CDPH
California Department of Public Health

Public Health Laboratory Experience with Whole Genome Sequencing (WGS) of Multidrug-Resistant Organisms (MDROs)

Healthcare-Associated Infections Program

Microbial Diseases Laboratory

May 14, 2025

Objectives

- Learn approaches that local public health laboratories use for WGS
- Understand applications of WGS for MDRO surveillance and epidemiological investigations

Housekeeping

Questions? Email us at HAIProgram@cdph.ca.gov or CARLProgram@cdph.ca.gov

Microbial Diseases Laboratory Updates

MDL Updates

- All CRE/CRPA/CRAB isolates now undergoing StarCarba testing
- Submitting isolates for sequencing:
  - Isolates submitted to MDL for sequencing must be submitted using the Antimicrobial Susceptibility Testing – AST form
  - Attach a printed copy of clinical ASTs and any isolate testing results when shipping to your local public health lab and/or MDL
    - **Clinical labs should submit isolates for sequencing to their local/county public health lab (LPHL)** to be sequenced there or forward to MDL; if you are not connected to an LPHL, reach out to CARLProgram@cdph.ca.gov
  - Do not send duplicate isolates without prior approval
    - Duplicate = same organism from the same patient within 12 months; do not submit if a carbapenemase mechanism was identified in the original or if the AST profile is not significantly different
  - Refer to MDL AR Testing Services document for submission criteria and additional instructions
- Reach out to HAI_AR@cdph.ca.gov, CARLProgram@cdph.ca.gov, and/or MDLBDS@cdph.ca.gov with any questions

ochealth CARE AGENCY

Orange County Whole Genome Sequencing

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Candida auris (*C. auris*) is a potentially multi-drug-resistant fungus

- Can cause outbreaks of nosocomial infection
- Infections can be severe for high-risk patients
  - Complex health conditions
  - Indwelling medical devices
  - Ventilator dependent
- Spread was identified in Orange County (OC) in 2019
  - The first local transmission seen in California
  - 3,300+ colonized & clinical cases as of April 2025

Identification of *C. auris*
(www.cdc.gov/candida-auris/hcp/laboratories/identification-of-c-auris.html)

C. auris WGS was of interest to supplement surveillance and investigations

- CDC lab sequenced initial 2019 isolates for Clade Identification
  - All Clade III
- WGS capacity through the OC Public Health Lab (PHL) was available December 2022
  - Clade Identification
    - 6 Clades total
    - Surveillance for emergence of other clades
  - FKS1 Gene Mutation Identification
    - Infers resistance to antifungal class: Echinocandins
  - SNP Analysis
    - Infer relationships and degree of relatedness
    - No threshold guidance available yet

Selecting a sample of isolates for WGS is both targeted & random

- Targeted
  - Multi-drug-resistant *C. auris*
  - Travel history
  - Outbreaks
  - Uncommon specimen collection settings (i.e. outpatient)
- Random
  - General acute-care hospitals (ACH)
  - Long-term acute care hospitals (LTACH)
  - Ventilator-equipped skilled nursing facilities (vSNF)

OC PHL Workflow for *C. auris* WGS

DNA Extraction
~1 hr
1-25 specimens

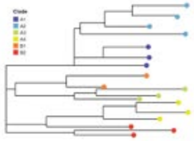
Library Prep
~5 hrs
8-96 specimens

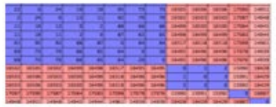
Sequencing
~40 hrs
8 specimens

Analysis
~3 hrs
8-16 specimens

Theiagen
GENOMICS

Data Visualization
~2 hrs





OC PHL Workflow for *C. auris* WGS

1. WGS *C. auris* ordered by Epi
2. DNA Extraction by Mycology Dept.
    - Qiagen EZ1 Advanced XL instrument
    - MasterPure Yeast DNA Purification Kit + EZ1 DNA Tissue Kit
3. WGS Library Prep by Molecular Dept.
    - Illumina DNA Prep Kit
    - Manual Prep or Automated Tecan Fluent Dream Prep
    - MiSeq v2 (500cycle)
    - Illumina MiSeq instrument
4. Analysis and reporting: Organism ID, Clade, detection for FKS1 gene mutation

Basespace → Theaigen workspace → TheiaEuk workflow → MycoSNP Variants workflow → Reporting

5. Additional Snippy Tree workflow
    - SNP/distance matrices
    - Nwk files for phylogenetic tree creation in Microreact application

Data sharing & communication between Epi & PHL

Regular email communication

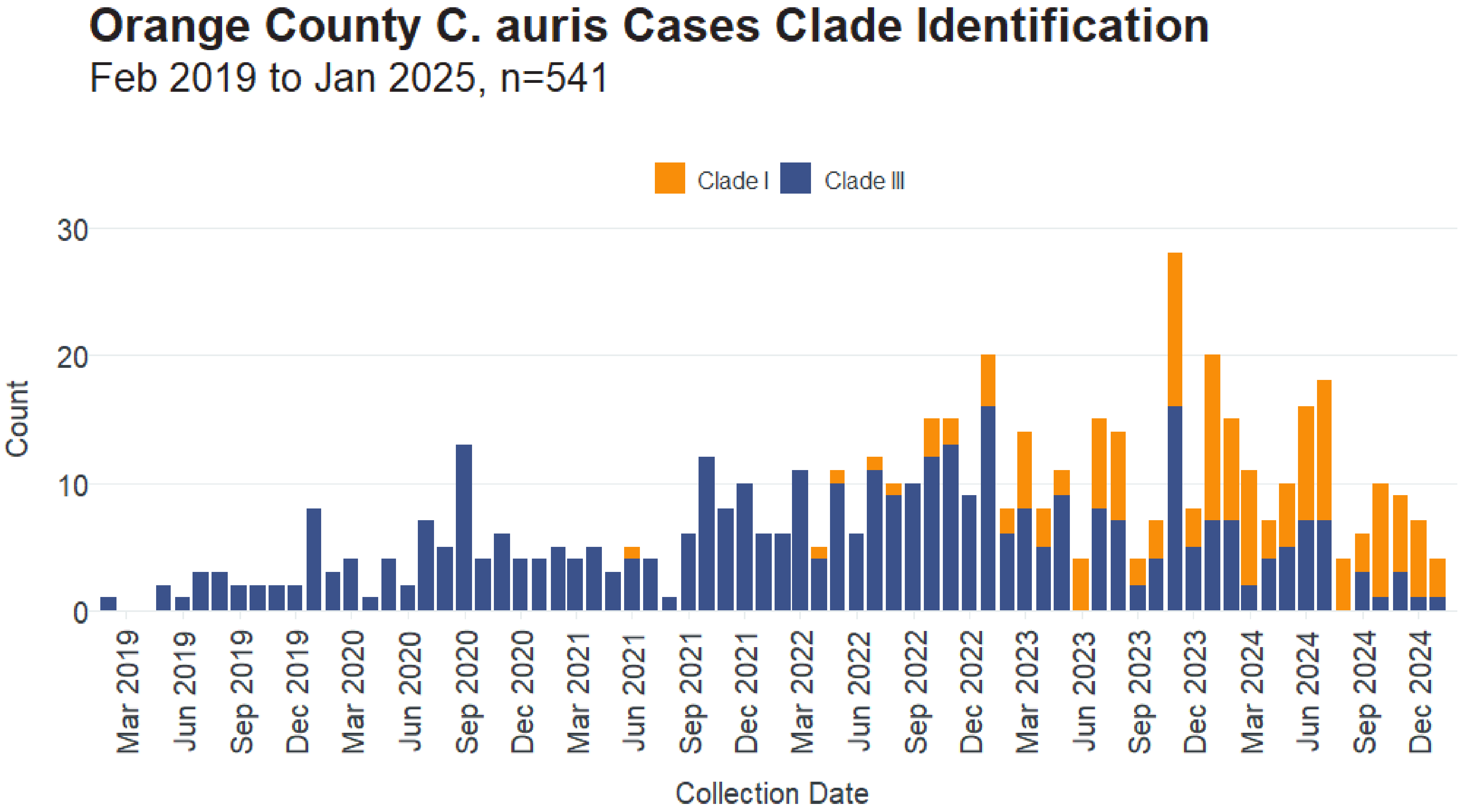
Bi-weekly meetings between HAI Epi & Lab

