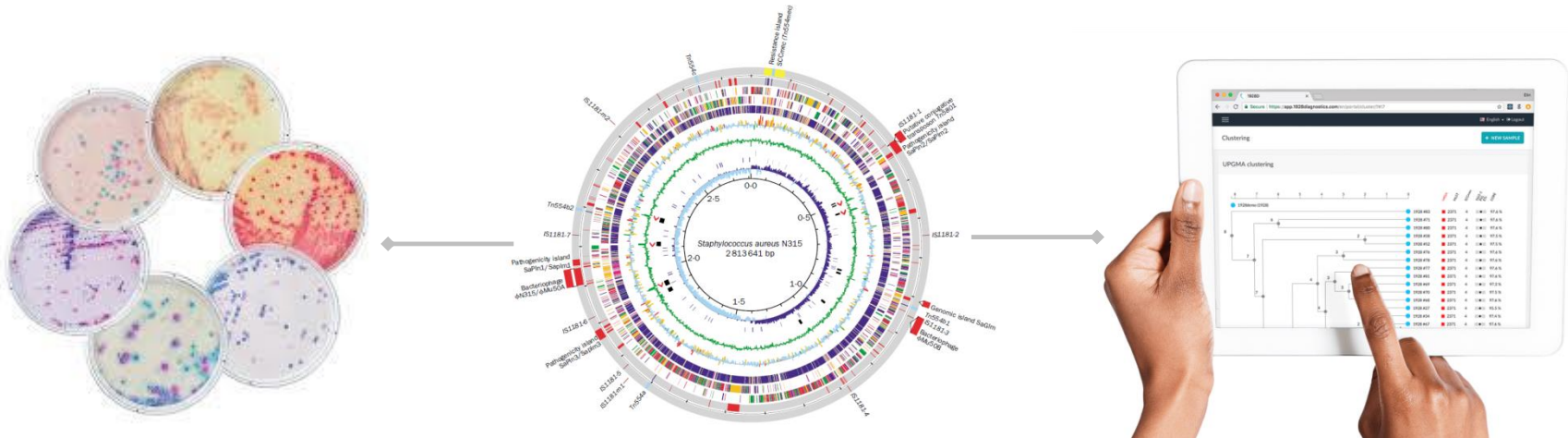




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## Precision AMR Programme at HSL



# Precision AMR Sequencing Facility

## Location

- Based within the diagnostic Infection Sciences and Molecular Pathology departments at the Halo building, 1 Mabledon Place.

### Microbiology (Level 3 & 4)

- Routine Bacteriology (Swabs & Urines)
- Blood Cultures
- Respiratory Pathogens (CL3)
- Tissues & Fluids
- Enteric Pathogens
- Hospital Acquired Infections
- Regional Mycology Unit
- Hospital of Tropical Diseases Parasitology

### Molecular Pathology (Level 5)

- Molecular Virology (including APDU)
- Molecular Microbiology
- Molecular Parasitology



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# Laboratory Scientific Team

## Our Senior Laboratory Team



**Dr Paul Grant**

- Lead Clinical Scientist, Molecular Virology



**Dr Alan Williams**

- Lead Clinical Scientist, Molecular Microbiology



**Dr Rebecca Gorton**

- Lead Clinical Scientist Molecular Microbiology



**Dr Jude Heaney**

- Lead Research Scientist, ADPU



**Dr Vicky Enne**

- Senior Research Associate, UCL



**New Precision AMR Clinical Scientist Post**



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# Exemplar Projects at HSL

## ***Dr Paul Grant/Dr Jude Heaney***

- *WGS for detection of drug resistance in herpes simplex virus*
- *WGS for detection of drug resistance in HIV-1*

## ***Dr Vicky Enne/Dr Alan Williams***

- *Potential of rapid direct from sample ONT MinION sequencing for prediction of antimicrobial resistance phenotypes and strain typing*
- *Combining long and short-read sequencing for mapping of carbapenemase-encoding plasmids from Gram-negative bacteria: an essential tool for tracing CPE outbreaks*

## ***Dr Rebecca Gorton***

- *WGS of pneumocystis for strain relatability and resistance profiling to Septrin (Cotrimoxazole) in chronic and acute PCP*
- *WGS of clinically significant aspergillus isolates to determine TLR gene profiling for Azole resistance*



# Clinical Laboratory Facilities

- Bacteriology



- High volume DNA/RNA Extraction



- Containment Level 3



- Clinical PCR & Sequencing



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# Sequencing Technology & Capabilities

## New Extraction Facilities

- Support for additional DNA/RNA extraction specifically within containment level 3.



