



Division of
**Consolidated
Laboratory Services**



Apples to Apples: Using Genomic Comparison to Aid in an Outbreak Investigation

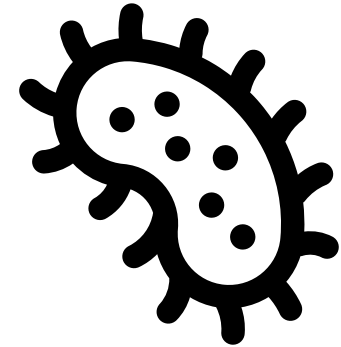
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October 25, 2023



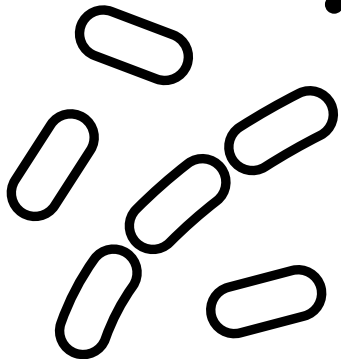
Objectives

- Improve understanding of whole genome sequencing (WGS)
- Explain how WGS can be used to assist in an antimicrobial resistant bacterial outbreak investigation
- Demonstrate DCLS WGS surveillance and reporting system

Carbapenem Resistant Organisms (CRO)



- Carbapenem-resistant Enterobacterales (CRE)
- Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA)
- Carbapenem-resistant *Acinetobacter baumannii* complex (CRAB)



modified Carbapenem Inactivation Method (mCIM)



PCR Testing

CRE, CRPA, CRAB:
KPC, NDM, VIM, IMP,
OXA-48

CRAB:
OXA-23, OXA-24/40,
OXA-58

Antimicrobial Susceptibility Testing (AST)