Shared data & nwk files

Tools used by the Epi team

Microreact

Orange County Case Study #1



Orange County Case Study #1

Introduction of Clade I – Case 1

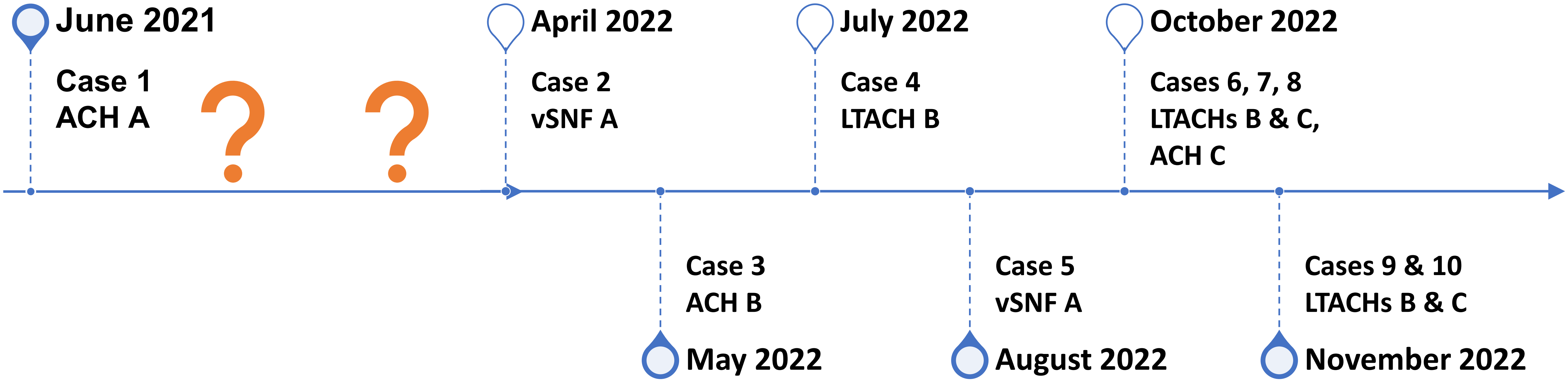
- International healthcare history 4/2021 - 6/2021
  - Hospitalized in Middle East

- Transferred to OC Hospital A 6/2021 - 7/2021
  - Admitted as known positive
  - Clade I specimen collected 6/2021

- Transferred to LTACH A 7/2021
  - Expired 9/2021

Orange County Case Study #1

Timeline of Early Clade I Cases



June 2021
Case 1
ACH A

? ?

April 2022
Case 2
vSNF A

May 2022
Case 3
ACH B

July 2022
Case 4
LTACH B

August 2022
Case 5
vSNF A

October 2022
Cases 6, 7, 8
LTACHs B & C,
ACH C

November 2022
Cases 9 & 10
LTACHs B & C

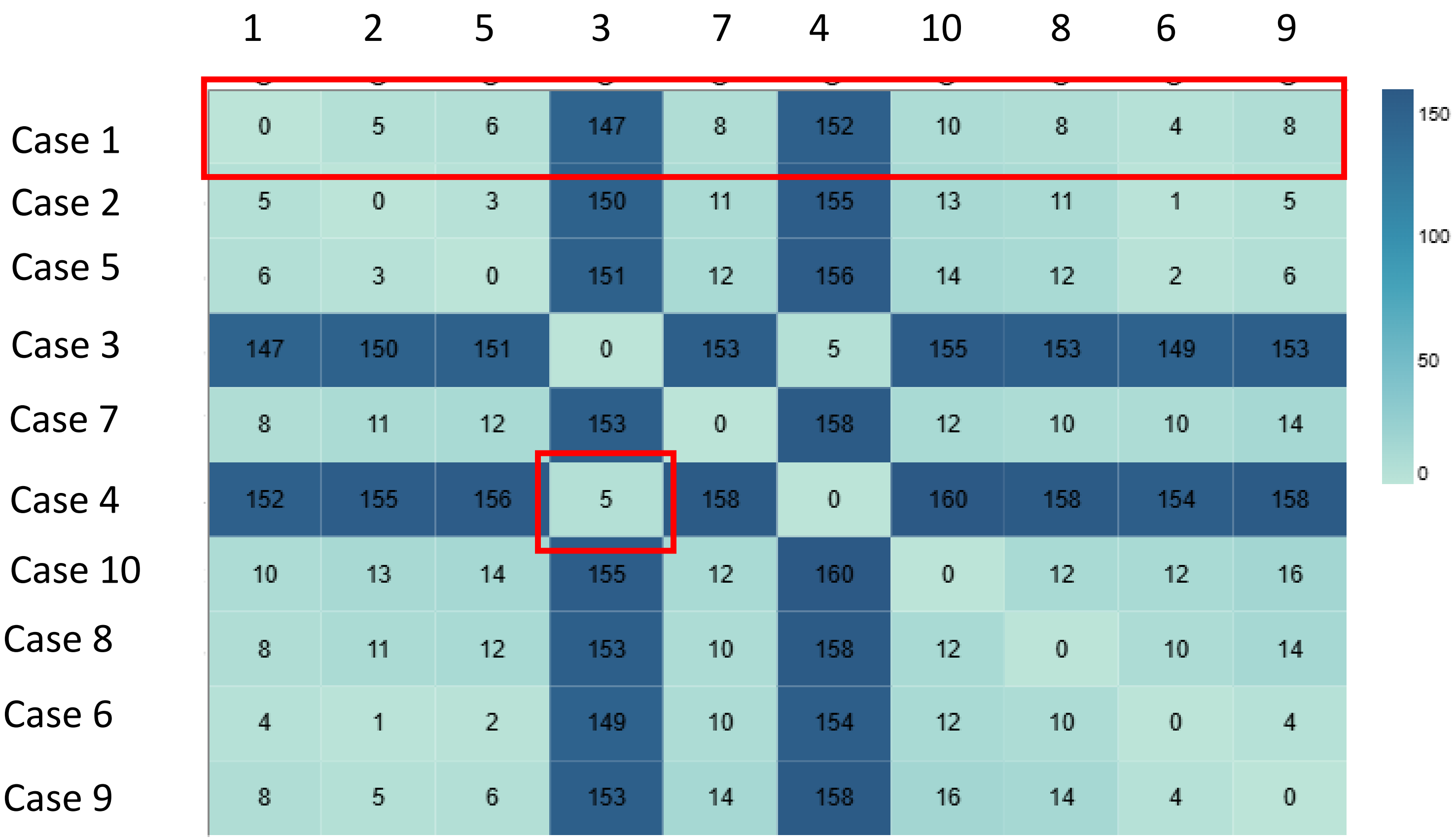
Orange County Case Study #1

Initial Epi Hypothesis

- Investigation found no links between Case 1 and subsequent Clade I cases
- No additional Clade I cases found at ACH A or LTACH A until 2023
- Our initial hypothesis
  - Case 1 was an isolated case with no transmission
  - Cases from early 2022 likely due to additional Clade I introduction(s) resulting in community spread
- ….Until additional WGS analyses
  - More data → more questions!