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# Sequencing Technology & Capabilities

## New Sequencing Facilities

**ThermoFisher**  
SCIENTIFIC



- Short-read
- Uni-directional

**illumina**<sup>®</sup>



- Short-read
- Bi-directional

**Oxford**  
**NANOPORE**  
Technologies



- Long-read
- Real time data



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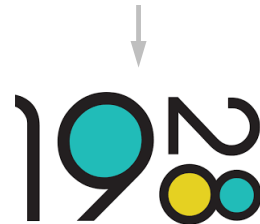
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# Informatics Pipelines & Data Analysis

- Led by Dr Eleni Nastouli and developed by UCLH and UCL Clinicians, Scientists & Bioinformaticians
- Validated extensively using HIV and Influenza and other viral and bacterial targets
- New bioinformatics framework partner with HSL for Bacterial Informatics
- Collaboration with Centre for Clinical Microbiology (UCL/RFL)
- Fungal Development Programme with HSL.
- Open access informatics tools available through Oxford Nanopore.
- Used by clinical and academic researchers across the North London Campus.



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# APDU Informatics Pipeline & Data Analysis

illumina®



Raw data .fastq

QC Phred QS >30

Filter for human reads

Reference database

Consensus genome

Resistance reporting

## GENOME SEQUENCING REPORT - HIV

UCLH - Advanced Diagnostics Pathogen Unit  
 Report Published: Thu 6 Jun 13:57:13 BST 2019  
 Website: www.uclh.nhs.uk Email: apdu@nhs.net  
 Address: 235 Euston Rd, Bloomsbury, NW12BU Telephone: 020 3456 7890



Patient Name:	Barcode:
Birth Date:	Patient ID:
Location:	Sample Type:
Sample Source:	Sample Collection Date:
Sequenced From:	Reporting Lab:
Requested By:	Requester Contact:

### Summary

The specimen was positive for Human Immunodeficiency Virus-1 (HIV1)  
 Subtype CRF02\_AG

### Drug Resistance

#### Nucleoside Reverse Transcriptase Inhibitors [NRTI]

abacavir (ABC):	SUSCEPTIBLE
zidovudine (AZT):	SUSCEPTIBLE
emtricitabine (FTC):	SUSCEPTIBLE
lamivudine (3TC):	SUSCEPTIBLE
tenofovir (TDF):	SUSCEPTIBLE

#### Non-nucleoside Reverse Transcriptase Inhibitors [NNRTI]

doravirine (DOR):	SUSCEPTIBLE
efavirenz (EFV):	SUSCEPTIBLE
etravirine (ETR):	SUSCEPTIBLE
nevirapine (NVP):	SUSCEPTIBLE
rilpivirine (RPV):	SUSCEPTIBLE

#### Protease Inhibitor

atazanavir/r (ATV/r):	SUSCEPTIBLE
darunavir/r (DRV/r):	SUSCEPTIBLE
lopinavir/r (LPV/r):	SUSCEPTIBLE

#### Integrase Strand Transfer Inhibitor

bictegravir (BIC):	SUSCEPTIBLE
dolutegravir (DTG):	SUSCEPTIBLE
elvitegravir (EVG):	POTENTIAL LOW-LEVEL RESISTANCE
raltegravir (RAL):	POTENTIAL LOW-LEVEL RESISTANCE

### Authorised

Signature	Name
Position	Date



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# Informatics Pipelines & Data Analysis

