1. Respiratory Tract Infections (RTIs) can be classified into three main categories:

   - Pneumonia
   - Ventilator-Associated Pneumonia (VAP)
   - Community-Acquired Pneumonia (CAP)

2. RTIs affecting the lung parenchyma may develop within 48 hours or more. RTIs are a major cause of morbidity and mortality worldwide. RTIs are easy to use, rapid, high performance alternative to traditional based techniques are often insensitive, laborious and inappropriate, unnecessary, or both. The consequences of inappropriate, unnecessary antibiotic treatment can lead to evolution of antibiotic resistance and unwanted side-effects (such as Clostridium difficile)

3. The INHALE Project: What have we learnt about the molecular diagnostics of HAP/VAP?

   Dr Vicky Enne
   University College London
   Twitter: @Beat_AMR_Bugs
   @HAP_Diagnostics
   Website: www.ucl.ac.uk/inhale-project

4. The RiD-RTI Consortium is a three year collaborative project based in eight hospitals from four countries, the aims of the RiD-RTI project have been to develop a one step, rapid molecular diagnostic for RTIs which will contribute to improved management of RTIs in adults and children alike. Specifically, it is designed to eliminate the current practice of empiric treatment with broad spectrum antibiotics whilst waiting for culture results.

5. Finally, the skills of individual Consortium Partners are being deployed to develop a rapid, easy to use, high performance alternative to traditional techniques for the rapid diagnosis of respiratory tract infections (RTIs).

6. The Project involved researchers from hospitals, healthcare providers, small and medium sized enterprises (SMEs), universities, and technological development and demonstration under grant agreement No 304865 of the European Community’s 7th Framework Programme (2007-2013) for research, technological development and demonstration (FP7).

   - This project has received funding from the European Union’s Seventh Programme for Research, Technological Development and Demonstration under grant agreement No 304865
   - The INHALE Team: University College London Hospitals, National University of Ireland, Galway, University of Leicester, University College London, Mobidiag, Genewave SAS, Zymo Research, NIVI, Vambach,

7. Flowchart showing intended diagnosis and management of pneumonia in immunosuppressed patients (Opportunistic RTIs or ORTIs).

   - If patients is suspected of having CAP, symptoms are developed on a hospital admission to hospital ventilator and viral pathogens are responsible for the rapid identification of respiratory tract infections (RTIs). The project has designed the diagnostic platform around a syndromic presentation rather than a test. Such empiric antibiotic therapy is necessary for the delivery of all the required steps from initial specimen collection, sample preparation, and viral pathogens targets) and molecular detection, identification, quantification (for selected targets) and molecular

8. The Impact of the INHALE Project:

   - The INHALE Project: What have we learnt about the molecular diagnostics of HAP/VAP?
   - Twitter: @Beat_AMR_Bugs
   - Website: www.ucl.ac.uk/inhale-project
Sample arrives in lab

Microscopy/Gram Stain (a few min)

Culture (16h-48h)

Identify growth: MALDI-TOF/Biochemical testing (a few min – 24h)

Antimicrobial susceptibility testing (24-48h)

Further testing / reference laboratory (up to 2 weeks)
# The Need for Rapid Diagnostics

<table>
<thead>
<tr>
<th><strong>TODAY</strong></th>
<th><strong>2-3 days</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physician checks patient for symptoms</strong></td>
<td><strong>Lab technician receives patient sample, pathogens are put on overnight incubation</strong></td>
</tr>
<tr>
<td><strong>In the absence of test results physician has to administer broad therapy</strong></td>
<td><strong>Pathogens are screened for morphological characteristics (eg Gram staining)</strong></td>
</tr>
<tr>
<td><strong>Broad-spectrum Therapy</strong></td>
<td><strong>Infected pathogens is identified and its susceptibility to antibiotics is determined</strong></td>
</tr>
<tr>
<td><strong>Results arrive from lab, specific therapy commences</strong></td>
<td><strong>Resistance</strong></td>
</tr>
</tbody>
</table>

Source: Biopest analysis
What Factors Contribute to the Adoption of Molecular Diagnostics?