Orange County Case Study #1

SNP Matrix for Early Clade I Cases



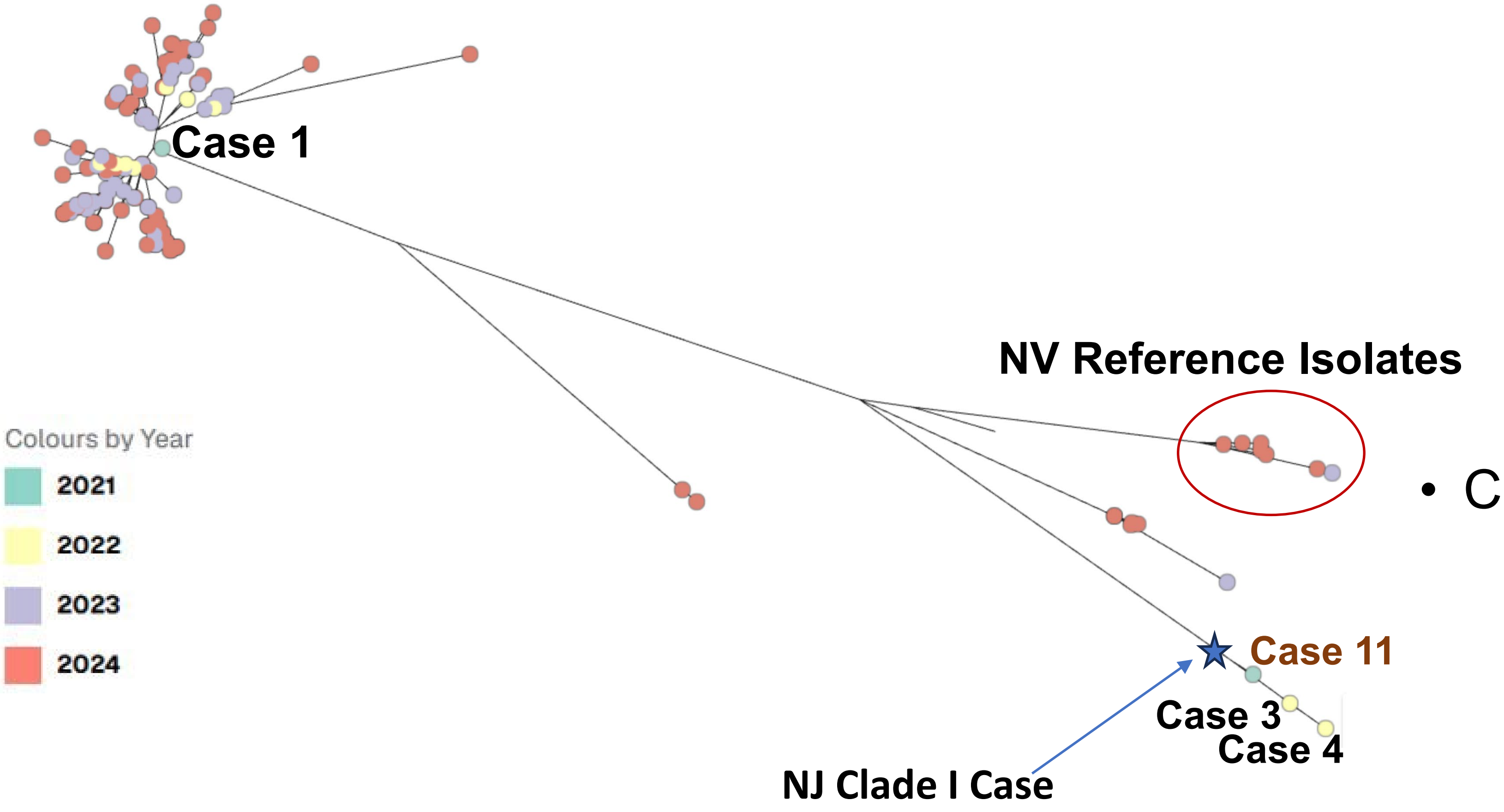
	1	2	5	3	7	4	10	8	6	9
Case 1	0	5	6	147	8	152	10	8	4	8
Case 2	5	0	3	150	11	155	13	11	1	5
Case 5	6	3	0	151	12	156	14	12	2	6
Case 3	147	150	151	0	153	5	155	153	149	153
Case 7	8	11	12	153	0	158	12	10	10	14
Case 4	152	155	156	5	158	0	160	158	154	158
Case 10	10	13	14	155	12	160	0	12	12	16
Case 8	8	11	12	153	10	158	12	0	10	14
Case 6	4	1	2	149	10	154	12	10	0	4
Case 9	8	5	6	153	14	158	16	14	4	0

- Case 1 is within 10 SNPs of early Clade I cases, except 3 & 4
- Why are 3 & 4 so different from all the others?

Orange County Case Study #1

More pieces of the puzzle

A second Clade I introduction from New Jersey



- Additional Clade I sequences from CDC
  - Known positive case from NJ admitted to LTACH C 12/2020
  - Case 11 positive 2/2021 at LTACH C
  - Closely related by WGS
- Cases 3 & 4
  - No epi links to Case 11 or NJ Case
  - Admitted to LTACH B 2 months apart

Orange County Case Study #1

Conclusions

- Importance of WGS + Epi
  - Both are necessary for the full story
- Contrasting outcomes from 2 introductions
  - 1 led to widespread community transmission
  - 1 appears contained with very few additional cases
    - Importance of proper infection control precautions!

Orange County Case Study #2

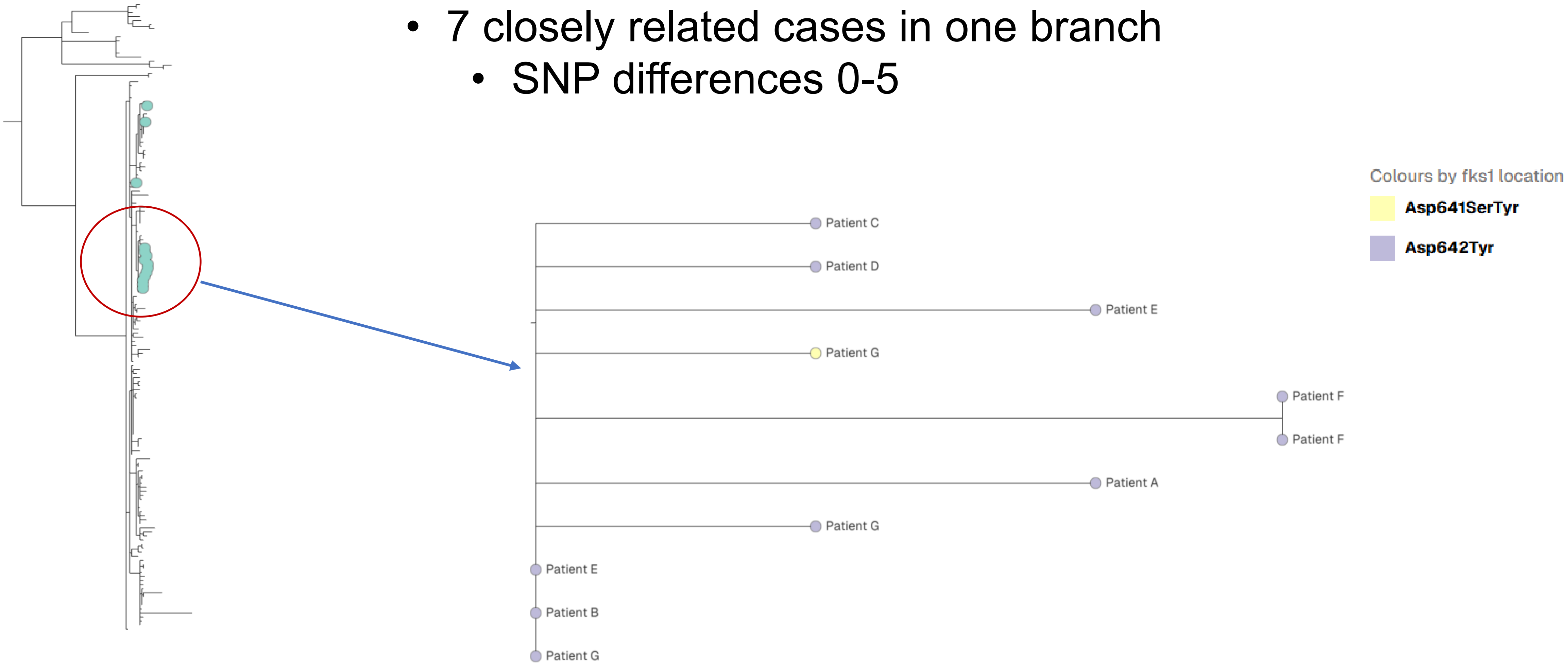
Echinocandin Resistance – FKS1 gene

- Three classes of antifungals available to treat *C. auris* infections
  - Azoles, Polyenes and Echinocandins
  - Echinocandins are the first choice to treat bloodstream infections
- Resistance to antifungals in *C. auris* isolates in the US
  - Azoles >90%
  - Polyenes 30%
  - Echinocandins 2%
- Mutations in the FKS1 gene are associated with resistance to Echinocandins

Antifungal Susceptibility Testing for *C. auris*
(cdc.gov/candida-auris/hcp/laboratories/antifungal-susceptibility-testing.html)

