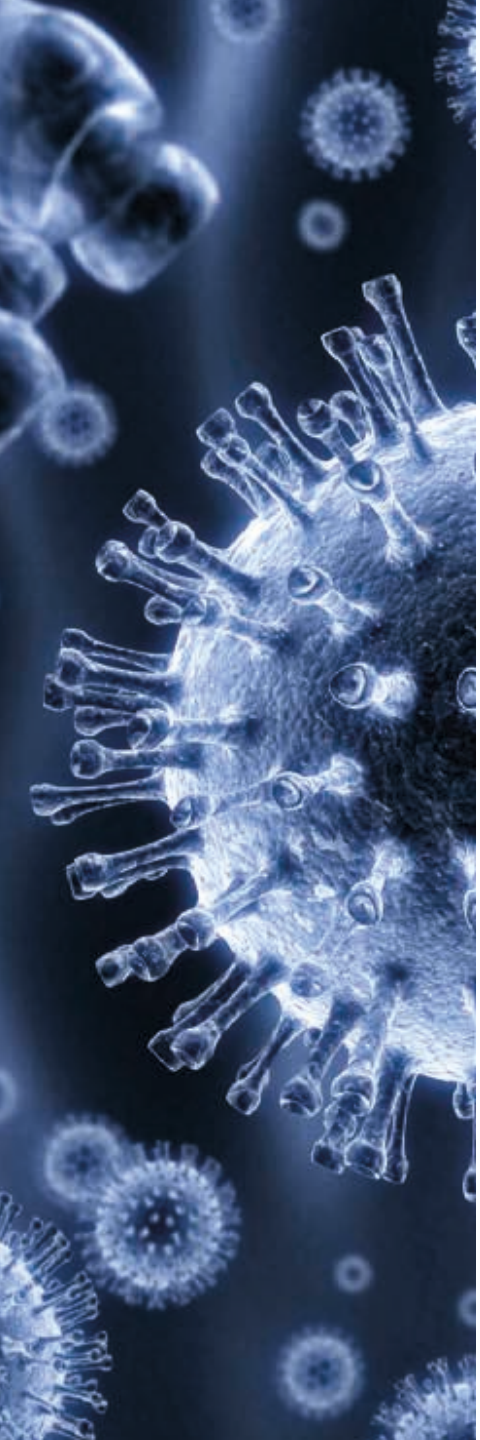

The INHALE Project: What have we learnt about the molecular diagnostics of HAP/VAP?

Dr Vicky Enne
University College London
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@HAP_Diagnostics
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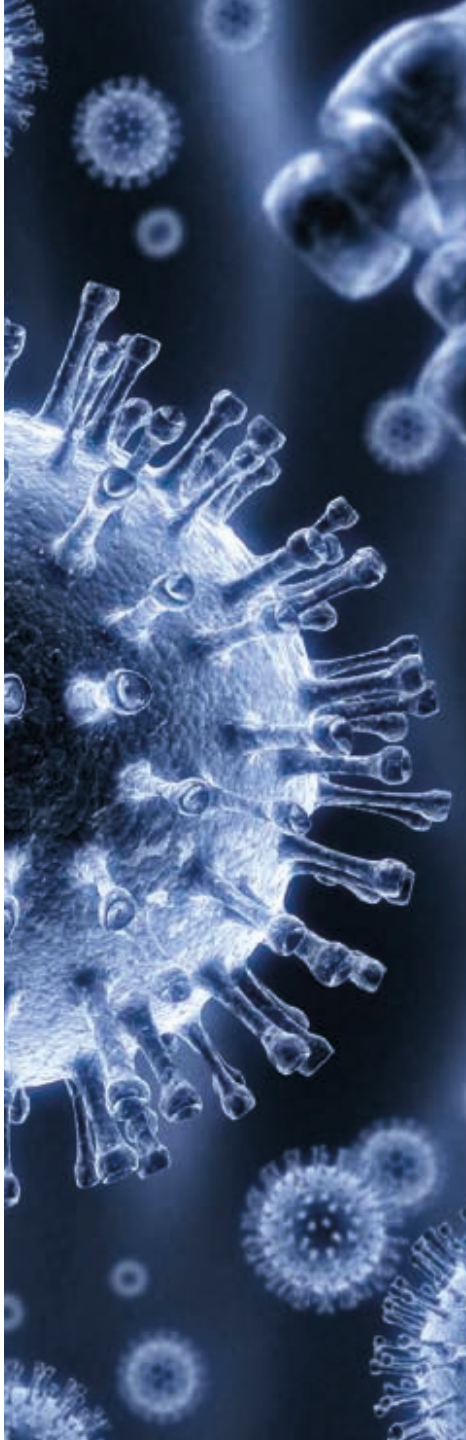
UCL

INHALE

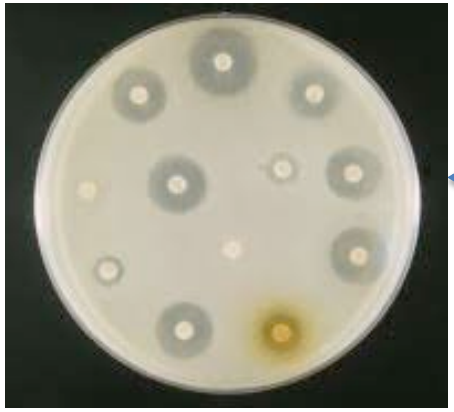


Sample arrives in lab

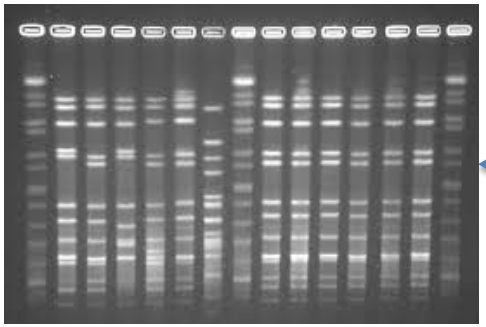
Microscopy/Gram Stain (a few min)

