

# Assessing the potential of targeted and shotgun metagenomics for the diagnosis of hospital acquired pneumonia

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# Conflicts of interest

- Dr Justin O'Grady
  - Received reagents free of charge from Oxford Nanopore®
  - Co-funded PhD student with Oxford Nanopore®

# Background

- Rapid diagnostics key in combating AMR
- Multiple molecular platforms available providing rapid and informative data to clinicians but are not without limitations



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## GLOBAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE

# Metagenomics

- Genomic analysis of all DNA sequences extracted directly from environmental or clinical samples – the metagenome
  - I.e. culture independent, unbiased
- Made possible by advances in next generation sequencing technology and bioinformatics

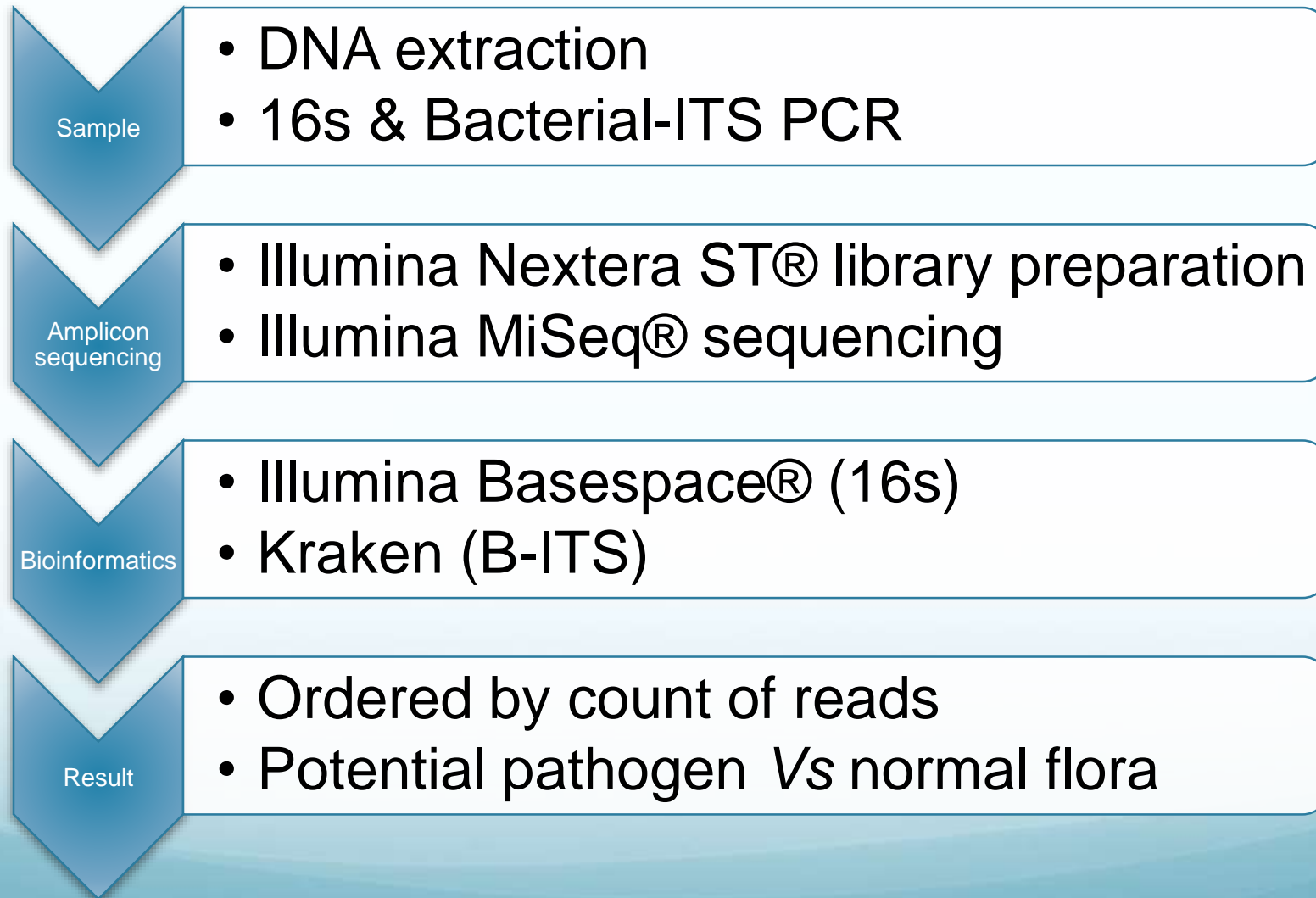
# Metagenomic approaches

	Targeted (amplicon based)	Whole genome shotgun
Advantages	<ul style="list-style-type: none"><li>• Straightforward</li><li>• No need for enrichment</li><li>• Rapid</li><li>• Relatively inexpensive</li></ul>	<ul style="list-style-type: none"><li>• Depth of information including the presence of resistance genes</li></ul>
Disadvantages	<ul style="list-style-type: none"><li>• Reliable only to the genus level</li><li>• No resistance information</li></ul>	<ul style="list-style-type: none"><li>• Requires human DNA depletion</li><li>• Linking plasmid born resistance to pathogen</li></ul>