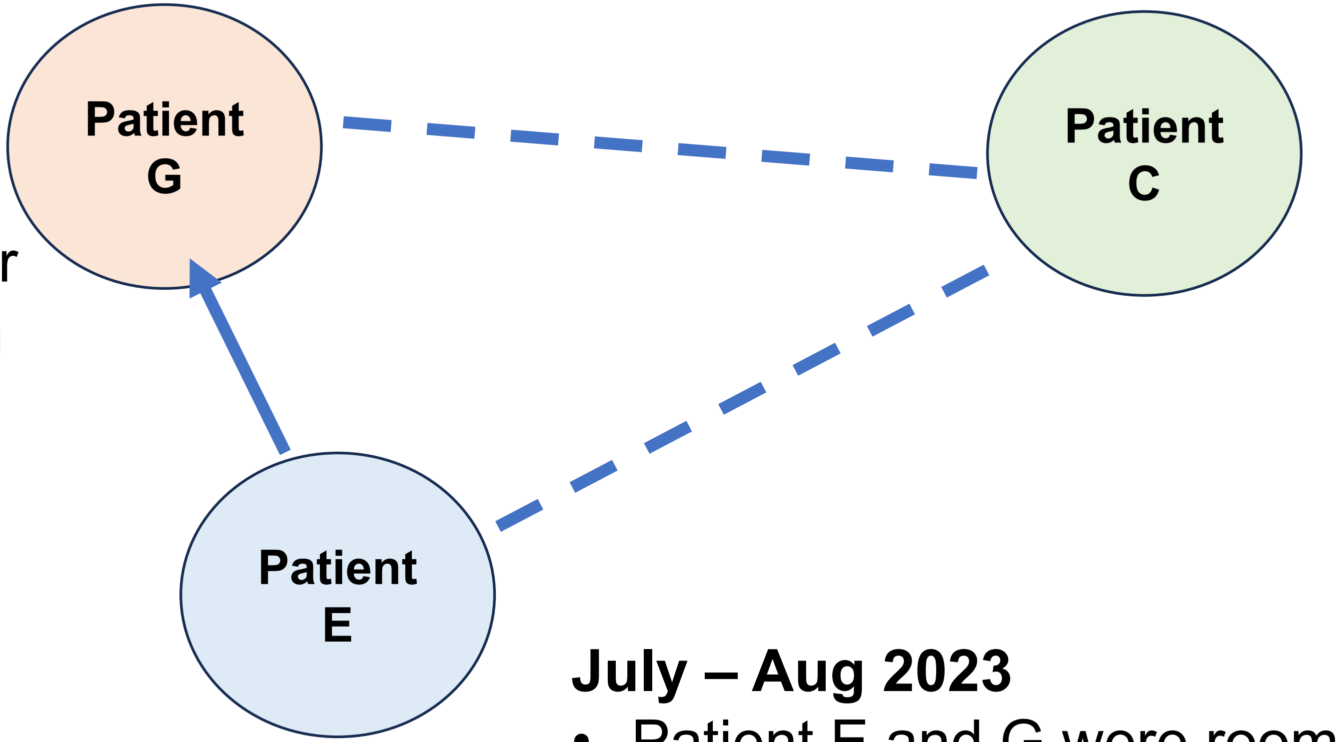
Orange County Case Study #2

- FKS1 mutations found in 14 Clade I isolates
- 7 closely related cases in one branch
  - SNP differences 0-5



Orange County Case Study #2

Outbreak Investigation at LTACH B



Nov 2023
Echinocandin Resistance alert from WA ARLN for 10/1/23 specimen from Patient G at LTACH B

Conducted response screenings and WGS for patients in LTACH B

Sept 2023
Negative admission screen at LTACH B

Nov 2023
Positive during response testing, FKS1+

July – Aug 2023

- Patient E and G were roommates at LTACH B
- Patient E positive at vSNF B 8/9/23 WGS found FKS1+

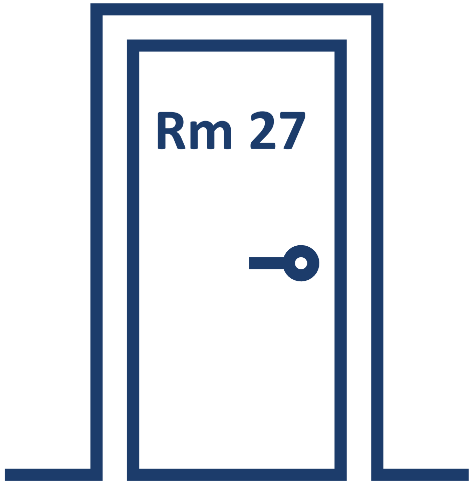
Orange County Case Study #2

Outbreak Investigation continues at vSNF B

Aug 2023

- Patient E admitted to vSNF B
- Positive 8/9/23, FKS1+

Patient E



Patient A

Apr 2024

- Patient A positive on admission to ACH A, FKS1+

March 2024

- Patient A admitted to room 27

Nov 2023

- Multiple rounds of response testing
- No other FKS1+ cases identified

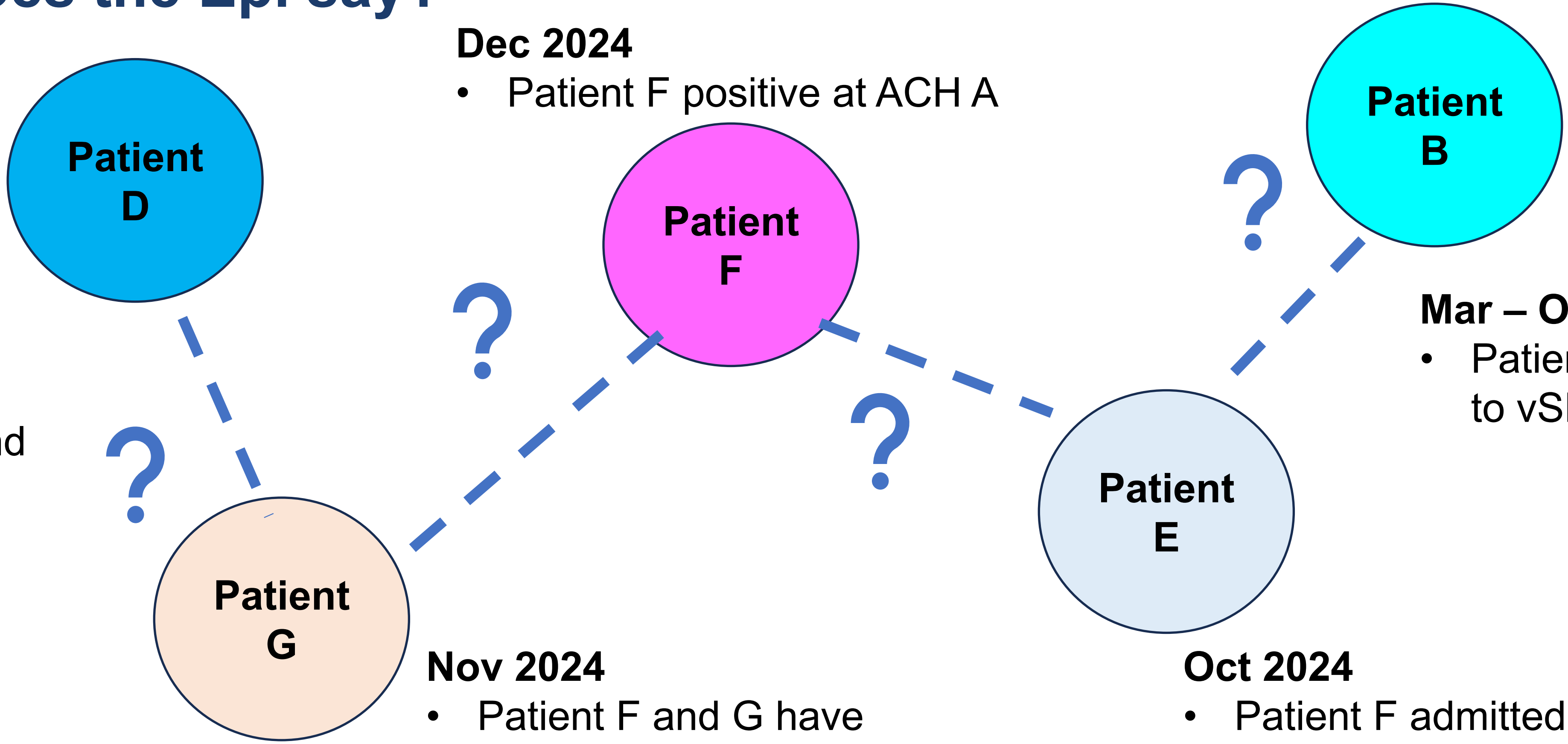
Patient G

Feb 2024

- Patient G admitted to vSNF B
- Shared room 27 with Patient E until Mar 2024

Orange County Case Study #2

WGS says cases are closely related.
What does the Epi say?



