to determine the presence of pathogens.

2. The GP uses its PDA to start the assay on the TheraPOC device. 20 minutes later, the GP uses the PDA again to check results: presence of pathogens, their type and antibiotic resistance characteristics.

3. TheraGUIDE is the patient finding out about the use and abuse of antibiotics. The system also sends reminders through the patient’s (or caregiver) cellular phone or digital TV set concerning the daily drug dosage intake, the next date for a visit to the GP, etc.

4. At the end of the visit, TheraGUIDE may synchronize with the Primary Care Practice general Information Mgmt System, to consolidate session data into the patient clinical records, enable session reimbursement, etc.

5. The GP uses the TheraGUIDE application on his PDA or PC to record the clinical episode, establish appropriate treatment and to hand out/submit a standard report to the patient including therapeutic guidance.

Broadband network / Interoperability Protocols (HL7, POCT1-A, UPnP, HTML, SMS, etc.)
Acknowledgements

• Laboratory of Medical Microbiology, UA
  – Liesbet van Heirstraeten
  – Jascha Vervoort
  – Muriel Gazin
  – Christine Lammens
  – Sabine Chapelle
  – Liesbet Bryssinck
  – Gert Leten
  – Anouk Vanderstraeten
  – Greetje Vercauteren
  – Margareta leven

Herman Goossens

• University of Geneva Hospitals, Switzerland
  – Stephan Harbarth
  – Andie Lee

• National Medicines Institute, Poland
  – Marek Gniadkowski

• MOSAR, Intopsens, and TheraEdge collaborators

• Companies for providing assays for evaluations