In the Clinical Laboratory

• Can it be integrated into the clinical pathway/laboratory workflow?
  • How much does it cost – who pays?
  • Is it easy to use, how much training is required?
  • How much space is needed?
  • How long does it take?
• Is the machine reliable
  • How often does it break down?
  • Is the customer support adequate?

At the Bedside

• Are the results accurate
  • Does the test do “what it says on the tin”?
  • Does is look for the important pathogens/resistance genes?
• Do doctors trust the results & know what to do with them?
• Does using the test actually make a difference:
  • To levels of antibiotic use?
  • To patient outcomes?
Hospital-acquired Pneumonia (HAP)

• Defined as pneumonia that occurs >48h after hospital admission
  • VAP occurs in ventilated patients
• 1.5% of inpatients in UK
• Approx. 200k patients/year
• Mortality rate for HAP/VAP is approx. 25-50%
• Increases to 75% if MDR pathogen
• HAP/VAP adds approx. 8 days to ICU stay
  • costs an additional $30,000 - $37,000 per patient
Project Participants

- UCLH/UCL/UEA (Lead organisations)
- 15 Critical Care Units in England representing diverse case-mix
INHALE WP1: Head to Head Comparison of 3 Rapid Molecular Tests for Pneumonia Diagnosis vs. Routine Microbiology Culture

Curetis Unyvero Pneumonia Test

Biofire FilmArray Pneumonia Panel

Oxford Nanopore Technologies MinION metagenomic sequencing
Clinical Evaluation Sample Characteristics

- 654 eligible samples collected from 15 hospitals
Oxford Nanopore MinION NGS Diagnostics

• Rapid, low-cost NGS sequencing based on nanopore technology
• Capability to obtain full organism & resistance gene profile – not limited to selected targets

• World first comprehensive trial of *rapid* metagenomics for the diagnosis of infection (336 specimens over 9 months)
INHALE Laboratory Custom Work Flow for MinION Processing: Total time to Result = approx. 7h

1. INHALE sputum sample arrives in research lab
2. Deplete human DNA with saponin based method (2h)
3. Extract pathogen DNA using robot & magnetic particles (1h)
4. PCR to tag DNA with barcodes (3h)
5. Fragment DNA & Set up Barcoding PCR (15 min)
6. Manually wash DNA with magnetic beads (30 min)
7. Pool and wash samples (30 min)
8. Library Prep (15 min)
9. Load onto flow cell & start sequencing (first results in 30 min)

INHALE sputum sample arrives in research lab
Routine Microbiology finds Organisms Commonly Associated with HAP/VAP

Median time to result from sample collection: 70.1 h
Median transport time to lab: 6.1h
## Example Results: UCLH Sample 111 (ETT)

<table>
<thead>
<tr>
<th>Method</th>
<th>Organism</th>
<th>Resistance Phenotype</th>
<th>Resistance genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine microbiology</td>
<td><em>Escherichia coli</em></td>
<td>Penicillins, amoxy-clav, aztreonam, 2/3/4 gen cephalosporins, co-trimoxazole, trimethoprim</td>
<td>NA</td>
</tr>
<tr>
<td>Unyvero</td>
<td><em>Escherichia coli</em> (+++)</td>
<td>Penicillins, aztreonam, 2/3/4 gen cephalosporins, sulphonamides <em>(predicted)</em></td>
<td><em>bla</em>&lt;sub&gt;TEM-1&lt;/sub&gt;, <em>sul1</em>, <em>bla</em>&lt;sub&gt;CTX-M&lt;/sub&gt;, GyrA wt</td>
</tr>
<tr>
<td>FilmArray</td>
<td><em>Escherichia coli</em> (&gt;1&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>Penicillins, aztreonam, 2/3/4 gen cephalosporins <em>(predicted)</em></td>
<td><em>bla</em>&lt;sub&gt;CTX-M&lt;/sub&gt;</td>
</tr>
<tr>
<td>MinION</td>
<td><em>Escherichia coli</em> (90.5% of reads)</td>
<td>Penicillins, aztreonam, 2/3/4 generation cephalosporins, <em>(predicted)</em></td>
<td><em>bla</em>&lt;sub&gt;TEM-1&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;CTX-M&lt;/sub&gt;</td>
</tr>
<tr>
<td>Method</td>
<td>Organism</td>
<td>Resistance Phenotype</td>
<td>Resistance genotype</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------------------</td>
<td>---------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Routine microbiology</td>
<td>Normal Respiratory Flora</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Unyvero</td>
<td><em>E. coli</em> (+++)</td>
<td>Penicillins, fluoroquinolones</td>
<td><em>bla</em>&lt;sub&gt;TEM-1&lt;/sub&gt;, GyrA83, GyrA87</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em> (+++)</td>
<td>(predicted)</td>
<td></td>
</tr>
<tr>
<td>FilmArray</td>
<td><em>E. coli</em> (&gt;10^7)</td>
<td>NA</td>
<td>None detected</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em> (&gt;10^7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MinION</td>
<td><em>E. coli</em> (14.5% reads)</td>
<td>Penicillins, (predicted)</td>
<td><em>bla</em>&lt;sub&gt;TEM-1&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em> (43.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>reads)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Example Results: CW Sample 5 (ETT)