Culture (16h-48h)

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

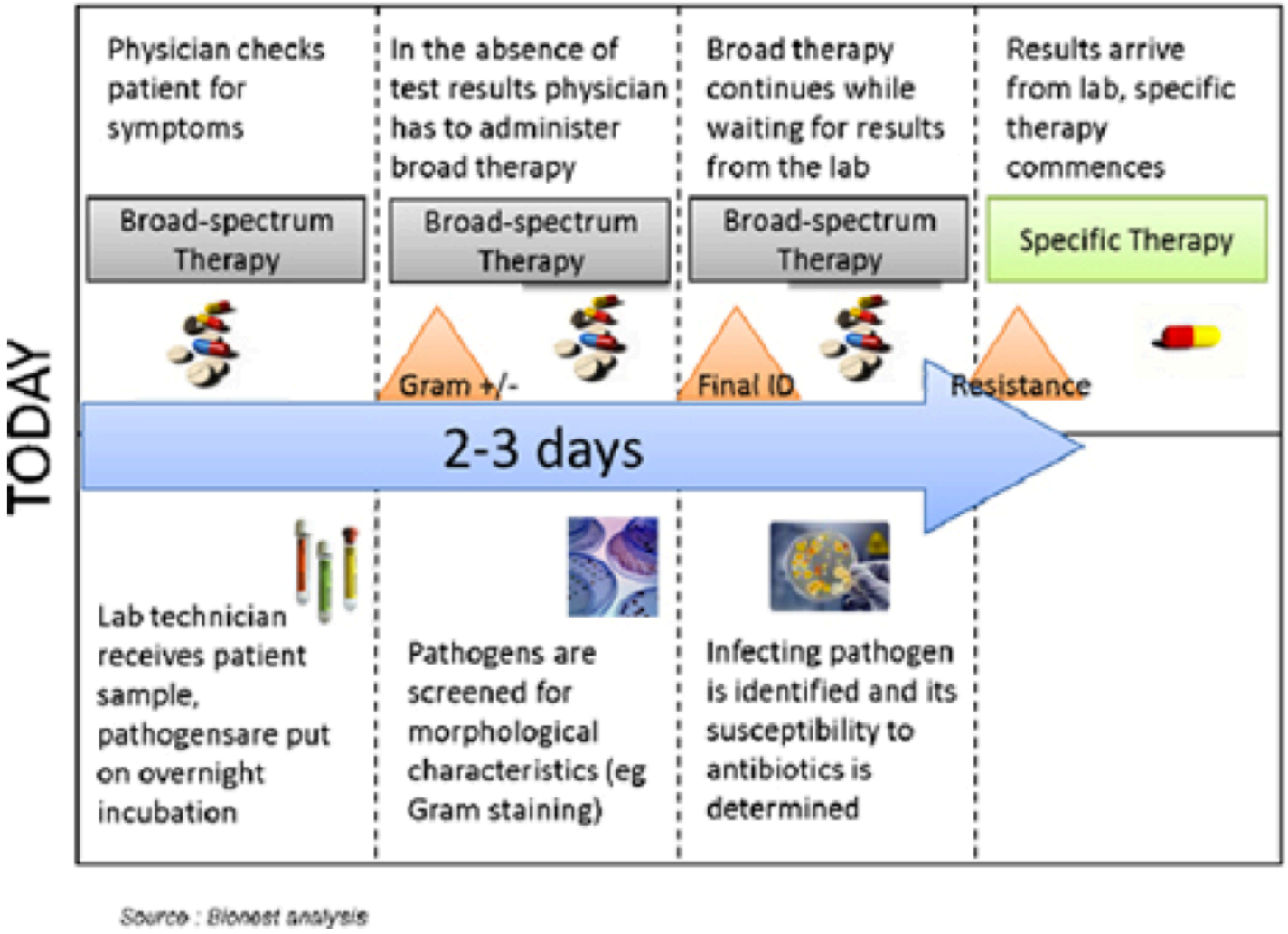


Antimicrobial susceptibility testing (24-48h)



Further testing / reference laboratory (up to 2 weeks)

The Need for Rapid Diagnostics



Source : Bionest analysis

What Factors Contribute to the Adoption of Molecular Diagnostics?

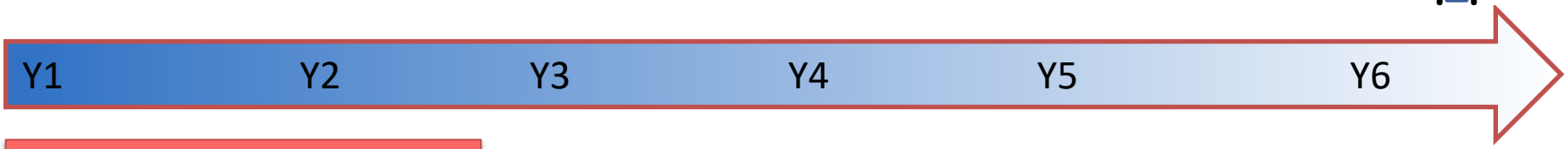
In the Clinical Laboratory

- 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?