Feb 2024
- Patient D positive at ACH A

Feb 2024
- Patient D and G at ACH D (no overlap)

Dec 2024
- Patient F positive at ACH A

Nov 2024
- Patient F and G have overlapping admissions at ACH E

Oct 2024
- Patient B positive at vSNF C

Mar – Oct 2024
- Patient E admitted to vSNF C

Oct 2024
- Patient F admitted to Rm 203 at ACH D the day Patient E is discharged

Orange County Case Study #2

Conclusions

- Investigation results found mixed support for transmission
  - Strong evidence
    - Known outbreak cases at LTACH B & vSNF B
      - Patients E, G, C
  - Some evidence
    - Admissions to the same facility or room, days or weeks apart
      - Patients A, B, D, F
- Implications of transmission of FKS1 mutations
  - Infection control precautions

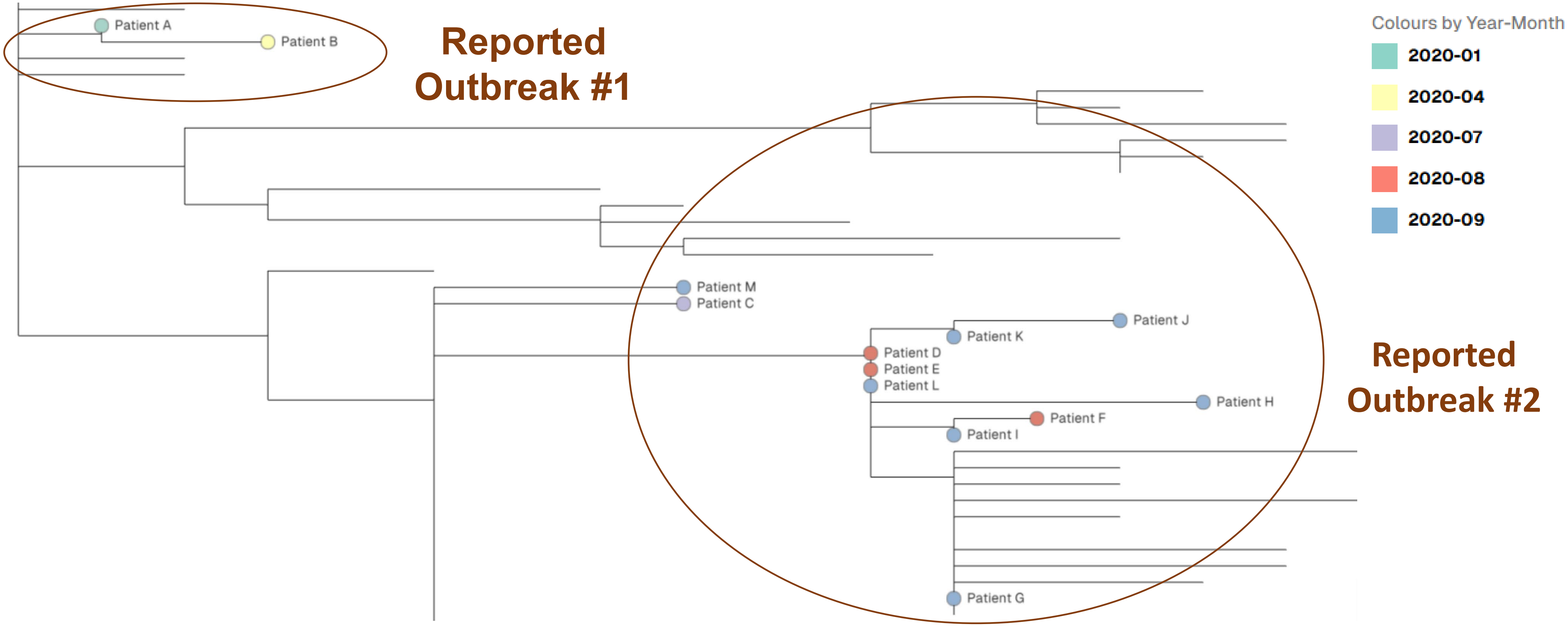
Orange County Case Study #3

Retrospective review of 2020 outbreak showed evidence of internal transmission in ACH D

- Reported hospital-acquired Outbreak #1 at Intensive Care Unit & Step-Down Unit end of 2019- Feb 2020
    - Point Prevalence Study (PPS), discharge tracking, and contact tracing testing did not identify additional cases
    - Admission lists of affected units reviewed
    - Infection prevention assessments conducted
- Reported hospital-acquired Outbreak #2 at Step-Down Unit, Cardiac Care, & Med Surg July 2020- Sep 2020
    - Additional cases identified through PPSs
- Epidemiology and investigation information at the time appeared to show 2 separate clusters

Orange County Case Study #3

Retrospective WGS review: the 2 reported HAI outbreaks may have been internal transmission from the same introduction (Clade III)

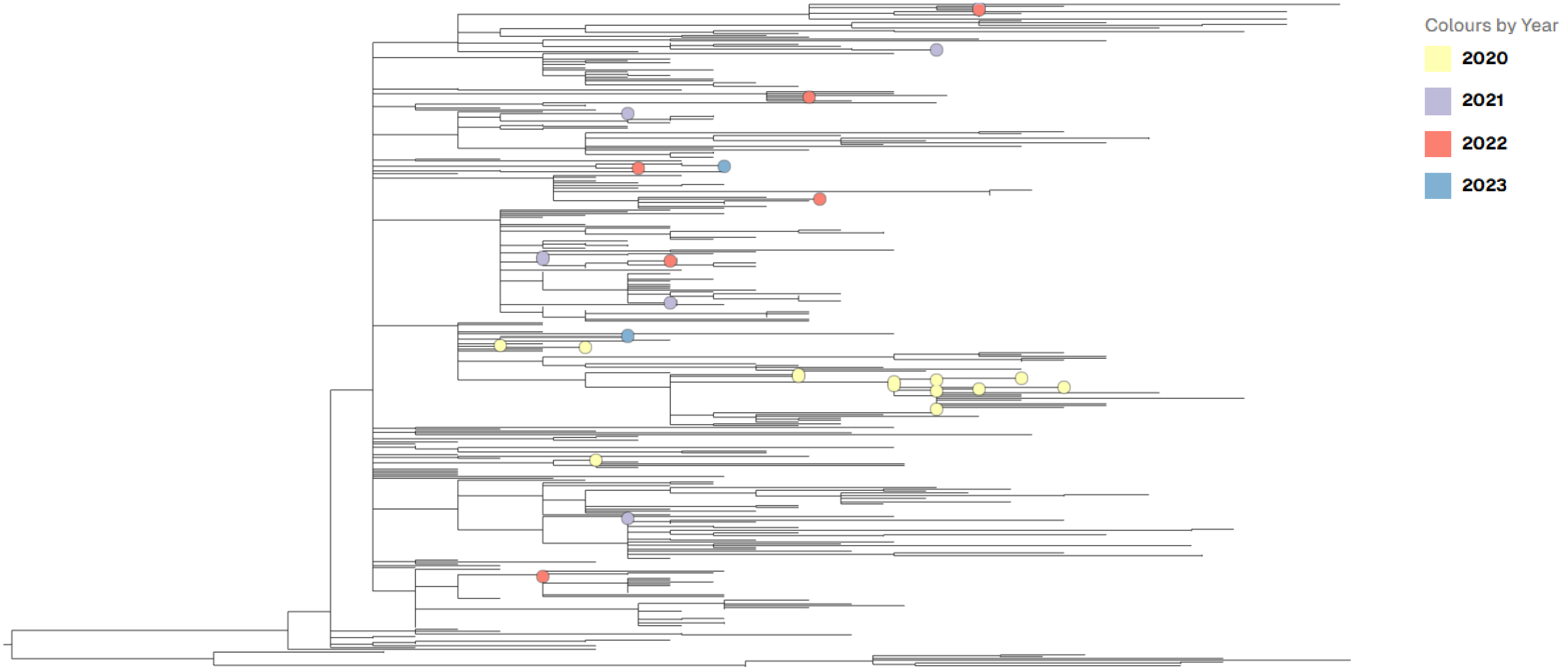


Reported Outbreak #1

Reported Outbreak #2

Orange County Case Study #3

After 2020, different introductions are observed in this hospital



Orange County Case Study #3

Conclusions

- WGS could be useful when investigating a high volume of cases with complicated epi exposures
- WGS supported evidence of ongoing internal transmission
- WGS also supported evidence of multiple introductions in a healthcare facility

Orange County Case Study #4

Using WGS to supplement an active outbreak reported by ACH A in 2024

- IP reported cluster of 8 newly colonized *C. auris* cases within 2 months
  - All had exposures in units involving cardiovascular care and overlapping hospitalizations
  - 3 had negative admission screenings