<table>
<thead>
<tr>
<th>Method</th>
<th>Organism</th>
<th>Resistance Phenotype</th>
<th>Resistance genotype</th>
</tr>
</thead>
</table>
| Routine microbiology  | *P. aeruginosa*  
                        | *K. pneumoniae*              | PA: none  
                        | KP: Gentamicin, trimethoprim | NA |
| Unyvero               | *P. aeruginosa* (+++)  
                        | *S. maltophilia* (+)         | Penicillins         | \( bla_{SHV} \) |
| FilmArray             | *P. aeruginosa* (>10^7)  
                        | *S. agalactiae* (10^5)       | NA                 | None detected |
| MinION                | *P. aeruginosa* (87.8% reads) | NA                           | None detected       |
Can MinION Metganomic Sequencing be used to predict AMR?

- Proof of concept study attempting to predict full antimicrobial susceptibility profiles directly from bacteria in INHALE samples
- Compared to full phenotypic susceptibility testing & troubleshooting using PCR, Sanger Sequencing & Illumina Sequencing
- Limited to *E. coli* and *K. pneumoniae* in the first instance
Results: AST phenotype vs MinION prediction

- Successfully identified 7 *E.coli* isolates and 3 *K. pneumoniae* isolates
- AMR Prediction Sensitivity: **71.2%**  AMR Prediction Specificity: **98.4%**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Predicted MinION Phenotype</th>
<th>MinION Genotype</th>
<th>Actual Resistance Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 E. coli</td>
<td>amoxicillin, co-trimoxazole, gentamicin, trimethoprim, tobramycin</td>
<td>bla\textsubscript{TEM-1}, aac(3)-II-1, sul1, dfrA12</td>
<td>amoxicillin, co-trimoxazole, gentamicin, piperacillin/tazobactam, tobramycin, trimethoprim, amoxicillin/clavulanate, cefepime</td>
</tr>
<tr>
<td>Sample 2 E. coli</td>
<td>amoxicillin, streptomycin, sulphamethoxazole</td>
<td>bla\textsubscript{TEM}, sul2, strA, strB</td>
<td>amoxicillin, streptomycin, sulphamethoxazole, tetracycline</td>
</tr>
<tr>
<td>Sample 3 K. Pneumoniae</td>
<td>amoxicillin, amoxicillin/clavulanate, ceftazidime, ciprofloxacin, gentamicin, piperacillin/tazobactam, streptomycin, trimethoprim, sulphamethoxazole, co-trimoxazole</td>
<td>bla\textsubscript{CTX-M}, aac-6'-1b, bla\textsubscript{TEM-1}, sul1, strA, strB, aadA1</td>
<td>amoxicillin, amoxicillin/clavulanate, ceftazidime, ciprofloxacin, gentamicin, piperacillin/tazobactam, streptomycin, trimethoprim, sulphamethoxazole, co-trimoxazole, tetracycline</td>
</tr>
</tbody>
</table>

Key: Agreement  Disagreement  Difficult to Interpret
Discrepancies between culture and MinION