INHALE Programme Outline



WP1 – Diagnostic evaluation

WP2 – ICU Epidemiology

WP3 – Preparation/analysis

WP3 RCT

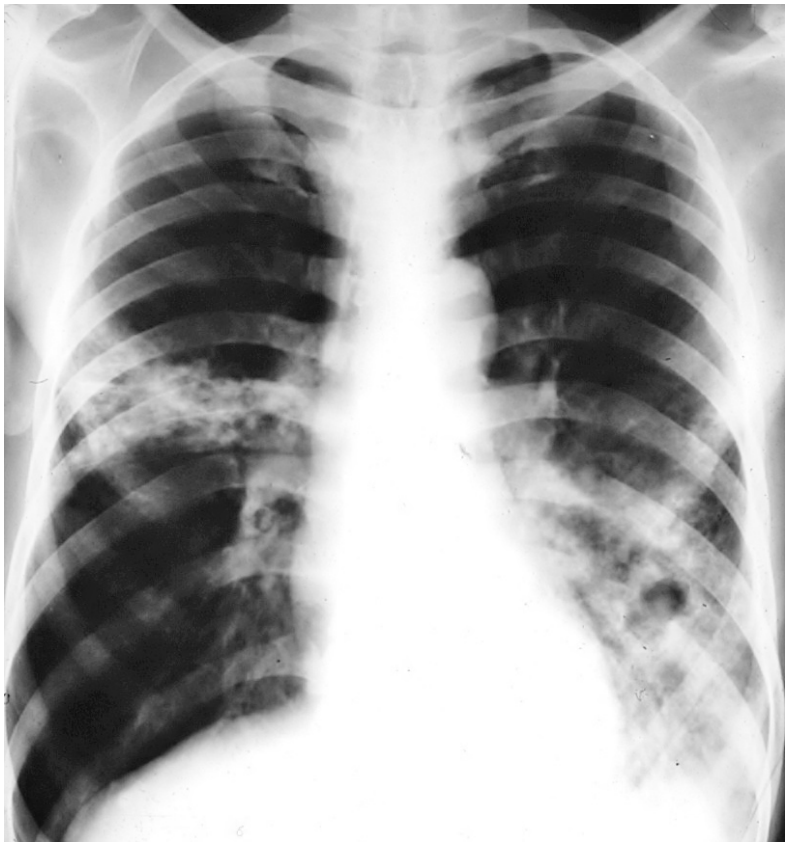
WP4 – Behaviour Study

WP5 – Health Economic Analysis

WP6 – Management and Dissemination

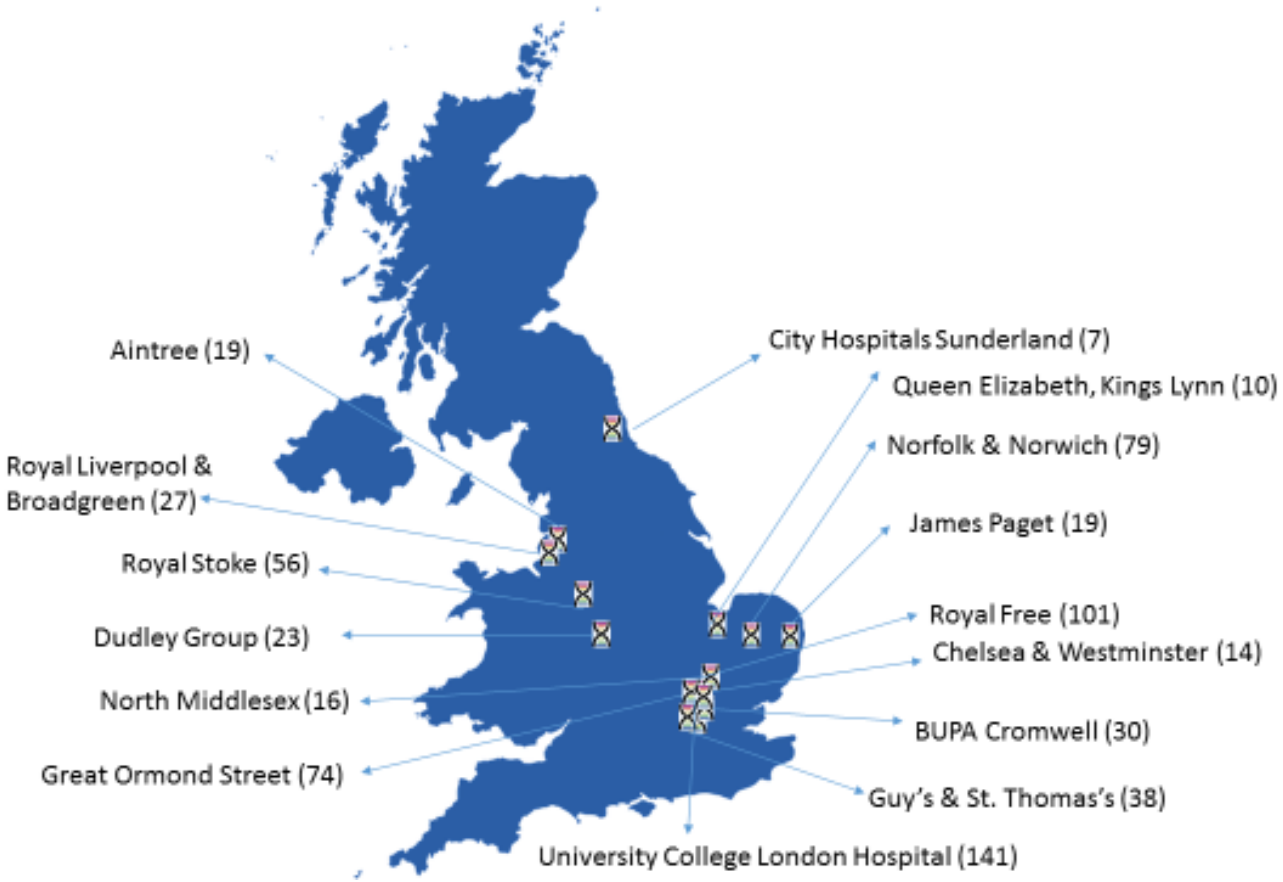
Hospital-acquired Pneumonia (HAP)