# Objectives

- Targeted metagenomics Vs current methods for pathogen identification in HAP/VAP
  - Culture
  - Curetis Unyvero P55 Pneumonia assay
- Whole genome shotgun metagenomics

# Targeted metagenomics

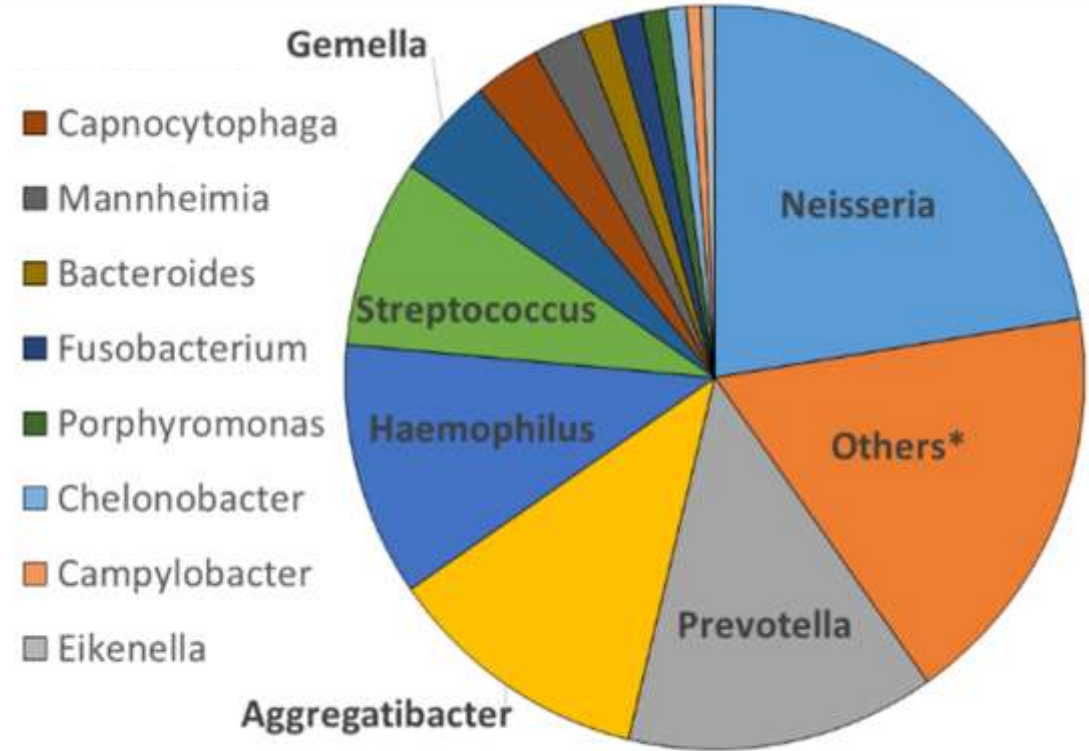
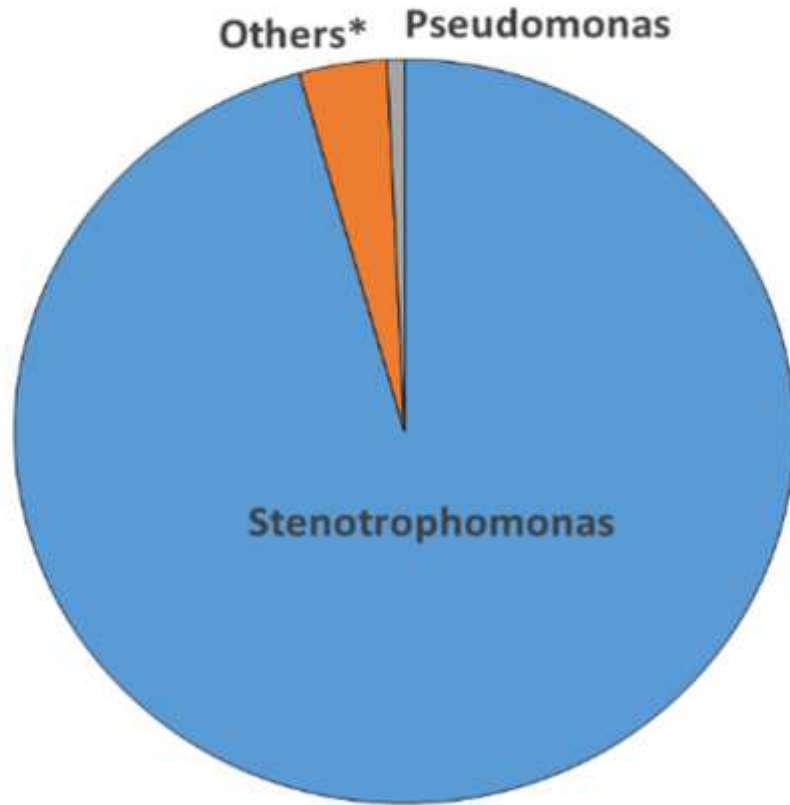


