Rapid metagenomic diagnosis of hospital acquired pneumonia

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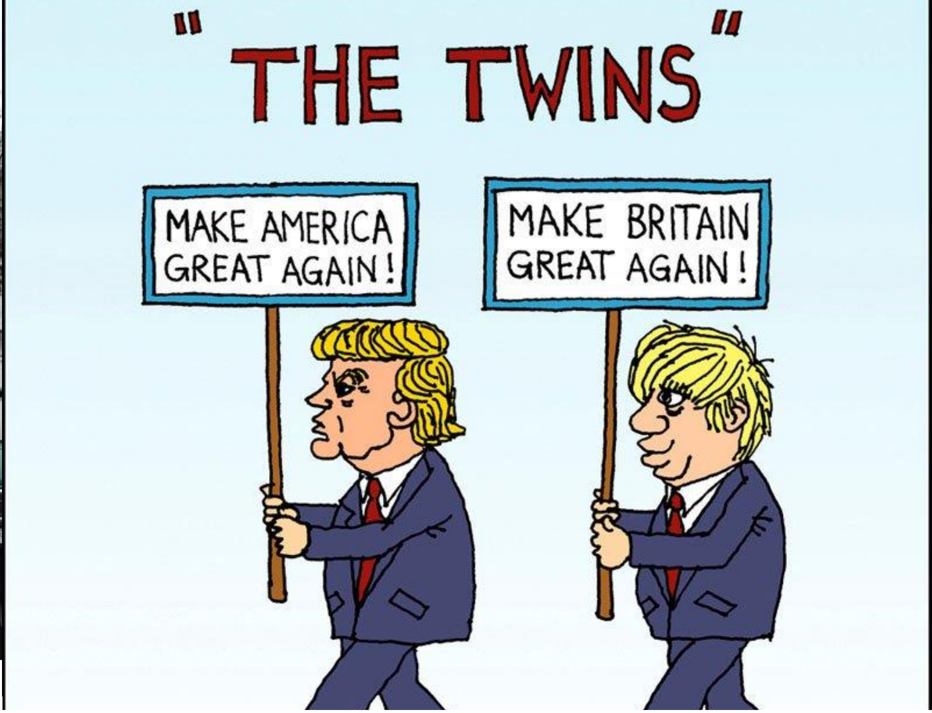
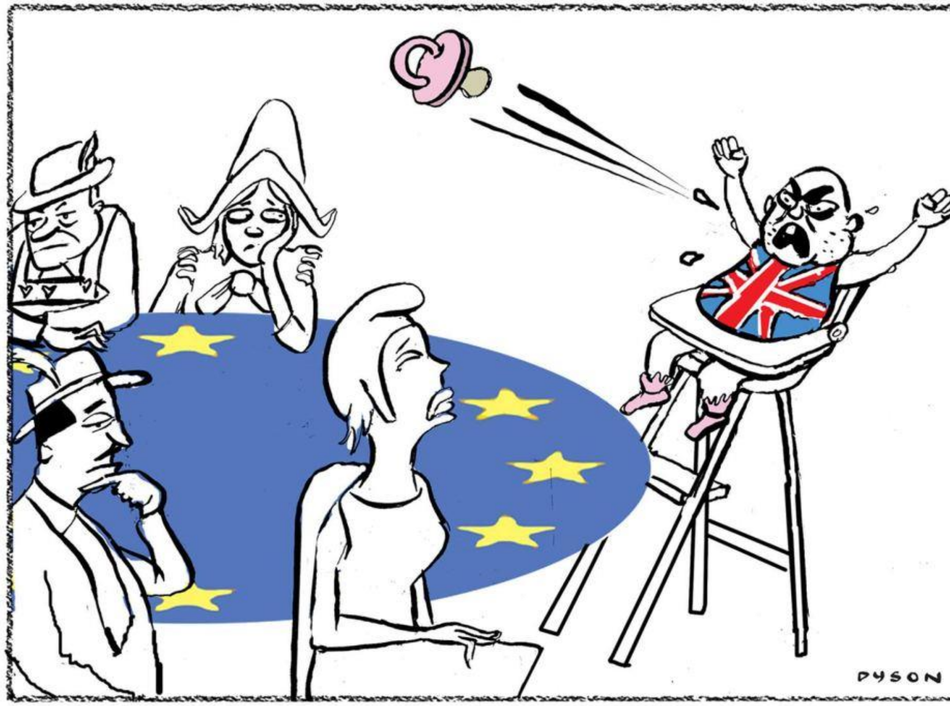
Conflict of interest statement

- I receive free flow cells and reagents on occasion as a new technology testing site for Oxford Nanopore
- I have received expenses for travel and accommodation to speak at Oxford Nanopore Conferences
- Oxford Nanopore co-fund a PhD student in my laboratory

Foto: Federico Gambarini/dpa





In a world of rising antimicrobial resistance, is culture based diagnosis fit-for-purpose?