## Validated Pathogens



Species	cgMLST	MLST	Other typing schemes	Markers of special interest	Antibiotic classes
<i>S. aureus</i>	●	●	SCCmec, spa sequences	TSST-1, PVL, ETs	13
<i>K. pneumoniae</i>	●	●	-	ESBL, CRE	25+
<i>E. coli</i>	●	●	phylogroup	ESBL, CRE	25+
<i>Enterococcus faecium</i>	●	●	-	Vancomycin	25+
<i>C. difficile</i>	●	●	-	Multiple	25+
<i>S. enterica</i>	●	●	-	Multiple	25+
<i>P. aeruginosa</i>	●	●	-	Multiple	25+
<i>N. gonorrhoeae</i>	●	●	-	Multiple	25+
<i>A. baumannii</i>	●	●	-	Multiple	25+



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# 1928 Informatics Pipeline & Data Analysis



## Reporting

### Staphylococcus aureus

#83 - P26 - 2017-09-27 13:55

Resistance markers for the following antibiotics have been identified: Ciprofloxacin, Clindamycin (Inducible), Erythromycin, Gentamicin, Isoxazolyl Penicillins, Penicillinase-labile Penicillins, Trimethoprim.

#### Identified resistance markers

Identified resistance markers confer a resistance level above EUCAST clinical breakpoints.

ANTIBIOTIC	GENES	MUTATIONS
Ciprofloxacin	Not supported	grlA (S80F) <sup>[1][2][3]</sup> , gyrA (S84L) <sup>[2][4][5]</sup> , grlA (S80F) + gyrA (S84L) <sup>[1][3][6][7][8][9]</sup>
Clindamycin (Inducible)	ermC <sup>[10][11][12]</sup>	Not supported
Erythromycin	ermC <sup>[13][14]</sup>	Not supported
Gentamicin	aac6-aph2 <sup>[15][16][17]</sup>	Not supported
Isoxazolyl Penicillins	mecA <sup>[18]</sup>	Not supported
Penicillinase-labile Penicillins	mecA <sup>[18]</sup> , blaZ <sup>[18]</sup>	Not supported
Trimethoprim	dhfrA <sup>[19][20][21]</sup>	No mutations found
Clindamycin	No genes found	Not supported
Fusidic Acid	No genes found	No mutations found
Mupirocin	No genes found	Not supported
Rifampicin (Rifampin)	Not supported	No mutations found
Tetracycline	No genes found	Not supported
Vancomycin	No genes found	Not supported

#### Typing

##### MLST - TYPE: 2371

arcC	258
aroE	6
gipF	1
gmk	5
pta	8
tpi	8
yqiL	6

##### SCCMEC - TYPE: IV

IS1272	✓
ccrA1	Not found
ccrA2	✓
ccrA3	Not found
ccrA4	Not found
ccrB1	Not found
ccrB2	✓
ccrB3	Not found
ccrB4	Not found
ccrB6	Not found
ccrC	Not found
mecA	✓
mecC	Not found

lukF-PVL	✓
lukS-PVL	✓

#### Sample quality

MEASURE	QUALITY
Sequence depth	83x
Average read length	151bp
Fraction of core genes identified	98.6%