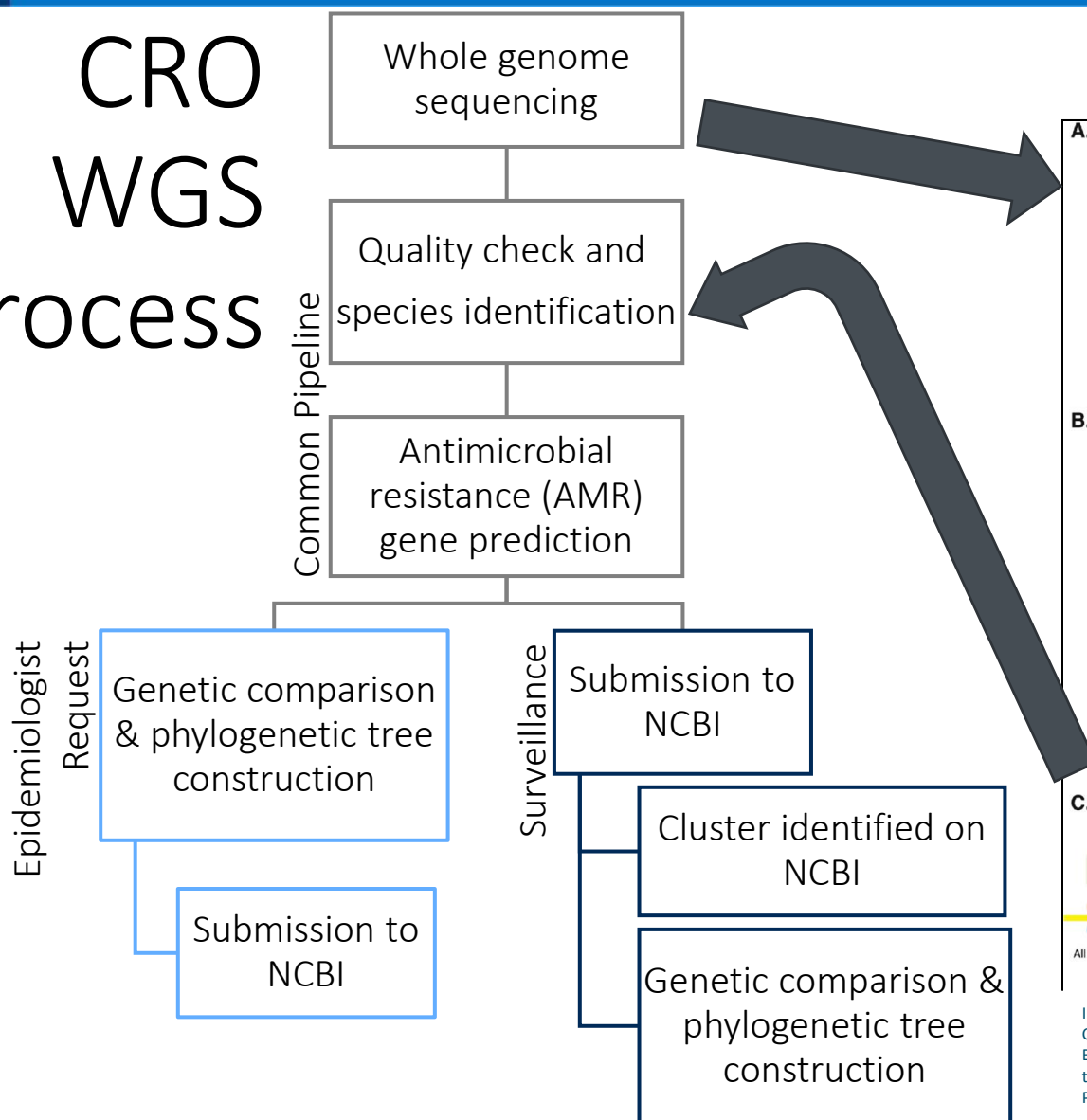
WGS Candidate Criteria

- ☐ *Enterobacterales* with a non-KPC gene by PCR
- ☐ Pan-non-susceptible
- ☐ 2 or more PCR genes detected
- ☐ Carbapenemase +/-PCR -
- ☐ Carbapenemase +/-PCR + CRPA
- ☐ PCR+ CRAB
- ☐ Pan- β -lactam resistant CRAB

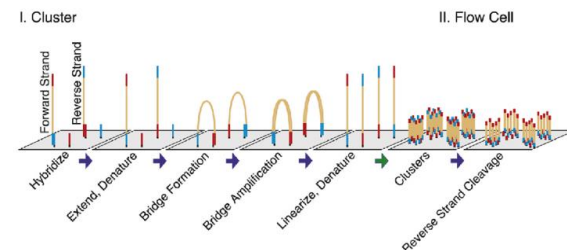
Introduction to HAI/AR WGS at DCLS

- Instruments Utilized
 - Illumina MiSeq (routine)
 - Oxford Nanopore MinION (validation pending)
- Purpose
 - Aid CDC in surveillance for novel resistance mechanisms
 - Support epidemiologic investigation by VDH