Current situation

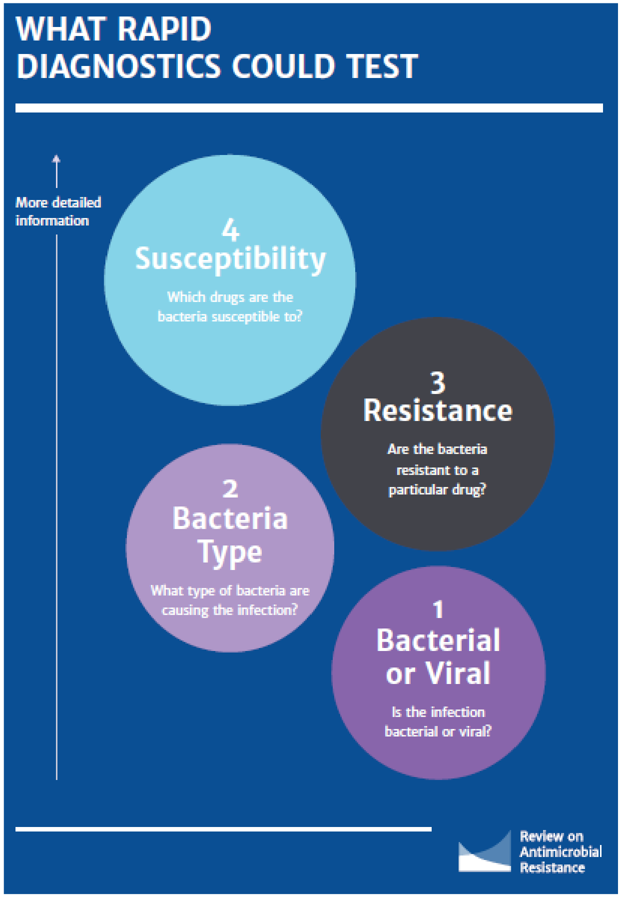
- Problems: e.g. UTIs
  - Trimethoprim replaced with nitrofurantoin, a worse agent
- E.g. Gonorrhoea
  - Ciprofloxacin replaced with cephalosporins
- Trimethoprim/Cipro could be used in 60-70% cases if susceptibility rapidly detected


Out of 40m people who get given antibiotics for respiratory issues, annually in the US:

O'Neill report Oct 2015: RAPID DIAGNOSTICS: STOPPING UNNECESSARY USE OF ANTIBIOTICS

Metagenomics sequencing based infection diagnostics