- 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

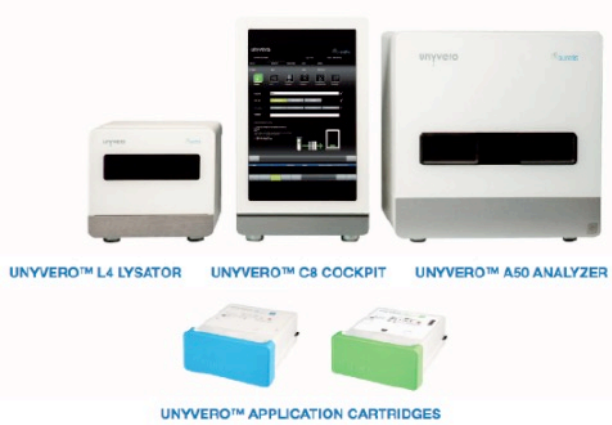


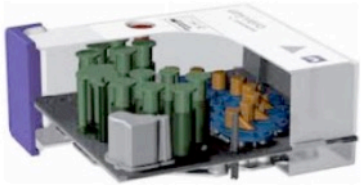
Project Participants

- UCLH/UCL/UEA (Lead organisations)
- 15 Critical Care Units in England representing diverse case-mix

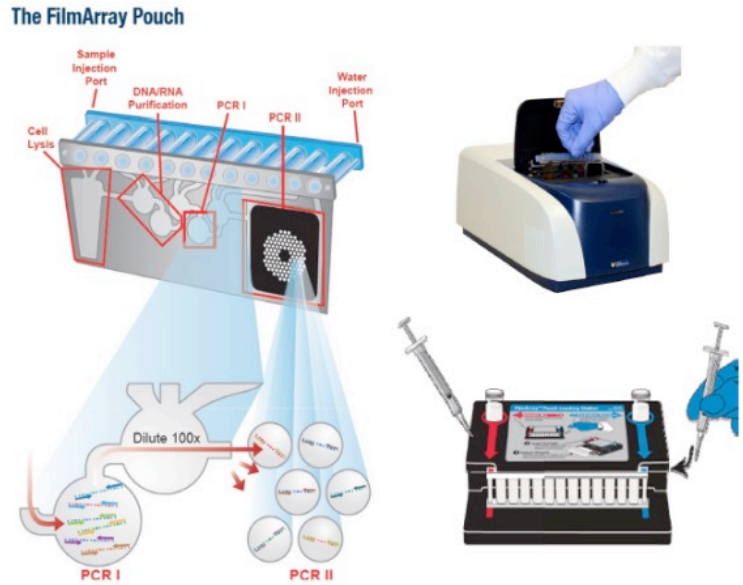


INHALE WP1: Head to Head Comparison of 3 Rapid Molecular Tests for Pneumonia Diagnosis vs. Routine Microbiology Culture