- False Positives
  - $bla_{TEM} n=1$
  - $sul2 n=1$
  - PCR unable to detect in isolate
  - Elsewhere in metagenome
  - Tetracycline $n=4$
  - $tetA / tetD$
  - Absent in CARD database
  - Amoxicillin $n=1$
  - Further susceptibility testing
  - Resolved
  - Co-amoxiclav $n=4$
  - Ciprofloxacin $n=2$
  - Unresolved
  - Multiple chromosomal mutations / Gene dosage

- False Negatives

- Demonstrates the future potential of MinION sequencing for the rapid identification of pathogenic bacteria and predicting resistance phenotypes. Sensitivity should be further improved by resolving database deficiencies and using enhanced bioinformatics.
WP3 INHALE RCT

Patient with suspected HAP/VAP at Participating ICU about to Receive Antibiotic

RANDOMISE
466 patients

Treatment Arm
- Test Respiratory Specimen using FilmArray in ICU

Control Arm
- Standard of Care Respiratory Specimen Sent to Micro Lab

FilmArray Result+
INHALE Prescribing Algorithm delivered to Treating Clinician

Assess appropriateness of antibiotics at 24h and clinical outcome at 14 Days

FilmArray Torch placed at Point of Care within 12 Critical Care Units
### INHALE Prescribing Algorithm

#### Key
- No known allergy to antibiotics
- Mild allergy to β-lactams i.e. rash
- Severe allergy to β-lactams, i.e. anaphylaxis
- Not applicable

**Table 2. Recommended treatment for combination of TWO or more organisms are detected by FilmArray**

PLEASE READ THIS TABLE FROM LEFT TO RIGHT

Coloured boxes refer to allergy status as in Table 1.

**Key:** + organism present, - organism absent, ± either present or absent

<table>
<thead>
<tr>
<th>First: What combination of bacteria have been found?</th>
<th>Second: Therapy if no resistance genes found</th>
<th>Third: if resistance genes found</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. baumannii</td>
<td>meca/C found</td>
<td>CTX-M found</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td>C. pneumoniae</td>
</tr>
<tr>
<td></td>
<td>H. influenzae/M. caterhalis</td>
<td>L. pneumophila</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>OR M. pneumoniae</td>
</tr>
<tr>
<td></td>
<td>S. agalactiae, S. pneumoniae or S. pyogenes</td>
<td>Carbapenemase found</td>
</tr>
</tbody>
</table>

Does the mixture include Acinetobacter? If YES; stay with this block; if NO, go to next block

<table>
<thead>
<tr>
<th>+</th>
<th>Any one or more second organism found</th>
<th>Meropenem&lt;sup&gt;6&lt;/sup&gt; OR Linezolid</th>
<th>-</th>
<th>Add Macrolide&lt;sup&gt;11&lt;/sup&gt; OR Levofloxacin or Ciprofloxacin</th>
<th>Discuss with Microbiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>±</td>
<td>Meropenem&lt;sup&gt;8&lt;/sup&gt; OR Linezolid</td>
<td>-</td>
<td>Add Macrolide&lt;sup&gt;11&lt;/sup&gt; OR Levofloxacin or Ciprofloxacin</td>
<td>Discuss with Microbiology</td>
</tr>
<tr>
<td>+</td>
<td>Add Levofoxacin or Ciprofloxacin&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Add Levofoxacin or Ciprofloxacin&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Add Glycopeptide&lt;sup&gt;10&lt;/sup&gt; OR Linezolid</td>
<td>Add Glycopeptide&lt;sup&gt;10&lt;/sup&gt; OR Linezolid</td>
<td>Colistin Combination</td>
</tr>
</tbody>
</table>
Consultant: I think time was a big thing because there were fewer people on at night, there is no boss. You’re racking around trying to get to see everyone with all the admissions coming in as well. And then you see, oh, he’s had a temperature and the nurse is flapping around and keeps phoning me about it, oh, give him some Augmentin and on we go. And perhaps I didn’t give it as much thought when I was pressured or rushed.
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Mr. Dewi Owen
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Dr. Federico Ricciardi
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The INHALE Study Team
& Staff at Participating Hospitals and Laboratories
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• The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.”