- Sequencing based diagnosis has great potential
  - Detection of bacterial, viral, fungal pathogens
  - Detection of antibiotic resistance markers
  - Epidemiology, infection control, transmission, virulence
  - Enable narrow spectrum antibiotic development, trial and use



WHAT RAPID DIAGNOSTICS COULD TEST

More detailed information

4
Susceptibility
Which drugs are the bacteria susceptible to?

3
Resistance
Are the bacteria resistant to a particular drug?

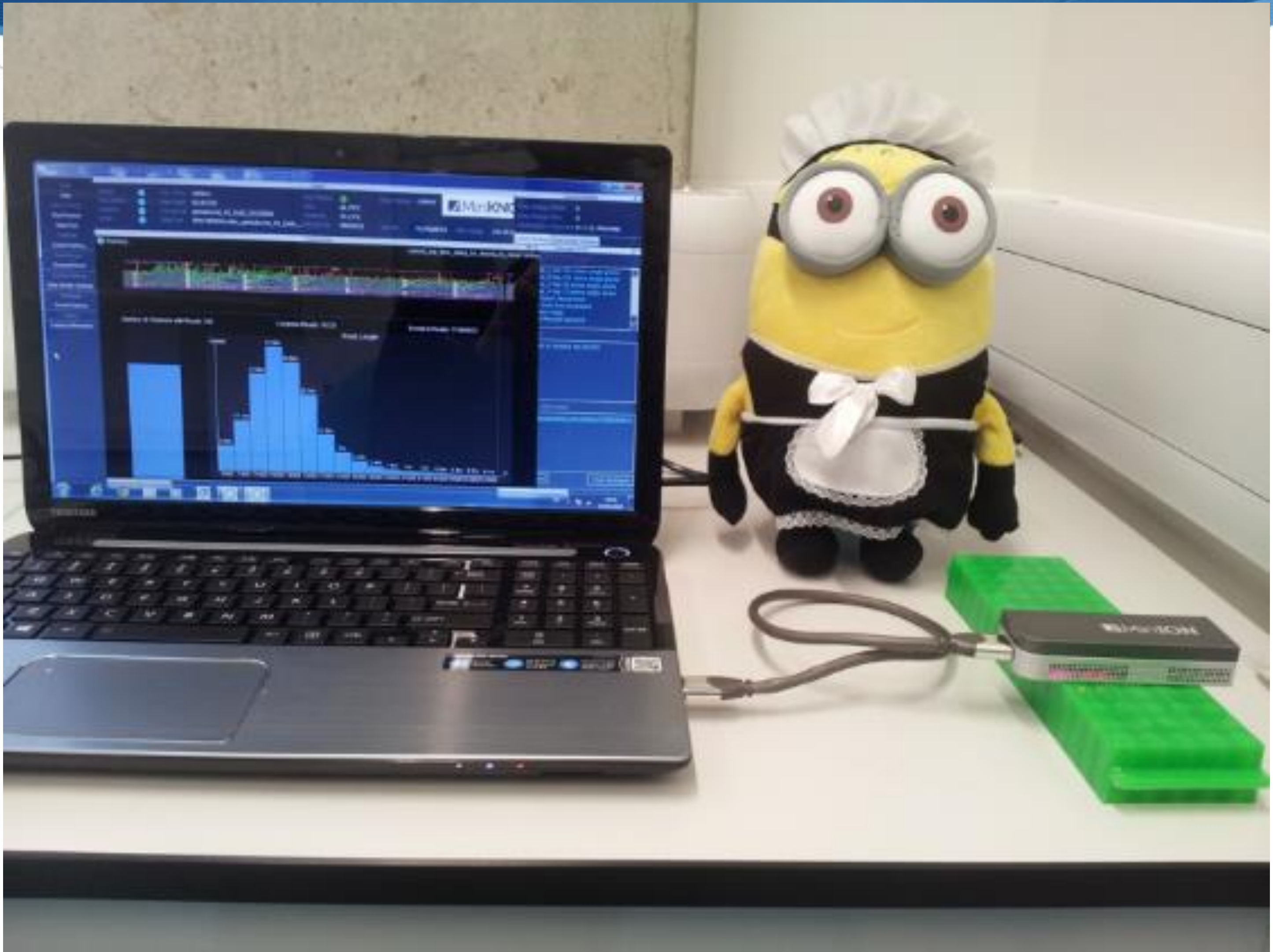
2
Bacteria Type
What type of bacteria are causing the infection?