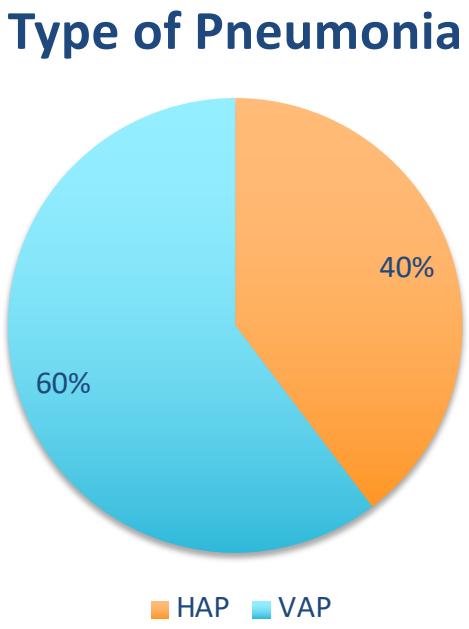
Curetis Unyvero Pneumonia Test

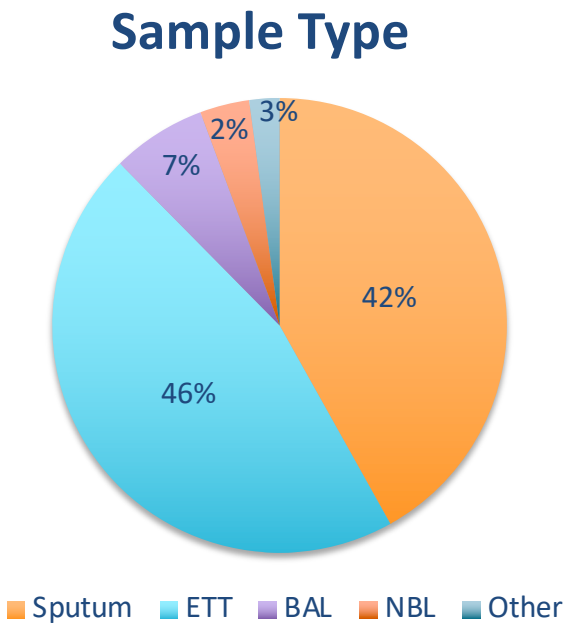


Biofire FilmArray Pneumonia Panel

Oxford Nanopore Technologies MinION metagenomic sequencing

Clinical Evaluation Sample Characteristics

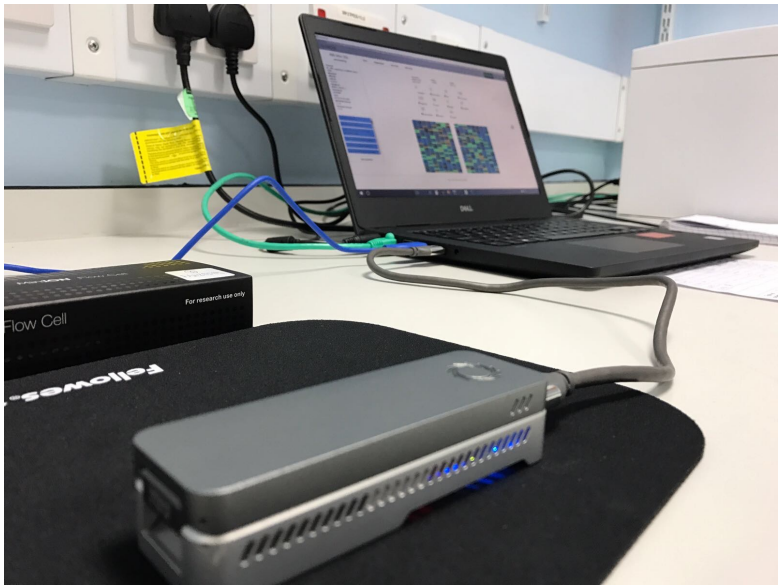




- 654 eligible samples collected from 15 hospitals

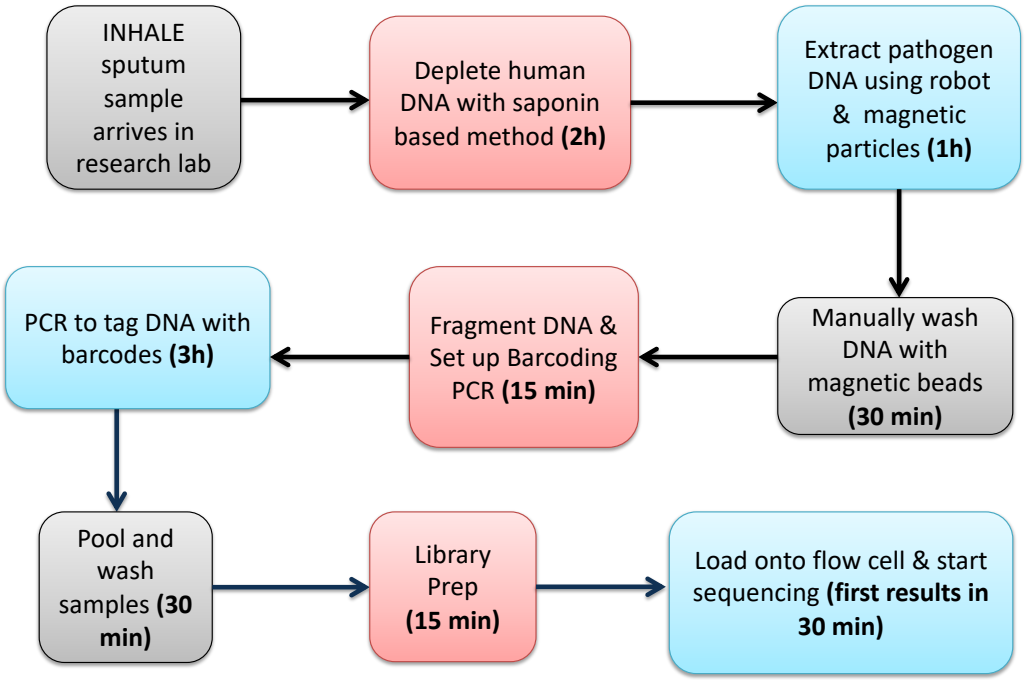
Oxford Nanopore MinION NGS Diagnostics

- 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

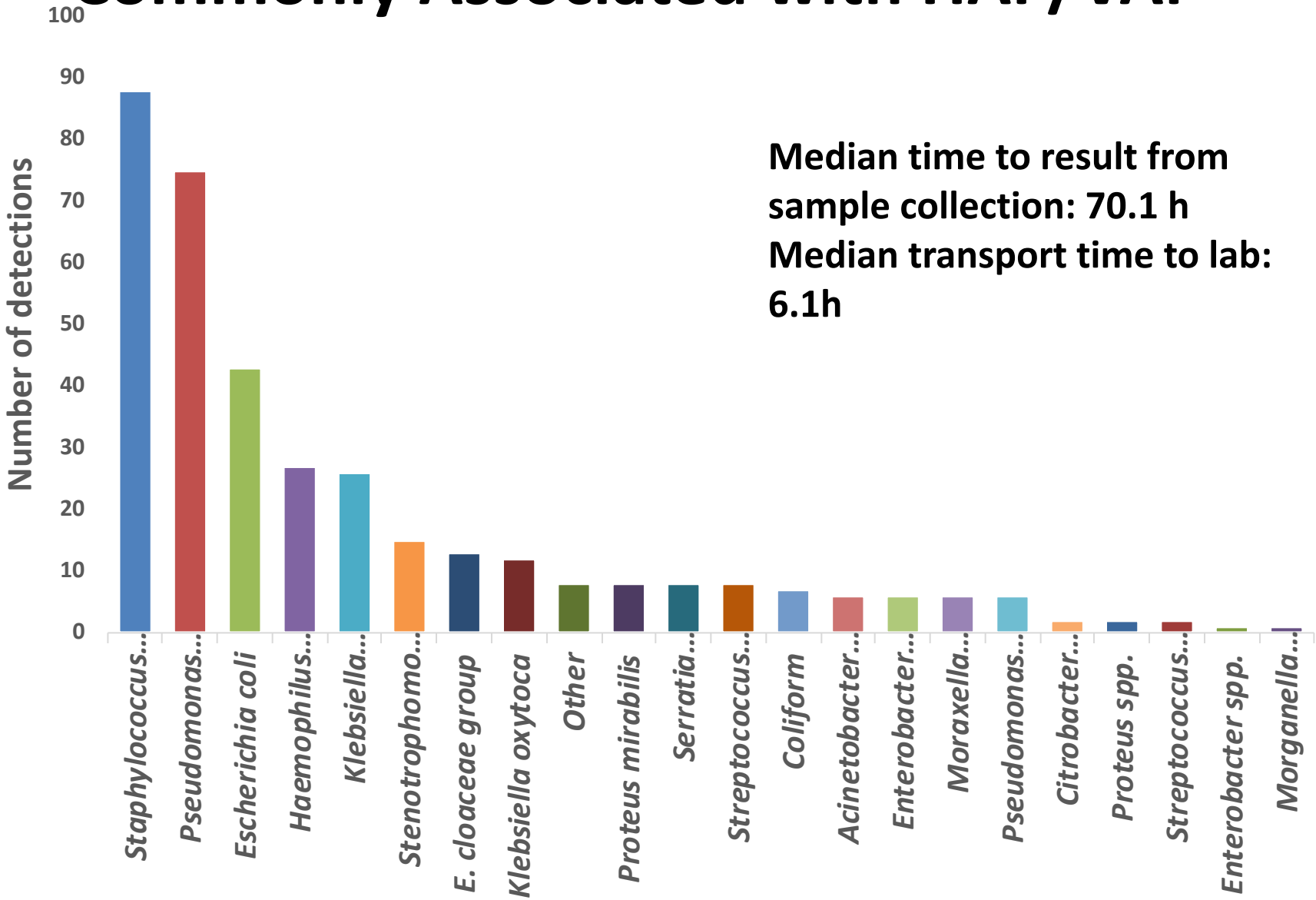


nature biotechnology
ARTICLES
https://doi.org/10.1038/s41587-019-0156-5

Nanopore metagenomics enables rapid clinical diagnosis of bacterial lower respiratory infection

Themoula Charalampous[1,8], Gemma L. Kay[1,2,8], Hollian Richardson[1,8], Alp Aydin[2], Rossella Baldan[1,3], Christopher Jeanes[4], Duncan Rae[4], Sara Grundy[4], Daniel J. Turner[5], John Wain[1,2], Richard M. Leggett[6], David M. Livermore[1,7] and Justin O'Grady[1,2*]

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)

Method	Organism	Resistance Phenotype	Resistance genotype
Routine microbiology	*Escherichia coli*	Penicillins, amoxy-clav, aztreonam, 2/3/4 gen cephalosporins, co-trimoxazole, trimethoprim	NA