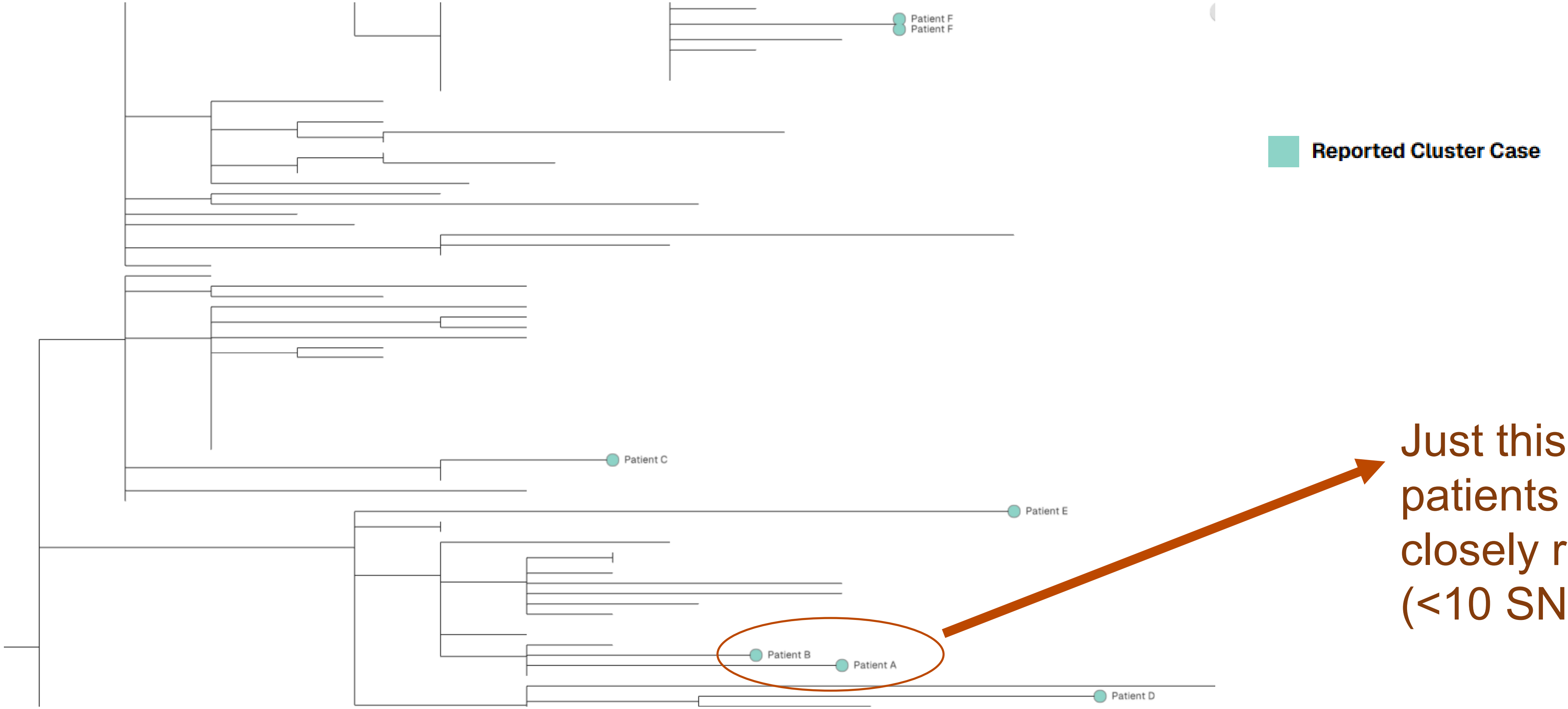
Orange County Case Study #4

Public Health Response

- Chart review & epi info collection
- Hospital conducted their own epidemiological investigation & series of PPS
- WGS Clade Identification
  - 6 Clade I
    - Review for relatedness
  - 1 Clade III
  - 1 did not have isolate submitted

Orange County Case Study #4

Reported cases do not appear to be a result of widespread transmission (Clade I)



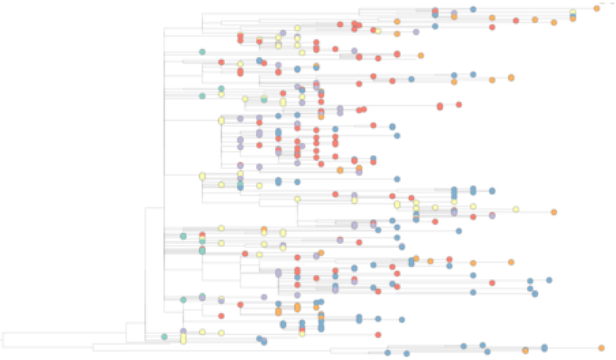
Just this pair of patients may be closely related (<10 SNPs apart)

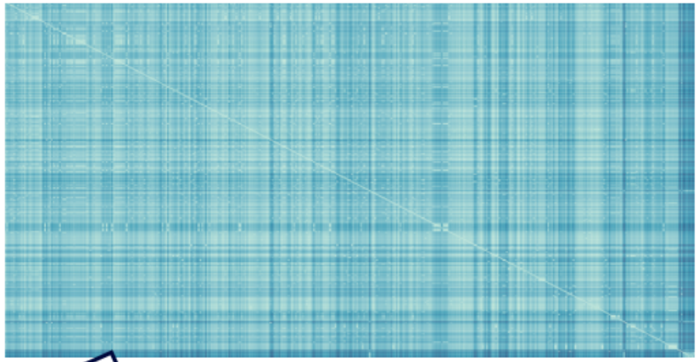
Orange County Case Study #4

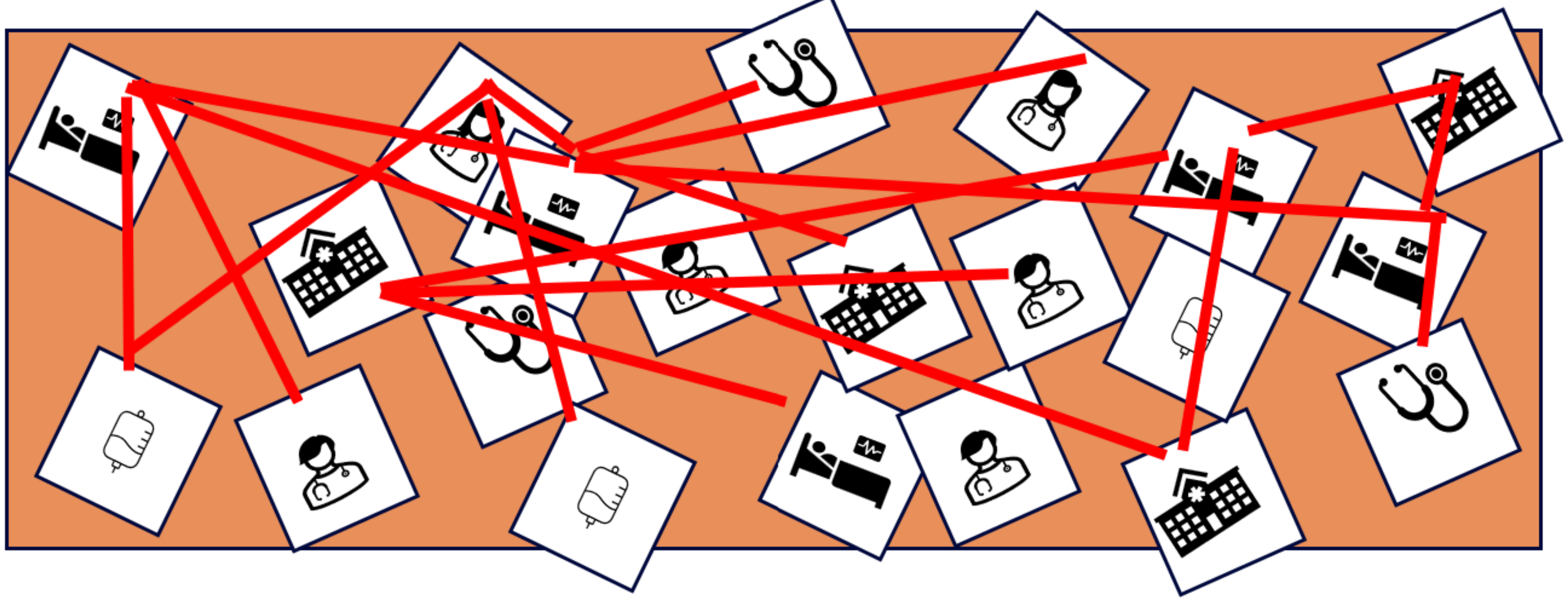
Conclusions

- Wide-spread transmission was ruled out
  - Even when epidemiology information makes it appear to be a cluster
- Helped save public health & hospital resources

Overall conclusions







Acknowledgements

- **OC Communicable Disease Control Division**
  - Public Health Nurses
- **OC Public Health Lab**
  - Victoria Buchanan (Supervising PH Microbiologist- Molecular Department)
- **Healthcare facility partners**
- **Theiagen Genomics**
- **Expert Stewardship, Inc.**
- **Washington Antimicrobial Resistance Lab Network**
- **CDPH**
- **CDC**