1
Bacterial or Viral
Is the infection bacterial or viral?

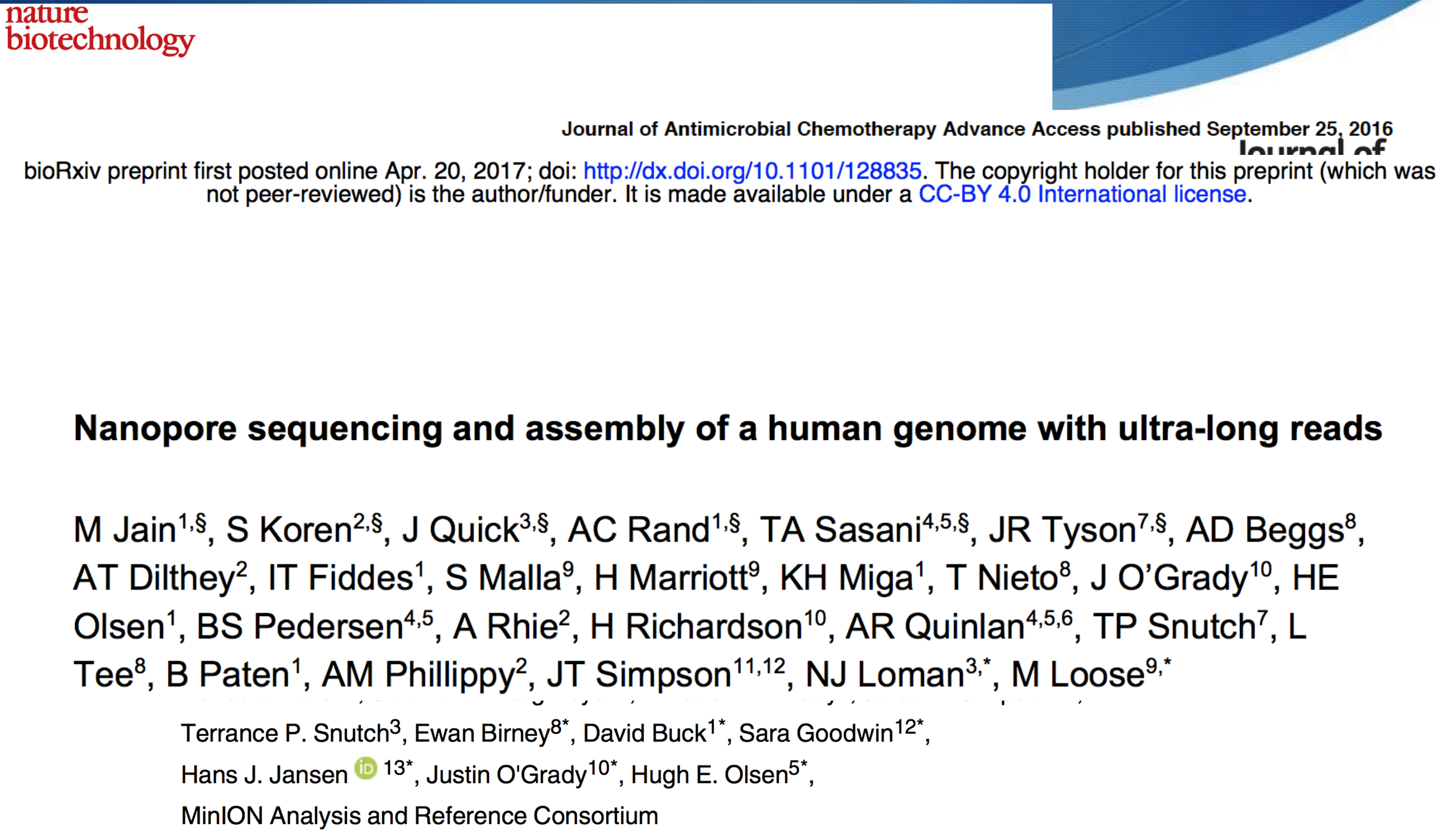
Review on Antimicrobial Resistance

O'Neill report Oct 2015: **RAPID DIAGNOSTICS:**
STOPPING UNNECESSARY USE OF ANTIBIOTICS

MinION



MinION papers



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Nanopore sequencing and assembly of a human genome with ultra-long reads

M Jain[1,§], S Koren[2,§], J Quick[3,§], AC Rand[1,§], TA Sasani[4,5,§], JR Tyson[7,§], AD Beggs[8], AT Dilthey[2], IT Fiddes[1], S Malla[9], H Marriott[9], KH Miga[1], T Nieto[8], J O'Grady[10], HE Olsen[1], BS Pedersen[4,5], A Rhie[2], H Richardson[10], AR Quinlan[4,5,6], TP Snutch[7], L Tee[8], B Paten[1], AM Phillippy[2], JT Simpson[11,12], NJ Loman[3,*], M Loose[9,*]

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Hans J. Jansen [13*], Justin O'Grady[10*], Hugh E. Olsen[5*],
MinION Analysis and Reference Consortium

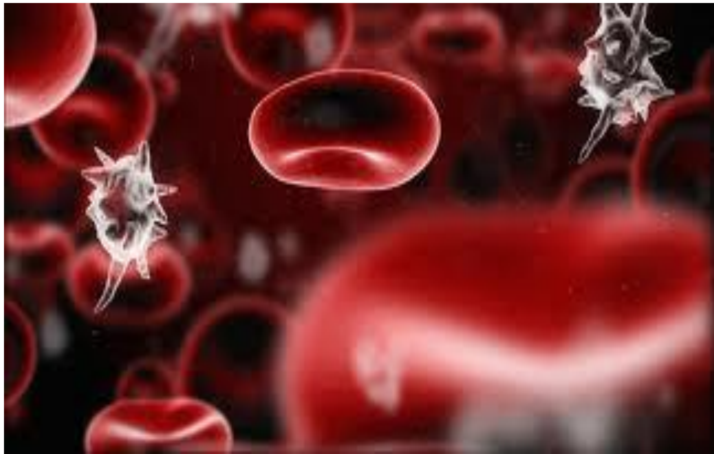
The need for host DNA depletion

- Major challenge
  - Sequencing the pathogen DNA in a vast excess of human DNA
  - Up to 10^5:1 human:bacterial DNA in respiratory samples