Unyvero	*Escherichia coli* (+++)	Penicillins, aztreonam, 2/3/4 gen cephalosporins, sulphonamides **(predicted)**	$bla_{\text{TEM-1}}$ *sul1* $bla_{\text{CTX-M}}$ GyrA wt
FilmArray	*Escherichia coli* (>10^7)	Penicillins, aztreonam, 2/3/4 gen cephalosporins (**predicted)**	$bla_{\text{CTX-M}}$
MinION	*Escherichia coli* (90.5% of reads)	Penicillins, aztreonam, 2/3/4 generation cephalosporins, (**predicted**)	$bla_{\text{TEM-1}}$ $bla_{\text{CTX-M}}$

Example Results: RFH Sample 72 (ETT)

Method	Organism	Resistance Phenotype	Resistance genotype
Routine microbiology	Normal Respiratory Flora	NA	NA
Unyvero	*E. coli* (+++) *P. aeruginosa*(+++)	Penicillins, fluoroquinolones **(predicted)**	$bla_{\text{TEM-1}}$ GyrA83, GyrA87
FilmArray	*E. coli* (>10^7) *P. aeruginosa* (>10^7)	NA	None detected
MinION	*E. coli* (14.5% reads) *P. aeruginosa* (43.4% reads)	Penicillins, (**predicted**)	$bla_{\text{TEM-1}}$

Example Results: CW Sample 5 (ETT)

Method	Organism	Resistance Phenotype	Resistance genotype
Routine microbiology	*P. aeruginosa* *K. pneumoniae*	PA: none KP: Gentamicin, trimethoprim	NA
Unyvero	*P. aeruginosa*(+++) *S. maltophila* (+)	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