Stay Connected: HAI_EPI@ochca.com

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Alameda County

Tyler Lloyd, Alameda Public Health Laboratory

Rachel Marusinec, Epidemiologist

Alameda County Whole Genome Sequencing

Program overview

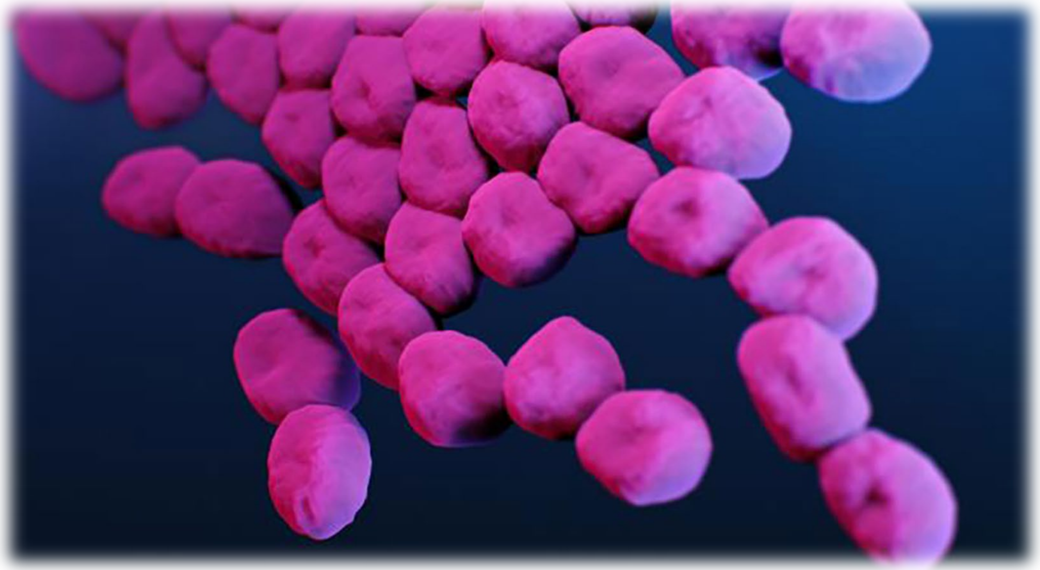
- Bacterial sequencing at the Alameda County Public Health Laboratory began in 2016.
- Pathogen focus – Bacterial Surveillance and Diagnostics
- Routine is Key – We sequence 1 to 2 times per week. 'CRO weather report' and STAT testing.

Alameda County Whole Genome Sequencing

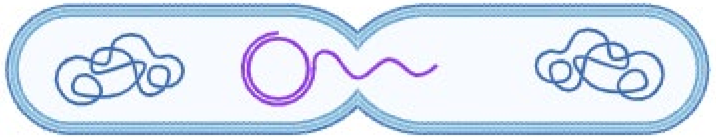
Program overview

- Why Surveillance?
  - Disease Burden
  - Outbreak Detection
  - Resource Allocation

- Why Diagnostics?
  - Emerging Diseases
  - Evolving Pathogens, Evolving Needs



Acinetobacter baumannii



Conjugation

Alameda County WGS Application

How we are using WGS to assist in MDRO Surveillance/Outbreaks

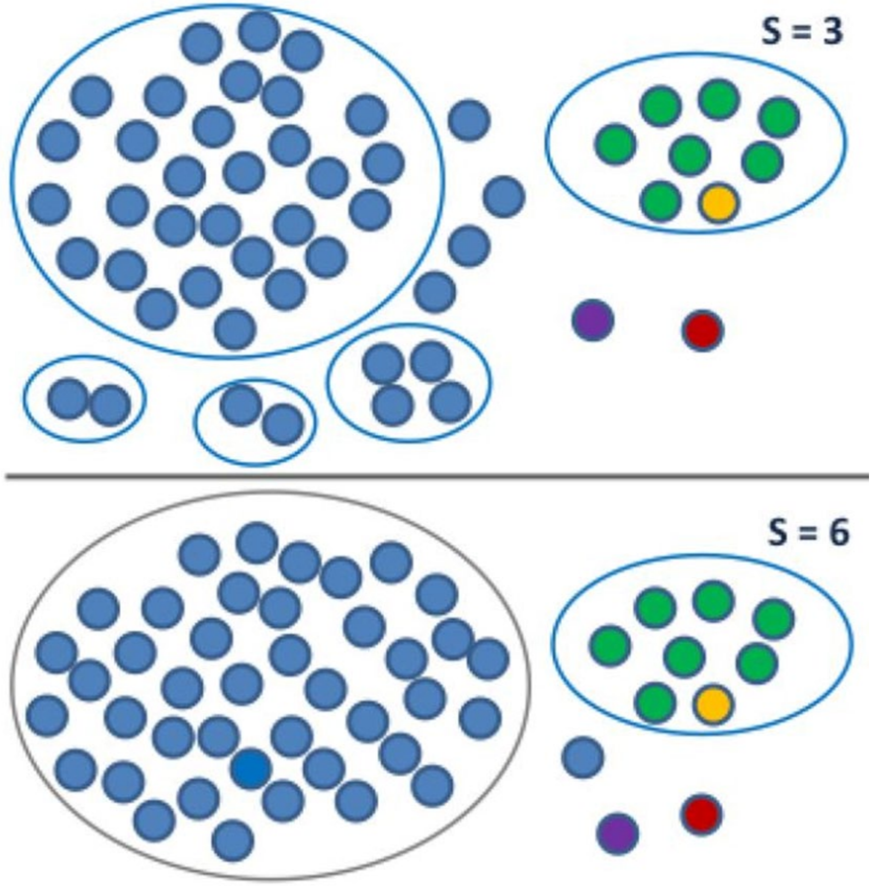
- The laboratory performs: identification, subtyping, resistance gene annotation, and other functions as requested (monthly meetings, shared spreadsheets!)
- Areas of focus – Carbapenem Resistant *Enterobacterales* Infections, and Emerging Pathogens.
- These efforts are possible because of CDPH's Terra contract and CAPHLD's LabAspire Fellowship program

Alameda County Case study

How the lab and epi teams worked together

- WGS can add evidence to possible links

- Successful analysis and investigation requires careful research and collaboration. SNPs ≠ linked isolates.