- Solution – host DNA depletion or pathogen DNA enrichment

- We have developed depletion methods

- Up to 99.9999% human DNA removal



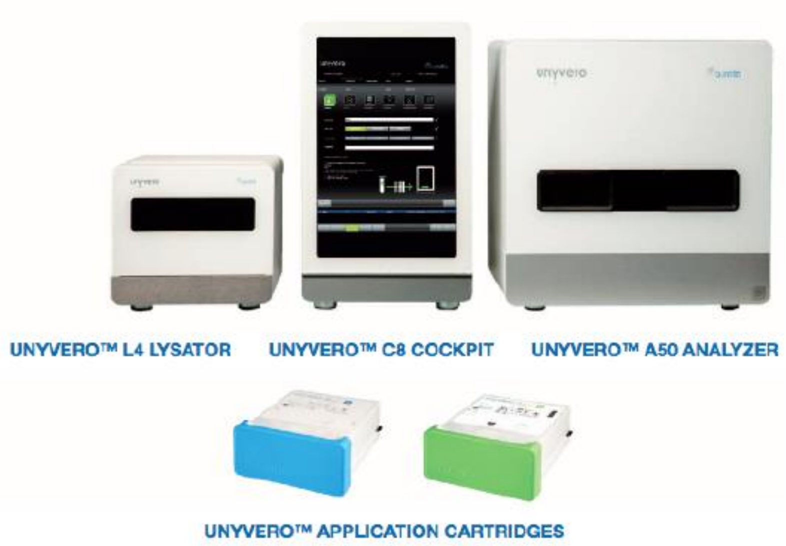
Hospital acquired pneumonia

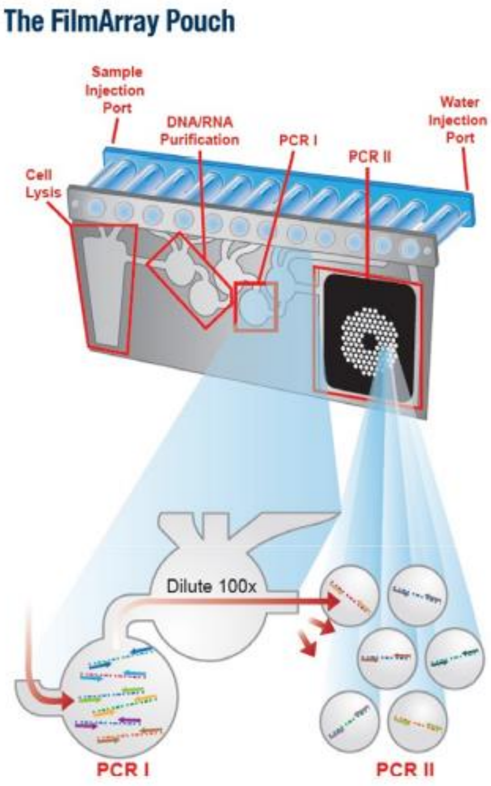
- HAP - respiratory infection > 48 h after admission
- 1.5% of inpatients in UK – approx 200k patients/year
- HAP associated with mechanical ventilation is VAP
- Mortality rate for HAP/VAP is approx 25-50%, which increases to 75% if MDR pathogen
- HAP/VAP adds approx 8 days to ICU stay costing an additional $30,000 - $37,000

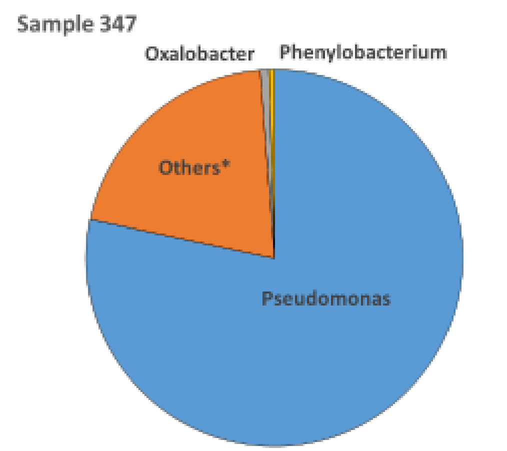
INHALE – Pneumonia diagnosis study

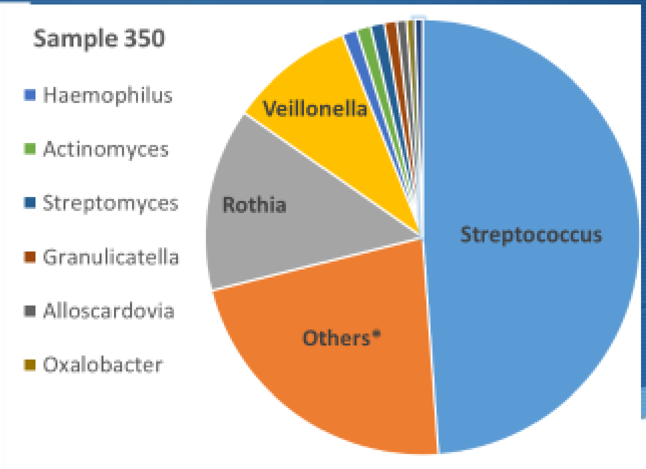
- 5 year NIHR programme grant on rapid diagnosis of HAP
- Diagnostics evaluation study followed by RCT
- Impact of rapid diagnosis on antibiotic stewardship
- 2 commercial PCR based tests
- Also assessing targeted/shotgun metagenomic sequencing

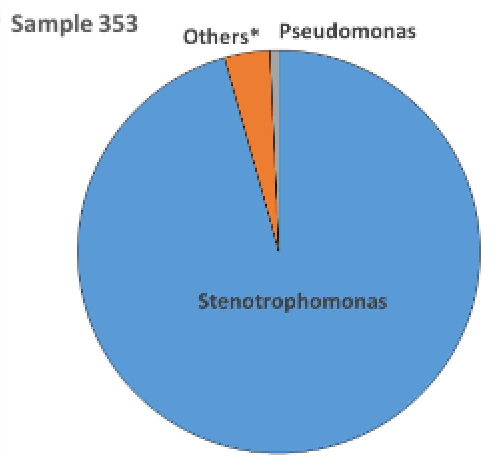
Curetis UnyVero and Biofire Filmarray Tests