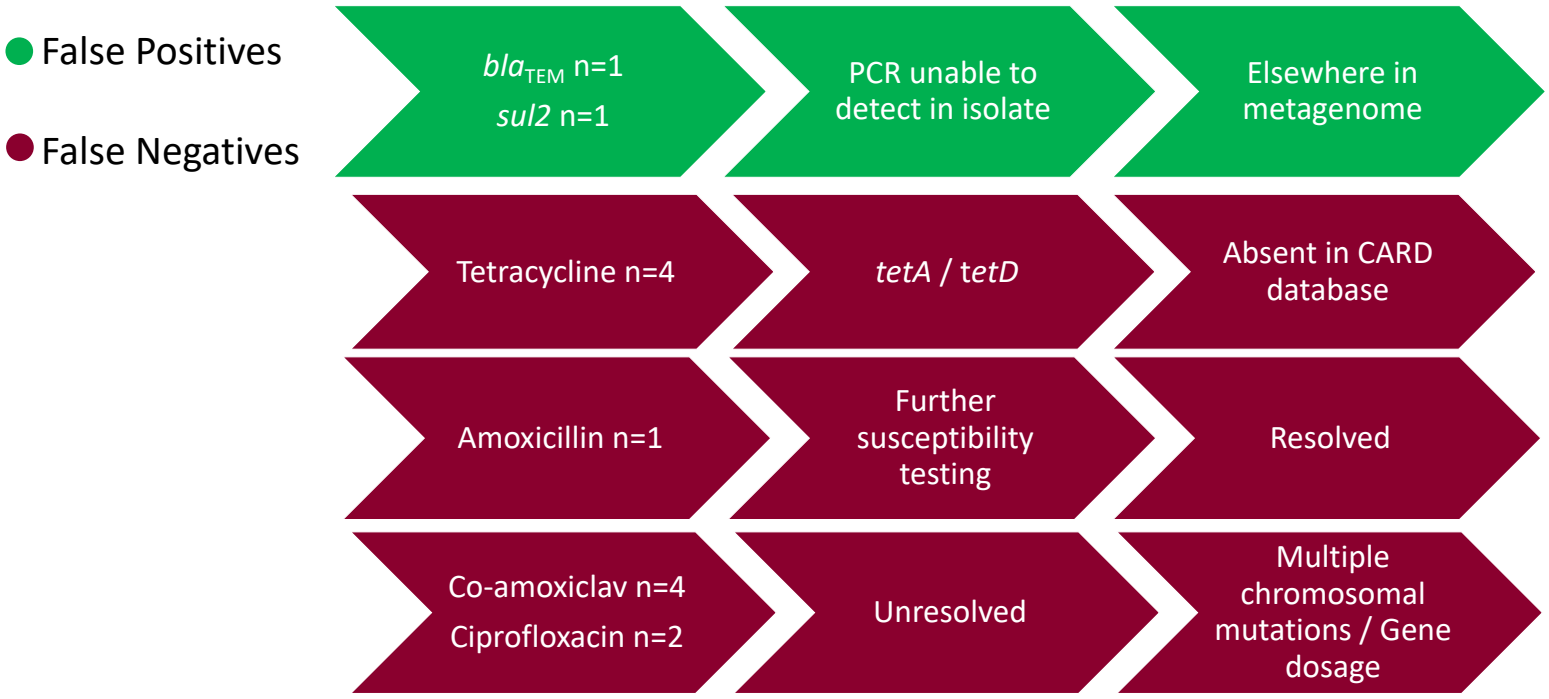
Results: AST phenotype vs MinION prediction

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

Strain	Predicted MinION Phenotype	MinION Genotype	Actual Resistance Phenotype
Sample 1 E. coli	amoxicillin co-trimoxazole gentamicin trimethoprim tobramycin	bla_{TEM-1} aac(3)-II sul1 dfrA12	amoxicillin, co-trimoxazole, gentamicin, piperacillin/tazobactam, co-trimoxazole, tobramycin, trimethoprim, amoxicillin/clavulanate, cefepime
Sample 2 E. coli	amoxicillin streptomycin sulphamethoxazole	bla_{TEM} sul2 strA strB	amoxicillin, streptomycin, sulphamethoxazole, tetracycline
Sample 3 K. Pneumoniae	amoxicillin, amoxicillin/clavulanate, ceftazidime, ciprofloxacin, gentamicin, piperacillin/tazobactam, streptomycin, trimethoprim, suphamethoxazole, co-trimoxazole	bla_{CTX-M} catB3 aac-6'-1b dfrA1 bla_{TEM-1} sul1 straA strB aadA1	amoxicillin, amoxicillin/clavulanate, ceftazidime, ciprofloxacin, gentamicin, piperacillin/tazobactam, streptomycin, trimethoprim, suphamethoxazole, co-trimoxazole, tetracycline