# Targeted metagenomics

Sample	Culture	Unyvero	16SrRNA	Bacterial-ITS
1 (Endotracheal aspirate)	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas sp.</i>	<i>Serratia sp.</i>
2 (Endotracheal aspirate)	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas sp.</i>	<i>Pseudomonas sp.</i>
3 (Sputum)	No significant growth	<i>Escherichia coli</i>	Normal respiratory flora	Normal respiratory flora
4 (Sputum)	No growth	Negative	Normal respiratory flora	Normal respiratory flora
5 (Sputum)	Normal respiratory flora	<i>Acinetobacter baumannii complex</i>	Normal respiratory flora	
6 (Endotracheal aspirate)	Normal respiratory flora	Negative	Normal respiratory flora	
7 (Endotracheal aspirate)	<i>Stenotrophomonas maltophilia</i>	<i>Stenotrophomonas maltophilia</i>	<i>Stenotrophomonas sp.</i>	<i>Stenotrophomonas sp.</i>
8 (Endotracheal aspirate)	Normal respiratory flora	Negative	Normal respiratory flora	Normal respiratory flora
9 (Sputum)	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella sp.</i>	<i>Klebsiella sp.</i>
10 (Endotracheal aspirate)	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas sp.</i>	<i>Pseudomonas sp.</i>
11 (Endotracheal aspirate)	Normal respiratory flora	<i>Stenotrophomonas maltophilia</i>	Normal respiratory flora	
12 (Endotracheal aspirate)	Normal respiratory flora	<i>Staphylococcus aureus</i>	<i>Staphylococcus sp.</i>	Normal respiratory flora
13 (Endotracheal aspirate)	Normal respiratory flora		<i>Klebsiella sp.</i>	<i>Klebsiella sp.</i>
14 (Sputum)	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas sp.</i>	
15 (Endotracheal aspirate)	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas sp.</i>	Negative
16 (Endotracheal aspirate)	Coliform		<i>Klebsiella sp.</i>	<i>Klebsiella sp.</i>
17 (BAL)	<i>Staphylococcus aureus</i>		<i>Staphylococcus sp.</i>	<i>Staphylococcus sp.</i>



# 16s metagenomics output



# MinION shotgun metagenomics

- Saponin based method for host DNA depletion
- Library preparation using Rapid Low Input kit
  - Transposon based library prep with long range PCR
  - 10ng DNA starting material required
  - Up to 3 million reads at 1-2kb average fragment length
- Good method for clinical samples
  - Can handle poor quality DNA
- < 8 hr turnaround time from sputum result (pathogen and resistance)