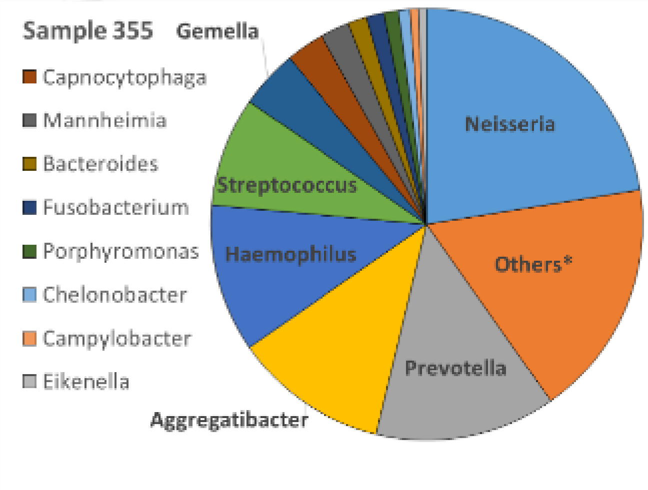


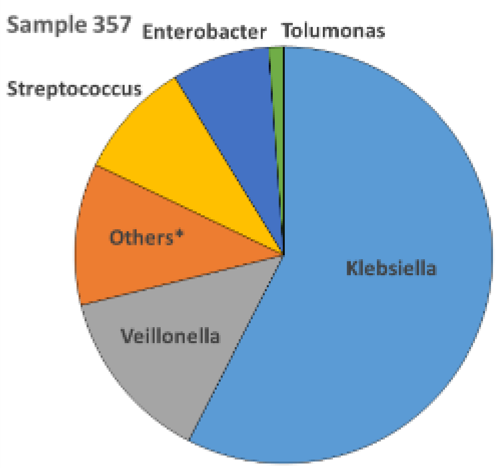






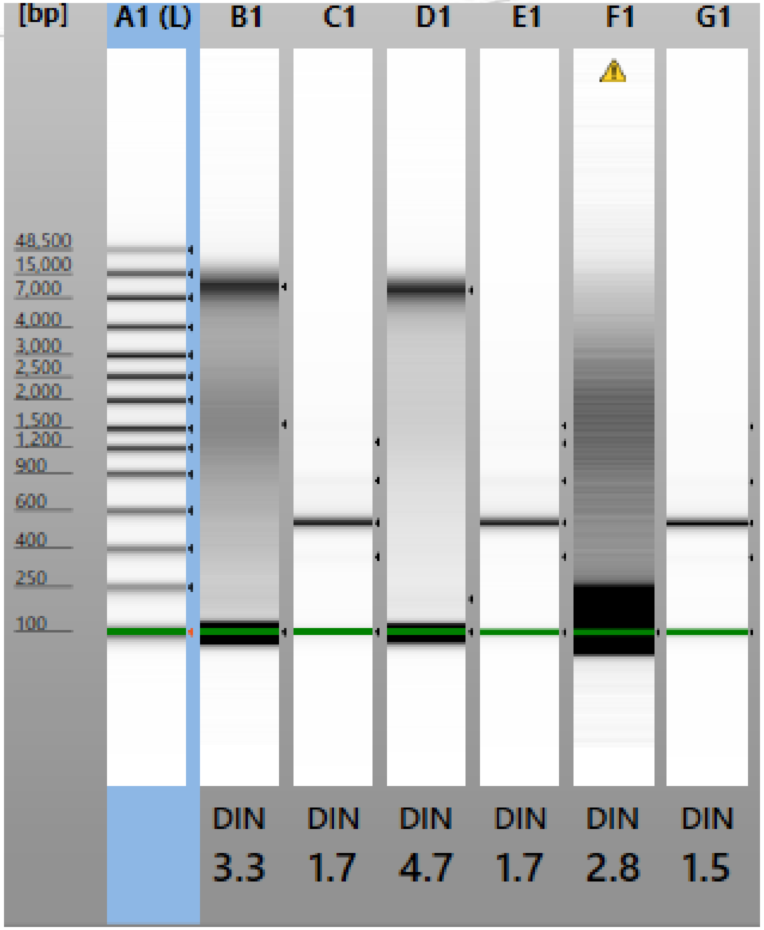






- 16S rDNA community profiles of endotracheal tube aspirates from suspected ventilator associated pneumonia patients (genera with reads <0.5% of total classified as others)

Respiratory sample DNA quality



INHALE

- World first comprehensive clinical evaluation of *rapid* metagenomics for the diagnosis of infection (600 specimens over 9 months)

Shotgun metagenomics of VAP

- Saponin based method for host DNA depletion (approx. 99.99% removal of human)

- Library preparation using Rapid Low Input kit
  - Transposon based library prep with long range PCR
  - As little as 10-100 pg DNA starting material required
  - Up to 3 million reads at 2-3 kb average fragment length

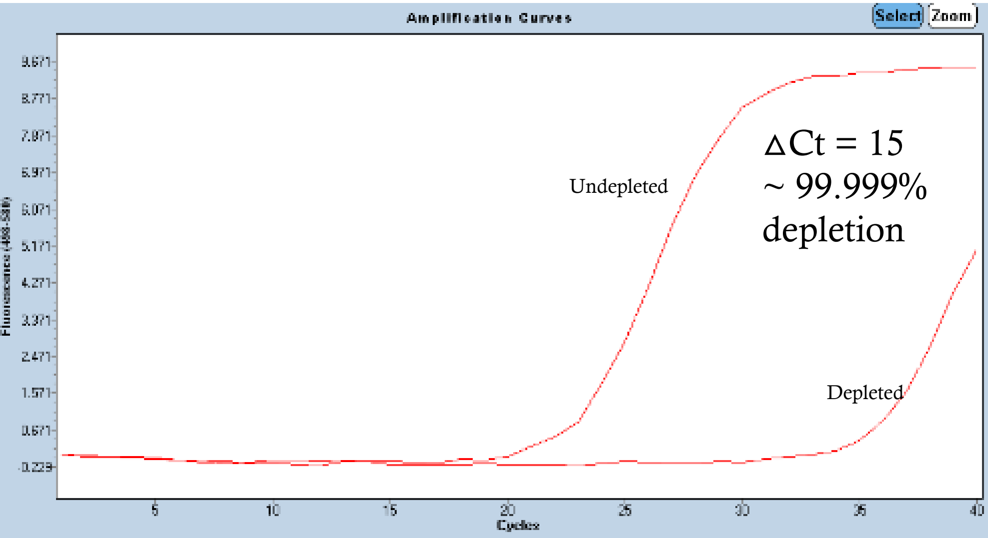
- Turnaround from sample to pathogen genome and AMR resistance results approx 8 hours