#### References

- [1] Susceptibility and resistance genes to fluoroquinolones in methicillin-resistant *Staphylococcus aureus* isolated in 2002. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/15848290\)](http://www.ncbi.nlm.nih.gov/pubmed/15848290)
- [2] Characterization of grlA, grlB, gyrA, and gyrB mutations in 116 unrelated isolates of *Staphylococcus aureus* and effects of mutations on ciprofloxacin MIC. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/9593159\)](http://www.ncbi.nlm.nih.gov/pubmed/9593159)
- [3] Mechanisms and frequency of resistance to premoxifloxacin in *Staphylococcus aureus*: novel mutations suggest novel drug-target interactions. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/2199694\)](http://www.ncbi.nlm.nih.gov/pubmed/2199694)
- [4] DNA gyrase gyrA mutations in ciprofloxacin-resistant strains of *Staphylococcus aureus*: close similarity with quinolone resistance mutations in *Escherichia coli*. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/2203254\)](http://www.ncbi.nlm.nih.gov/pubmed/2203254)
- [5] GyrA sequence analysis of *Staphylococcus aureus* and methicillin-resistant *S. aureus* strains selected, in vitro, for high-level ciprofloxacin resistance. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/7902228\)](http://www.ncbi.nlm.nih.gov/pubmed/7902228)
- [6] Development of resistance to ciprofloxacin, rifampin, and mupirocin in methicillin-susceptible and -resistant *Staphylococcus aureus* isolates. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/11036061\)](http://www.ncbi.nlm.nih.gov/pubmed/11036061)
- [7] Extended spectrum of quinolone resistance, even to a potential latter third-generation agent, as a result of a minimum of two GrlA and two GyrA alterations in quinolone-resistant *Staphylococcus aureus*. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/2040724\)](http://www.ncbi.nlm.nih.gov/pubmed/2040724)
- [8] Topoisomerase mutations that are associated with high-level resistance to earlier fluoroquinolones in *Staphylococcus aureus* have less effect on the antibacterial activity of besifloxacin. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/2199694\)](http://www.ncbi.nlm.nih.gov/pubmed/2199694)
- [10] Testing for induction of clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus*. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/1581499\)](http://www.ncbi.nlm.nih.gov/pubmed/1581499)
- [11] Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. [Link \(http://www.ncbi.nlm.nih.gov/pubmed/1453221\)](http://www.ncbi.nlm.nih.gov/pubmed/1453221)

- Resistance Markers



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- Typing

- Quality Metrics

- Clinical References



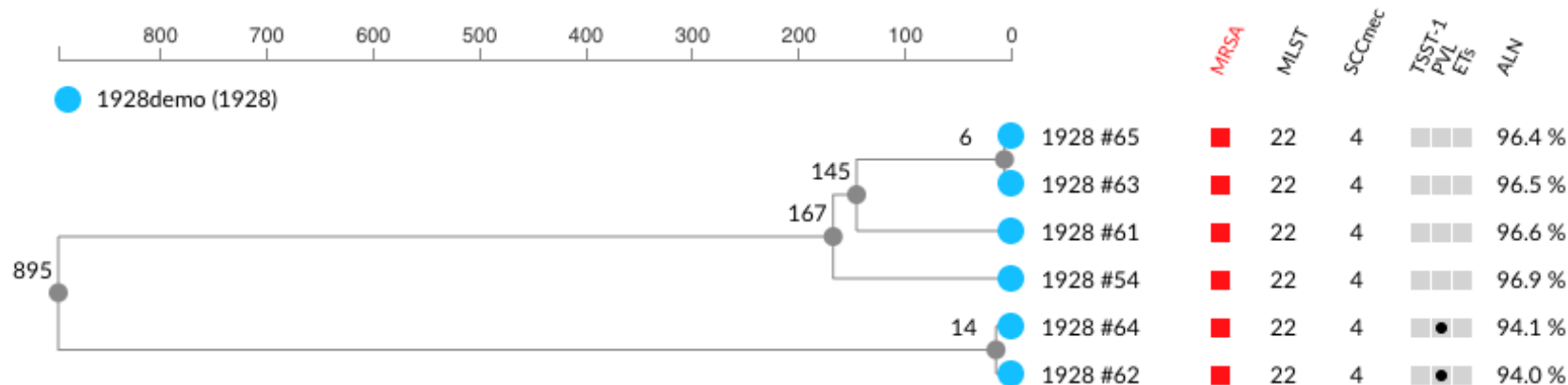
# Nanopore Informatics Pipeline & Data Analysis

## Phylogenetic Analysis (cgMLST / SNP clustering)



### UPGMA clustering

Reference: *Staphylococcus aureus* subsp. *aureus* HO 5096 0412 complete genome (NC\_017763.1) [Link](#)