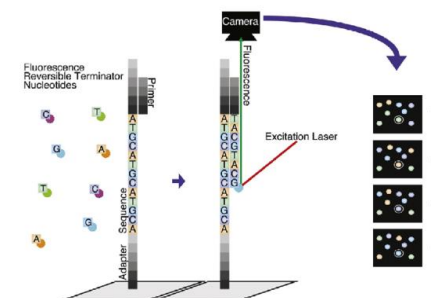
CRO WGS Process



A. Clustering



B. High-throughput sequencing



C. Demultiplexing samples and read mapping

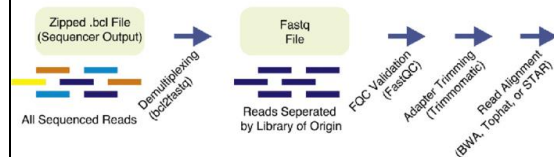
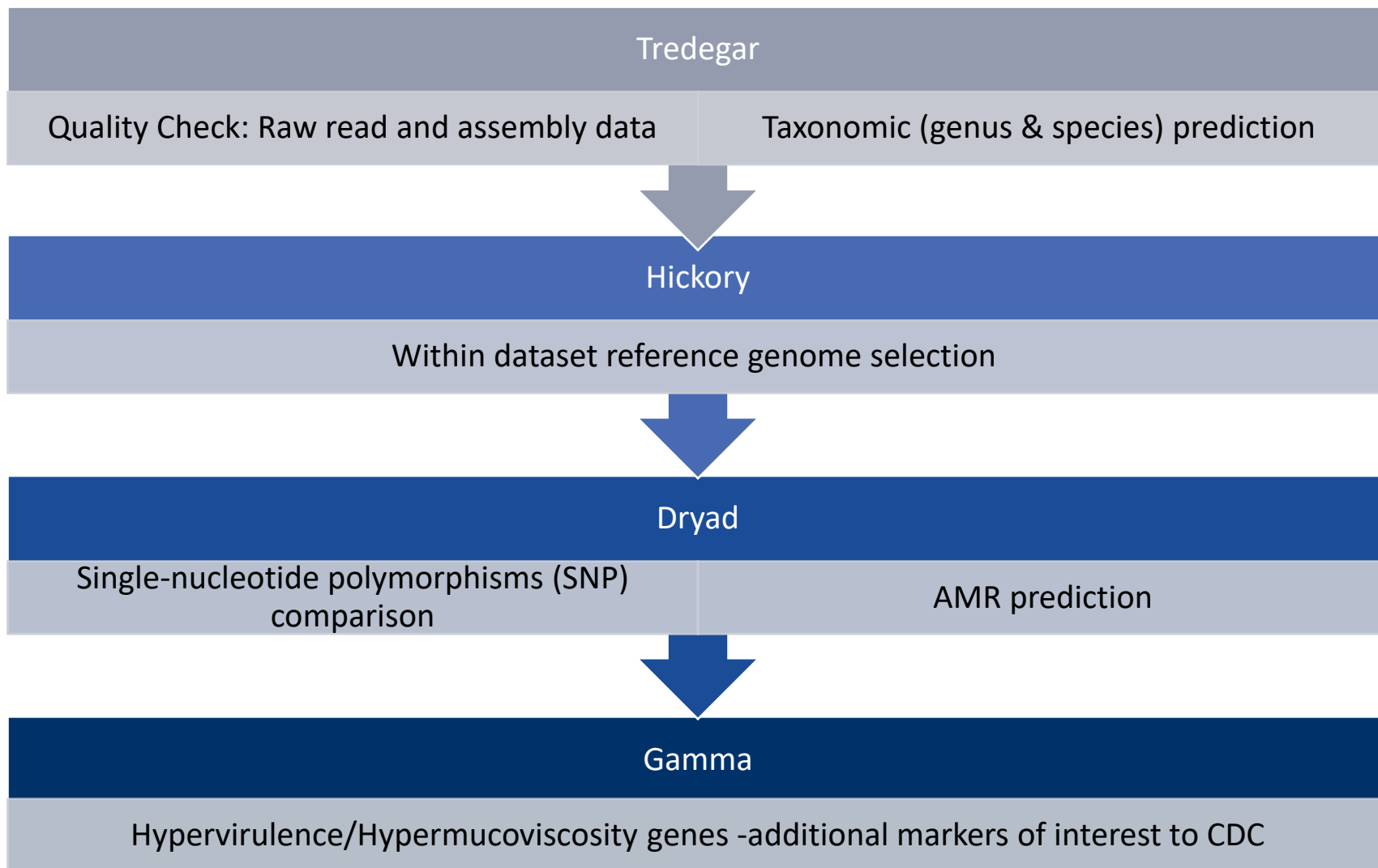


Image:

Chaitankar, Vijender & Karakulah, Gökhan & Ratnapriya, Rinki & Giuste, Felipe & Brooks, Matthew & Swaroop, Anand. (2016). Next generation sequencing technology and genomewide data analysis: Perspectives for retinal research. Progress in retinal and eye research. 55. 10.1016/j.preteyeres.2016.06.001.