HAP/VAP diagnostics workflow

- Human DNA depletion – 1 hr
- DNA extraction (MagNA Pure, Roche) – 40 min
- RLB001 library preparation – 4 hours
- MinION sequencing – 2 hrs
- Albacore basecalling, WIMP, ARMA – real-time

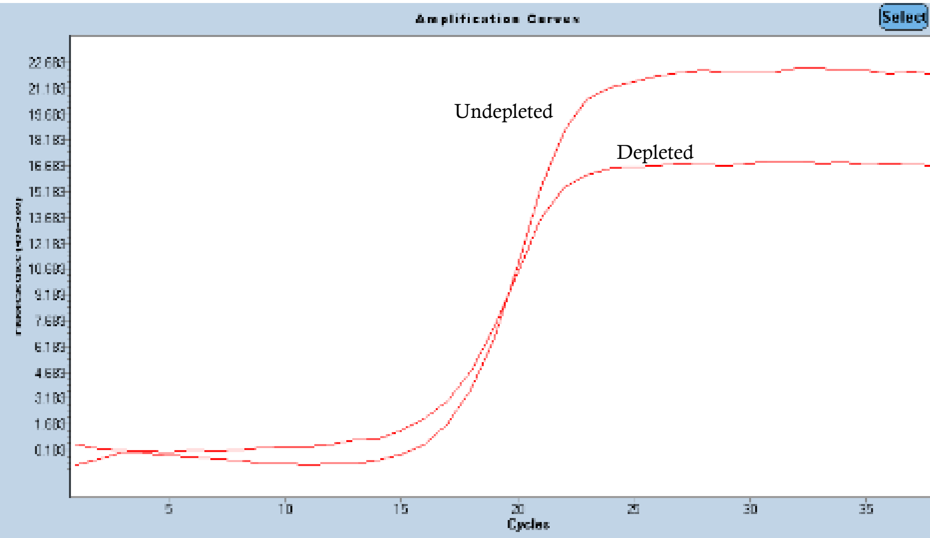
~8 hour turnaround time

Good human depletion



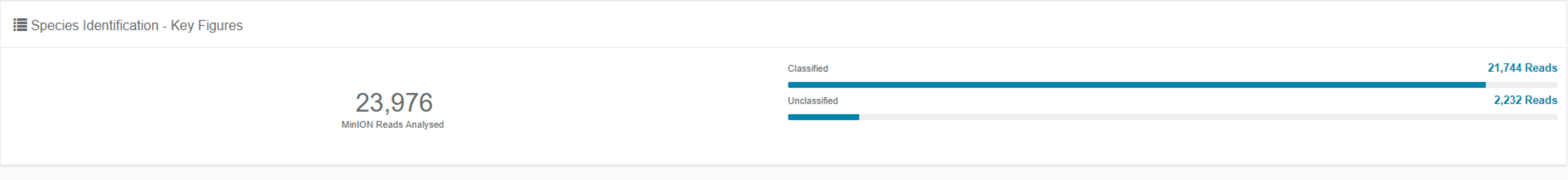
ΔCt = 15
~ 99.999%
depletion

Human qPCR assay



Bacterial qPCR assay (16S rDNA V3-V4 region)