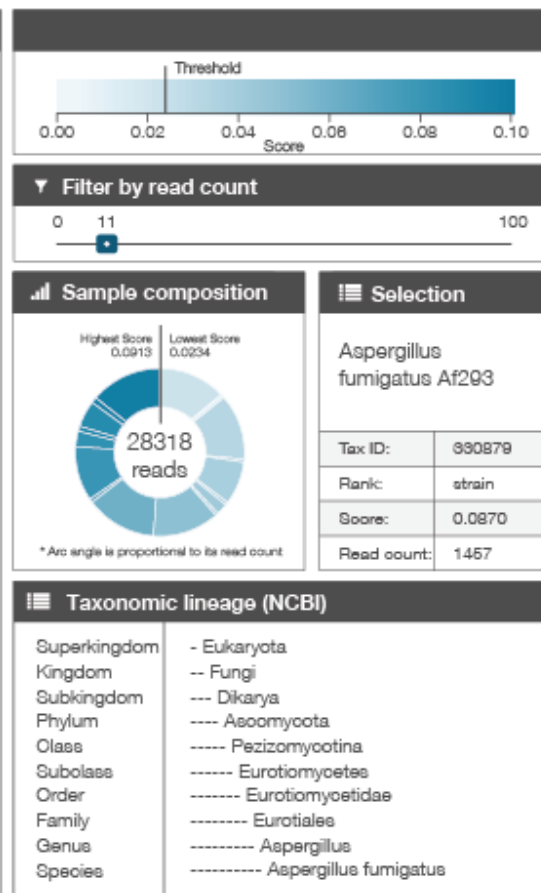
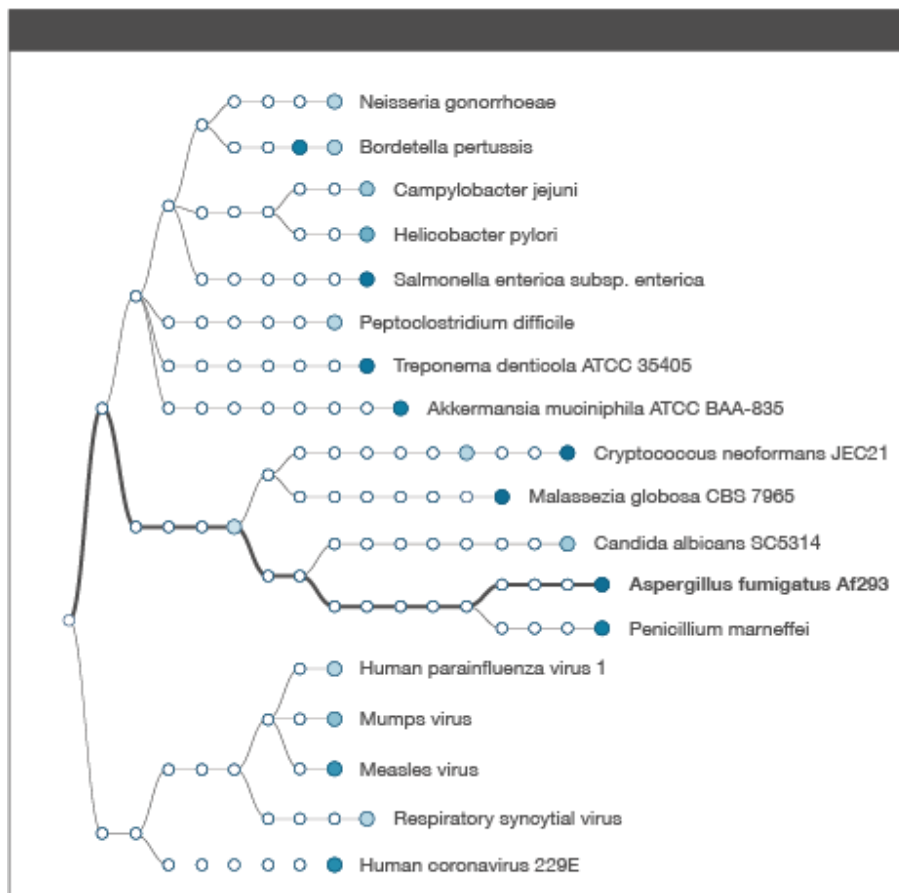


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# Nanopore Informatics Pipeline & Data Analysis



WIMP report, shown for a sample containing bacteria, viruses and fungi



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***Thankyou***



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