Key: Agreement Disagreement Difficult to Interpret

Discrepancies between culture and MinION



- 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

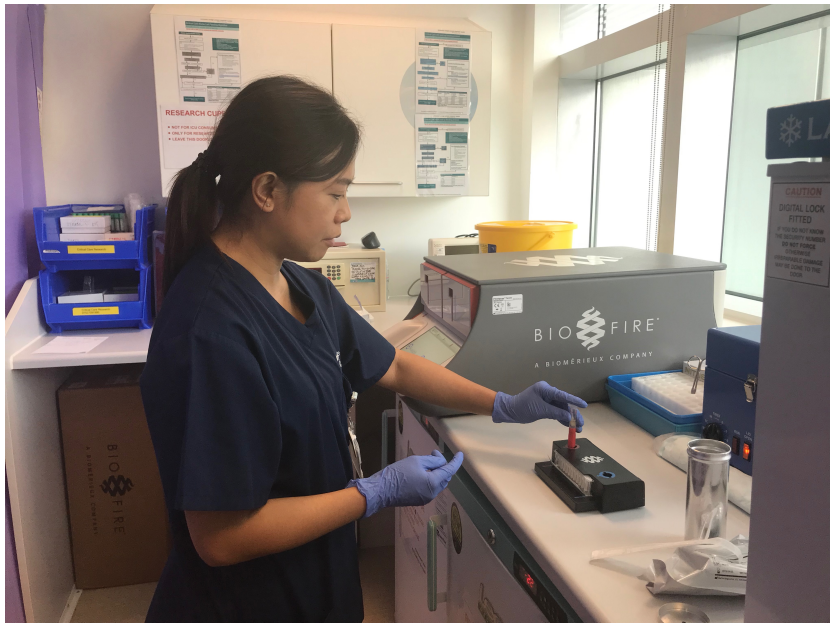
Test Respiratory Specimen using FilmArray in ICU

FilmArray Result+ INHALE Prescribing Algorithm delivered to Treating Clinician

Control Arm

Standard of Care Respiratory Specimen Sent to Micro Lab

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

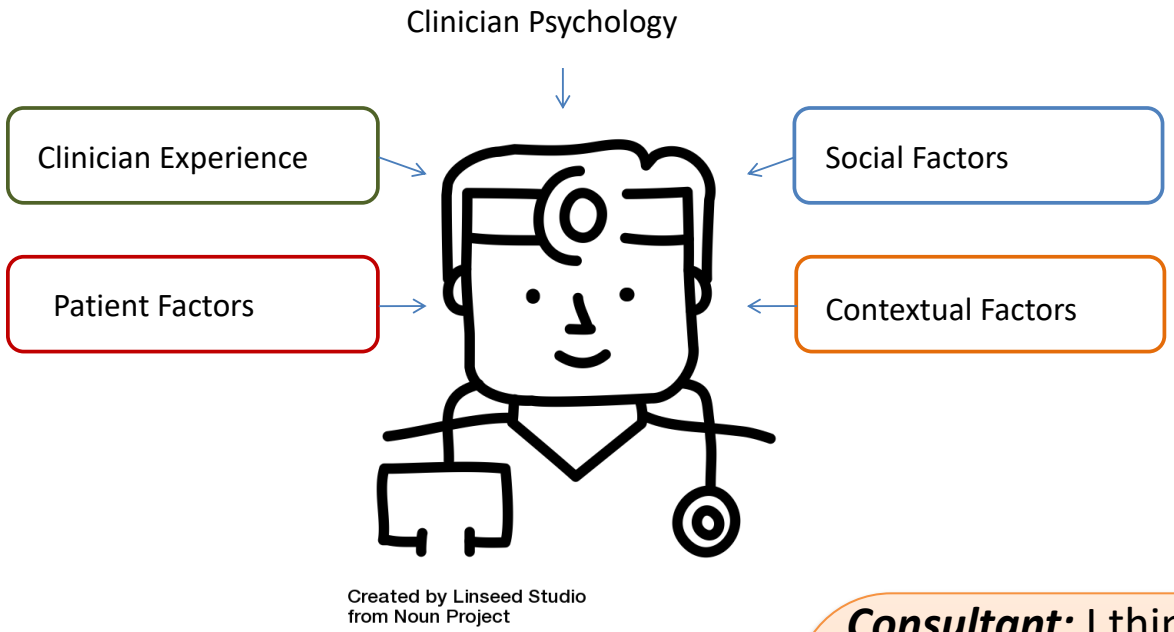
PLEASE READ THIS TABLE FROM LEFT TO RIGHT

Coloured boxes refer to allergy status as in Table 1.

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

First, What combination of bacteria have been found?						**Second: Therapy if no resistance genes**	**Third: if resistance genes found**			
A. baumannii	*Enterobacteriales: E. aerogenes, E. cloacae, E. coli, K. pneumoniae ,K. oxytoca, Proteus* sp., *S. marcescens*	*P. aeruginosa*	*H. influenzae/M. catarrhalis*	*S. aureus*	*S. agalactiae, S. pneumoniae* or *S. pyogenes*		*mecA/C* found	CTX-M found	*C. pneumoniae, L. pneumophila* OR *M. pneumoniae*	Carbapen-emase found
Does the mixture include *Acinetobacter*? If YES ; stay with this block; if NO, go to next block										
+	Any one or more second organism found					Meropenem[8]	Add Glycopeptide[10] OR Linezolid	-	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
+	± Any one or more second organism found					Meropenem[8]	Add Glycopeptide[10] OR Linezolid	-	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology
+	Add Levofloxacin or Ciprofloxacin[9]	Add Levofloxacin or Ciprofloxacin[9]	Add Levofloxacin or Ciprofloxacin[9]	Add Glycopeptide[10] OR Linezolid	Add Glycopeptide[10] OR Linezolid	Colistin Combination	Add Glycopeptide[10] OR Linezolid	Discuss with Micro-biology	Add Macrolide[11] OR Levofloxacin or Ciprofloxacin	Discuss with Micro-biology

WP4: Behavioral Study



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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The INHALE Study Team & Staff at Participating Hospitals and Laboratories

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UEA University of East Anglia

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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."*