Bioinformatics Software and Pipelines



How to Request WGS Analysis

- R numbers (DCLS sample ID #) or patient information (name and DOB)
- Genus/species
- Outbreak ID (if available)
- Any additional suspected outbreak details
- **Send request to dcls_ngsrequest@dgs.virginia.gov**



Outbreak Background Information

| Specimen Collection Date | WGS ID | Specimen Source | Species ID | CRO PCR | Resistance Genes |
|--------------------------|---------------------|---------------------------------------|------------------------------|-------------|------------------|
| 12/22/2022 | Patient 1 Isolate 1 | Peritoneal fluid | <i>Escherichia coli</i> | OXA-48-like | OXA-181* |
| | | | | NDM | NDM-5 |
| 01/04/2023 | Patient 1 Isolate 2 | Peritoneal fluid | <i>Klebsiella pneumoniae</i> | OXA-48-like | OXA-181* |
| | | | | NDM | NDM-5 |
| 02/23/2023 | Patient 2 | Urine | <i>Klebsiella pneumoniae</i> | OXA-48-like | OXA-181* |
| | | | | NDM | NDM-5 |
| Unknown | Patient 3 | Rectal swab-colonization screening ** | <i>Klebsiella pneumoniae</i> | NDM** | NDM-7 |

*OXA-181 is an OXA-48-like gene

**Colonization swab testing and sequencing performed by Maryland Department of Health Laboratories Administration

Genomic Comparison

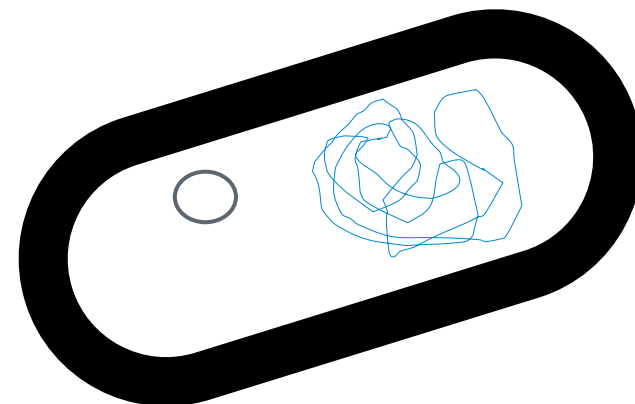
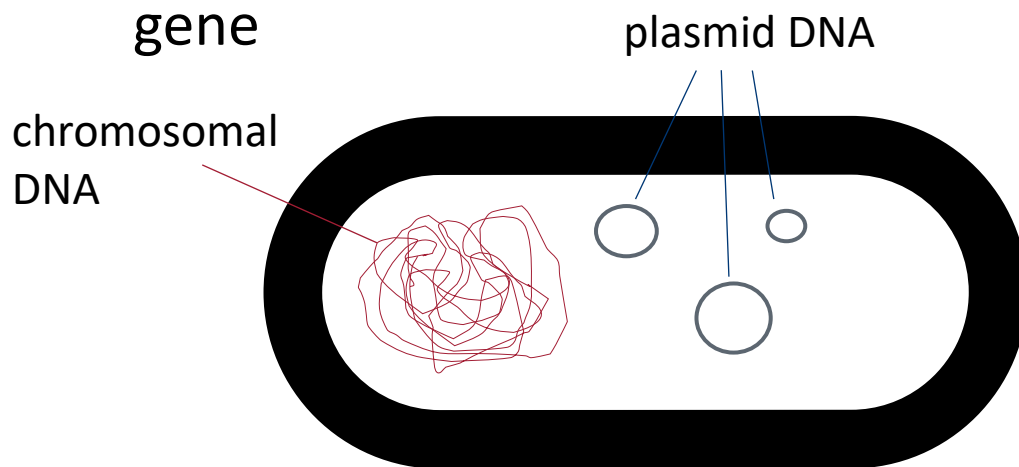
- Can include colonization screening samples
- Must be same species or harbor the same plasmid-mediated resistance gene



Klebsiella pneumoniae



Escherichia coli



What is a Single Nucleotide Polymorphism (SNP)?