# 464,408

MinION Reads Analysed

Unclassified

419,841 Reads

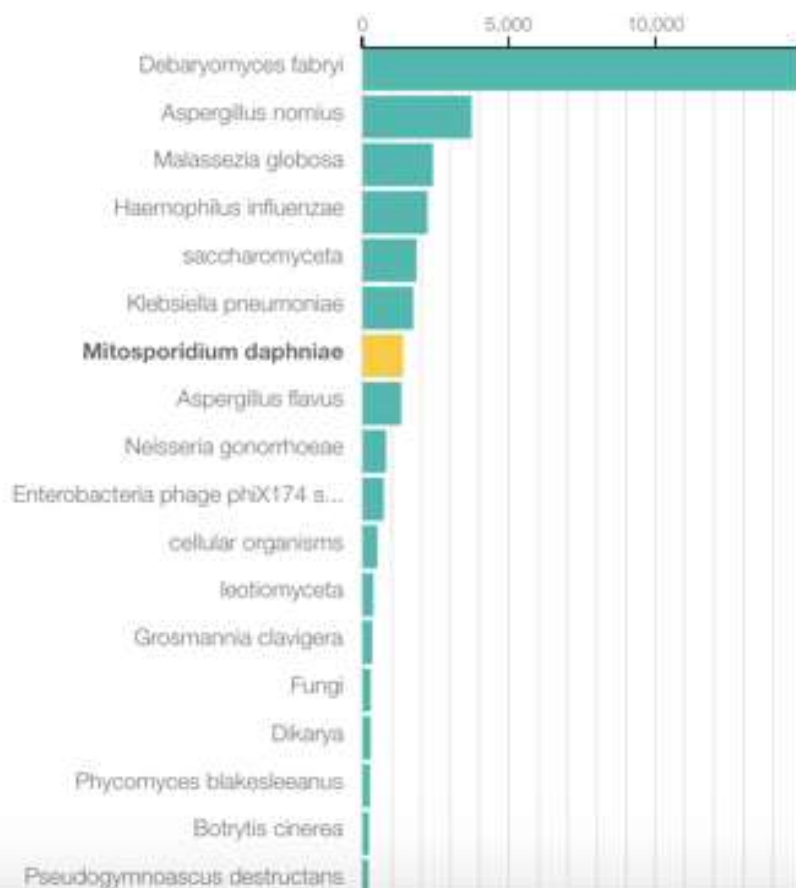
Classified

44,567 Reads

## Top Classifications



Classification Count  
where Quality Score > 10

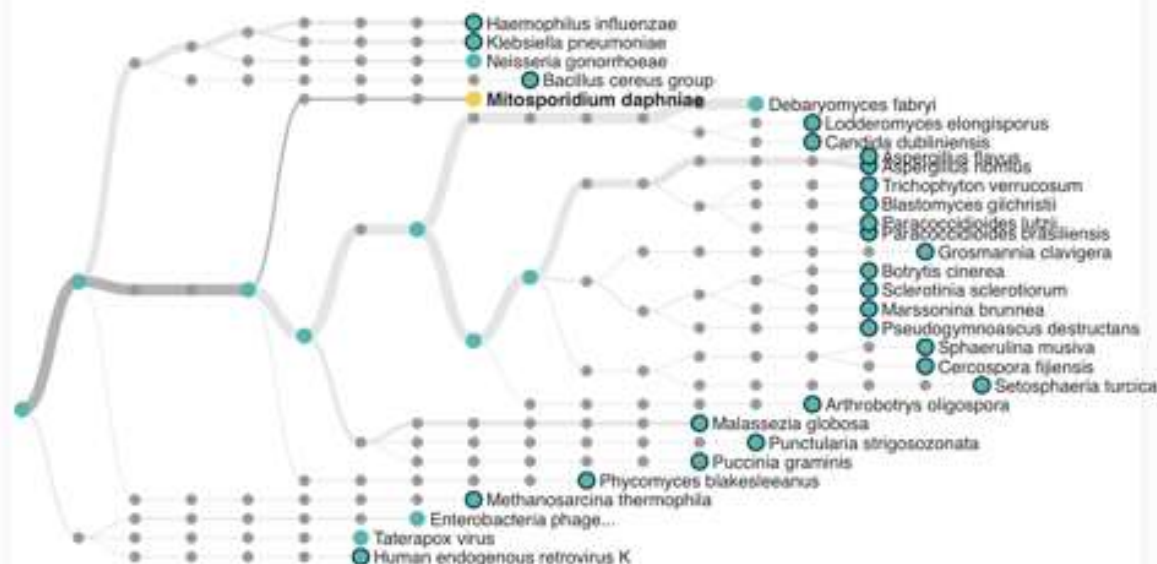


## Selection Summary

## NCBI Taxonomy



Collapse at Rank: Phylum Class Order Family Genus Species All

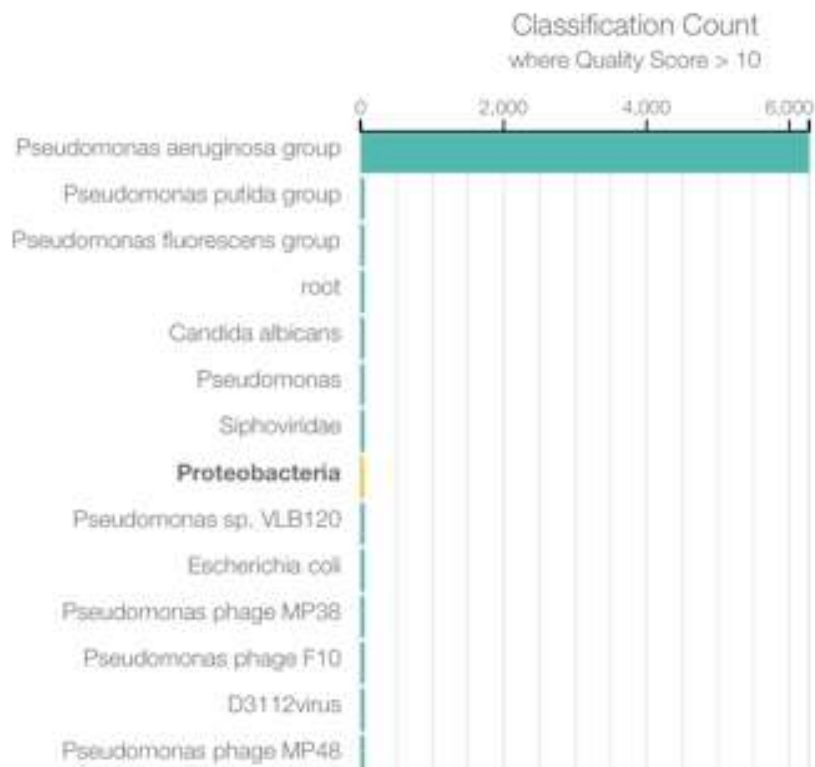


- Taxonomy Node
- Classification
- Classification - Collapsed

7,993  
MiniON Reads Analysed



### Top Classifications

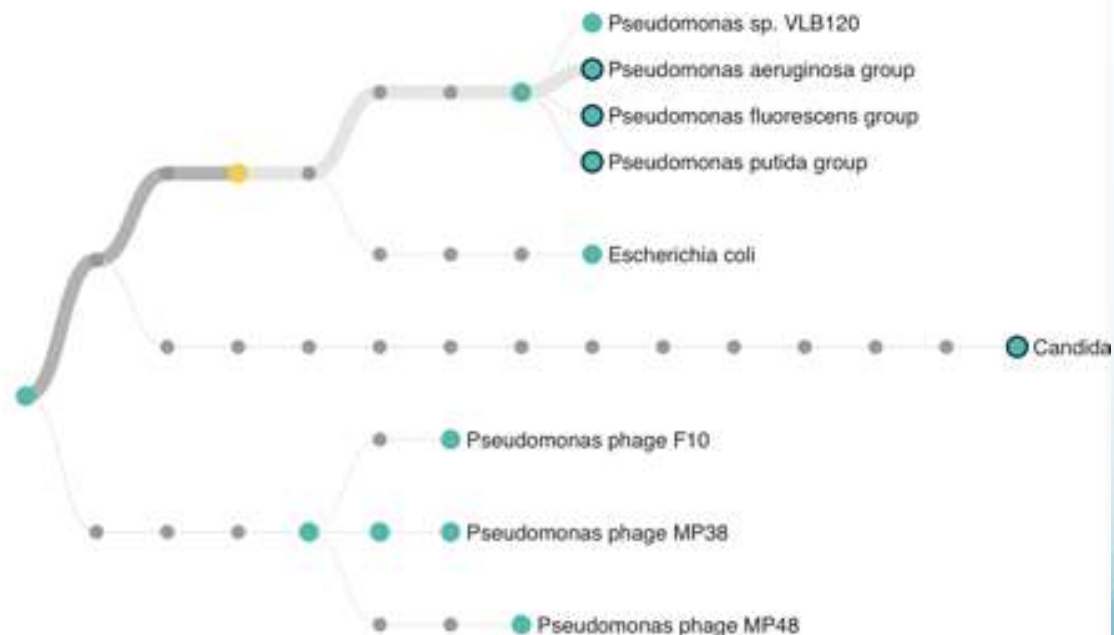


Taxon shown in the Selection Summary panel to the right. Click to select a row

### Selection Summary

### NCBI Taxonomy

Collapse at Rank:  Phylum  Class  Order  Family  Genus  Species  All



- Taxonomy Node
- Classification
- Classification - Collapsed

# Conclusion

- Targeted metagenomics
  - Useful description of the microbial community
- Whole genome shotgun metagenomics
  - Accurate speciation and depth of information
  - Human DNA depletion
- Future challenge
  - Genotypic Vs. Phenotypic resistance

# Acknowledgements

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