Human DNA depletion

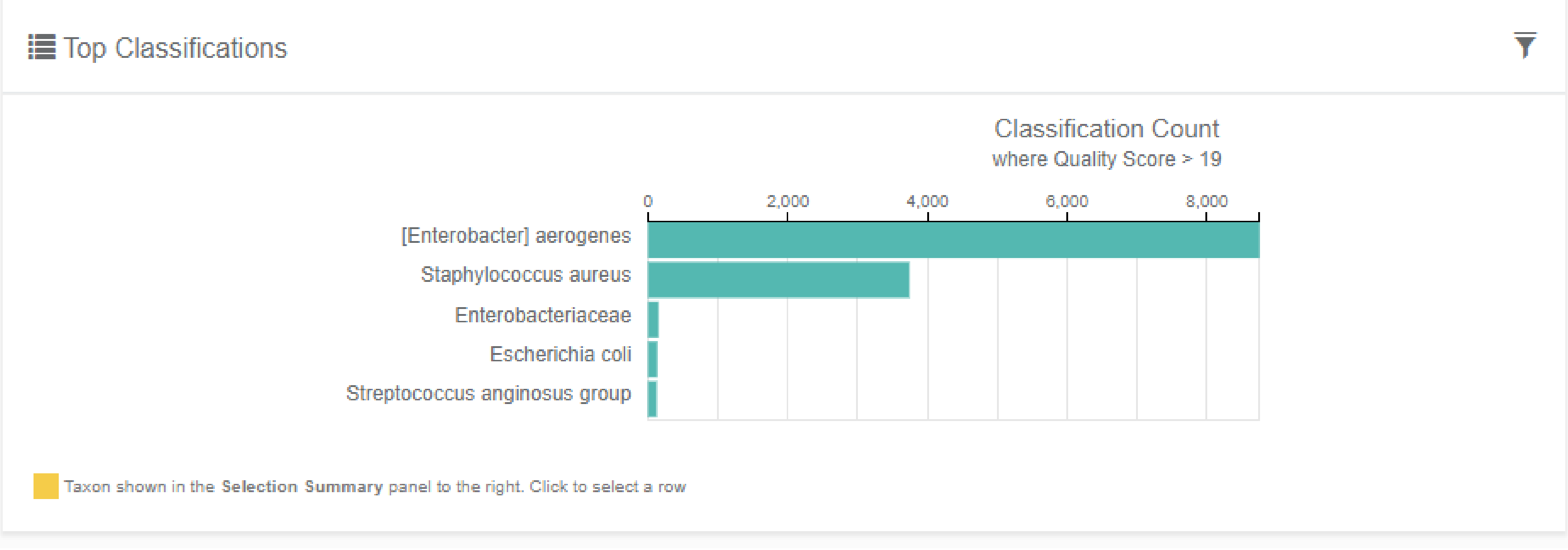


Species Identification - Key Figures

23,976
MinION Reads Analysed

Classified 21,744 Reads

Unclassified 2,232 Reads



Top Classifications

🟨 Taxon shown in the **Selection Summary** panel to the right. Click to select a row

Pilot study

- 42 suspected bacterial pneumonia samples
- Sputum, bronchoalveolar lavage (BAL) and endotracheal tube aspirates
- Human DNA depleted and sequenced on the MinION
- 1-6 samples per flowcell
- Approx cost per sample - $120

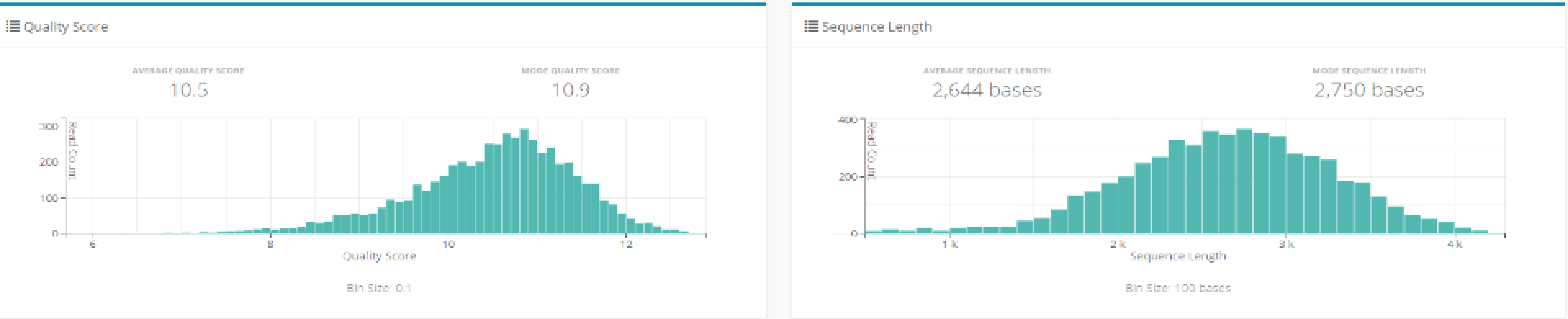
Optimisation

- Introduced bead-beating instead of enzyme cocktail for lysis
- Streamlined enrichment procedure to reduce time
- Reduced PCR elongation time from 6 to 4 minutes
- Optimised PCR cycle number for low input samples (BAL)
- Optimised sample storage conditions
  - Fresh vs frozen
  - -20C vs -80C
  - Glycerol and PEG

PCR extension time optimisation

S. aureus positive sputum - 20 PCR cycles with 6 minute extension

S. aureus positive sputum - 20 PCR cycles with 4 minute extension



HAP/VAP diagnostics workflow

- Human DNA depletion – 45 min
- DNA extraction (MagNA Pure, Roche) – 40 min
- RLB001 library preparation – 3 hours
- MinION sequencing – 1-2 hrs
- Albacore basecalling, WIMP, ARMA – real-time

~5-6 hour turnaround time

Summary

- Metagenomic diagnosis of HAP is feasible
- Sample preparation is key - host depletion makes metagenomic diagnosis faster and cheaper
- Real-time pathogen ID and resistance marker detection
- Percision medicine - modify empiric treatment before second dose

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- John Wain
- Vanya Gant
- Vicky Enne

orthopaedic research UK

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