TTGATGCTGAGCGGGTGCATGCCCGGTGAAATCCGCCCGACGATTGGCCAGCAAATGGAA
 |||||
 TTGATACTGAGCGGGTGCATGCCCGGTGAAATCCGCCCGACGATTGGCCAGCAAATGGAA



| | Patient 1 | Patient 2 | Patient 3 |
|-----------|-----------|-----------|-----------|
| Patient 1 | | 2 | 25738 |
| Patient 2 | 2 | | 25900 |
| Patient 3 | 25738 | 25900 | |

What makes a cluster of isolates?

Typical cluster criteria $\sim \leq 10$
 SNPs of the millions compared



Antimicrobial Resistance Gene Prediction

| Patient 1 <i>E. coli</i> (Gene, % Coverage and % Identity) | Patient 1 <i>K. pneumoniae</i> (Gene, % Coverage and % Identity) | Patient 2 <i>K. pneumoniae</i> (Gene, % Coverage and % Identity) | Patient 3 <i>K. pneumoniae</i> (Gene, % Coverage and % Identity) | Sequence name | | Subclass |
|--|--|--|--|---|-------------|-------------|
| blaOXA-181 (100%, 100%) | blaOXA-181 (100%, 100%) | blaOXA-181 (100%, 100%) | | OXA-48 family carbapenem-hydrolyzing class D beta-lactamase | BETA-LACTAM | BETA-LACTAM |
| blaNDM-5 (100%, 100%) | blaNDM-5 (100%, 100%) | blaNDM-5 (100%, 100%) | blaNDM-7 (100%, 100%) | subclass B1 metallo-beta-lactamase | BETA-LACTAM | CARBAPENEM |

DCLS has not validated reporting of AMR observations for clinical use

Same allele types = possible plasmid transfer

Different allele types = less likely to be a plasmid outbreak



AST Results

| Antimicrobial | Patient 2 <i>K. pneumoniae</i> | Patient 1 <i>K. pneumoniae</i> | Patient 1 <i>E. coli</i> |
|-------------------------------|-----------------------------------|-----------------------------------|-----------------------------|
| Amikacin | Resistant | Resistant | Resistant |
| Ampicillin | Resistant | Resistant | Resistant |
| Ampicillin/Sulbactam | Resistant | Resistant | Resistant |
| Aztreonam | Resistant | Resistant | Resistant |
| Cefepime | Resistant | Resistant | Resistant |
| Cefazolin | Resistant | Resistant | Resistant |
| Ceftazidime | Resistant | Resistant | Resistant |
| Ceftazidime/Avibactam | Resistant | Resistant | Resistant |
| Ceftolozane/Tazobactam | Resistant | Resistant | Resistant |
| Ceftriaxone | Resistant | Resistant | Resistant |
| Ciprofloxacin | Resistant | Resistant | Resistant |
| Ertapenem | Resistant | Resistant | Resistant |
| Gentamicin | Resistant | Resistant | Resistant |
| Imipenem | Resistant | Resistant | Resistant |
| Levofloxacin | Resistant | Resistant | Resistant |
| Meropenem | Resistant | Resistant | Resistant |
| Piperacillin/Tazobactam | Resistant | Resistant | Resistant |
| Tetracycline | Intermediate | Intermediate | Resistant |
| Tobramycin | Resistant | Resistant | Resistant |
| Trimethoprim/Sulfamethoxazole | Resistant | Resistant | Resistant |

Summary

- Patient 1 & 2 were genetically similar and had an epidemiologic link
- WGS data supported suspicion of transfer event
- Patient 3 was genetically distinct

Building Testing Capacity

- Oxacillinase (OXA) gene PCR testing for CRAB (implemented in August 2023)
- Colonization screening and sequencing of CRO isolates (validation in process)
- Long-read sequencing (development in process)
- *Candida auris* sequencing and colonization screening (development in process)



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Questions