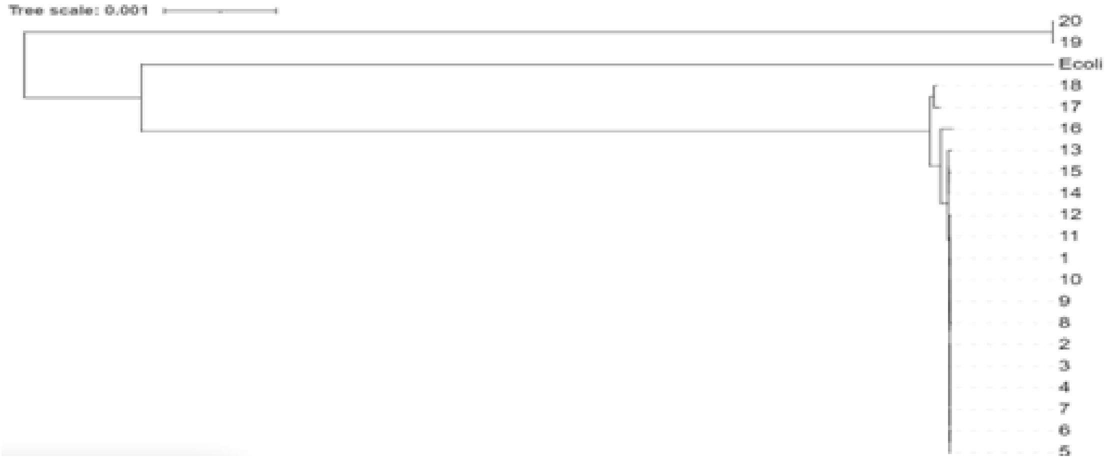


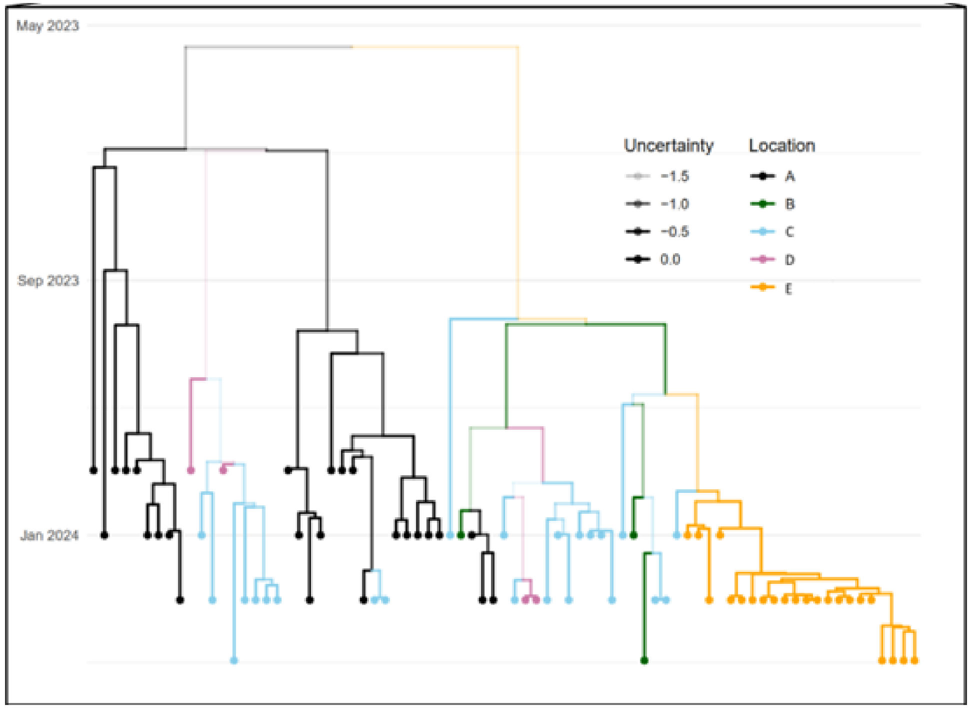
Stimson et al. mol.bio and evolution, 2019

Alameda County Case study

How the lab and epi teams worked together

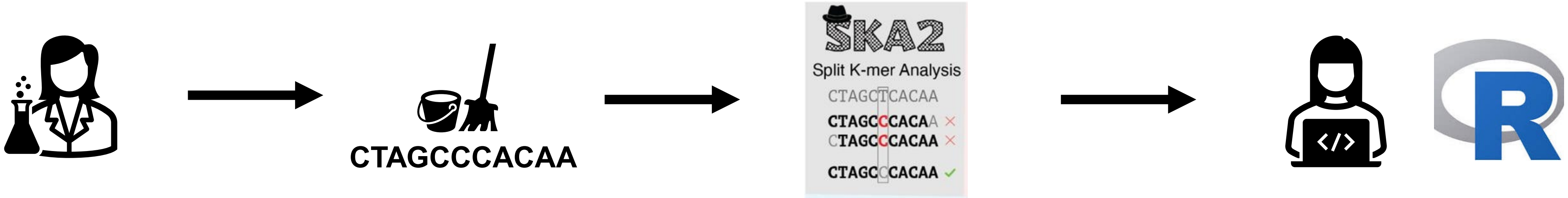
- Advanced phylogenetics can help resolve outbreak details when sequence data alone is insufficient.





Alameda County Case study

Genomic Epidemiology Methods and Tools in Alameda County



Lab sequences isolate and sends fastq file to epidemiologist. Lab also works with ARLN or other entities to get sequences run outside of our PHL

Epi cleans fastq files using fastp (academic.oup.com/bioinformatics/article/34/17/i884/5093234) program

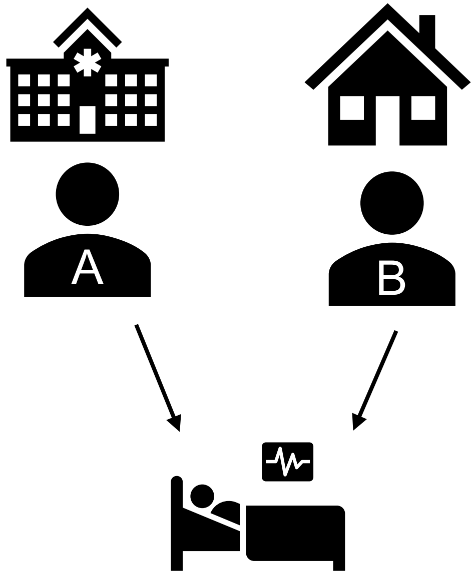
Use split K-mer Analysis (SKA) to generate sequence alignment, pairwise distances, and create .dot file for clustering

Use R to create cluster diagram, and tree (packages Rgraphviz, seqinr, ape, ggtree)

Alameda County Case study

CRAB NDM/OXA transmission Background

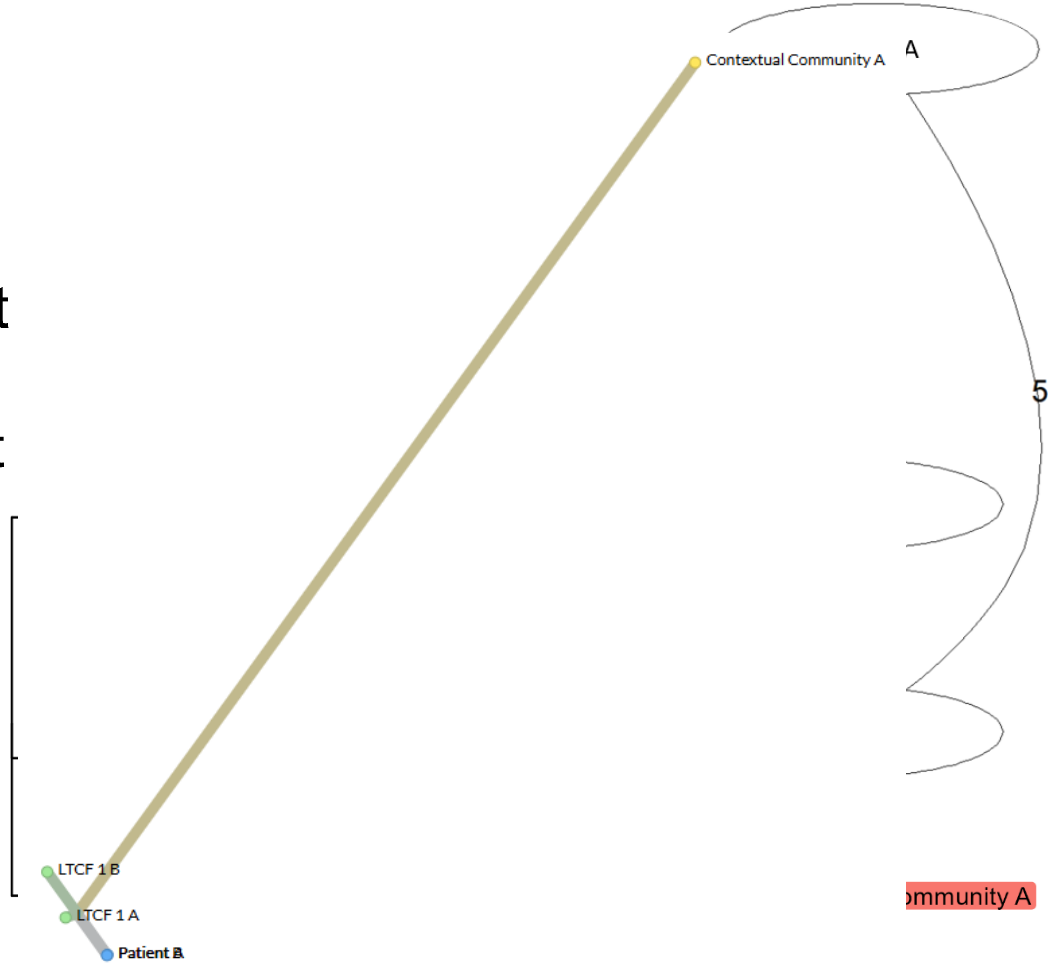
- Patient A was admitted to Hospital from LTCF 1
  - Already identified other CPO so on contact precautions
- Patient B was admitted to Hospital, no previous LTACH/SNF admissions
  - No admission screening; prior negative wound culture
  - No risk factors, surgery and wound vac at hospital
- Patients overlapped in ICU for 1.5 days
  - Had shared nursing, wound care, and RT's
- Patient B tested positive > 14 days after admission
- **Potential Transmission?**



Alameda County Case study

Investigation Findings

- Sequencing showed us 0 SNP differences between Patient A and B
- Similar to sequences from patients at Patient A's LTCF
- Very different from same organism at a different LTCF in county (~2000 SNPs)
- Could look at data by clustering or tree
    - Note: for SKA unrooted tree is better for visualizing



Alameda County Case study

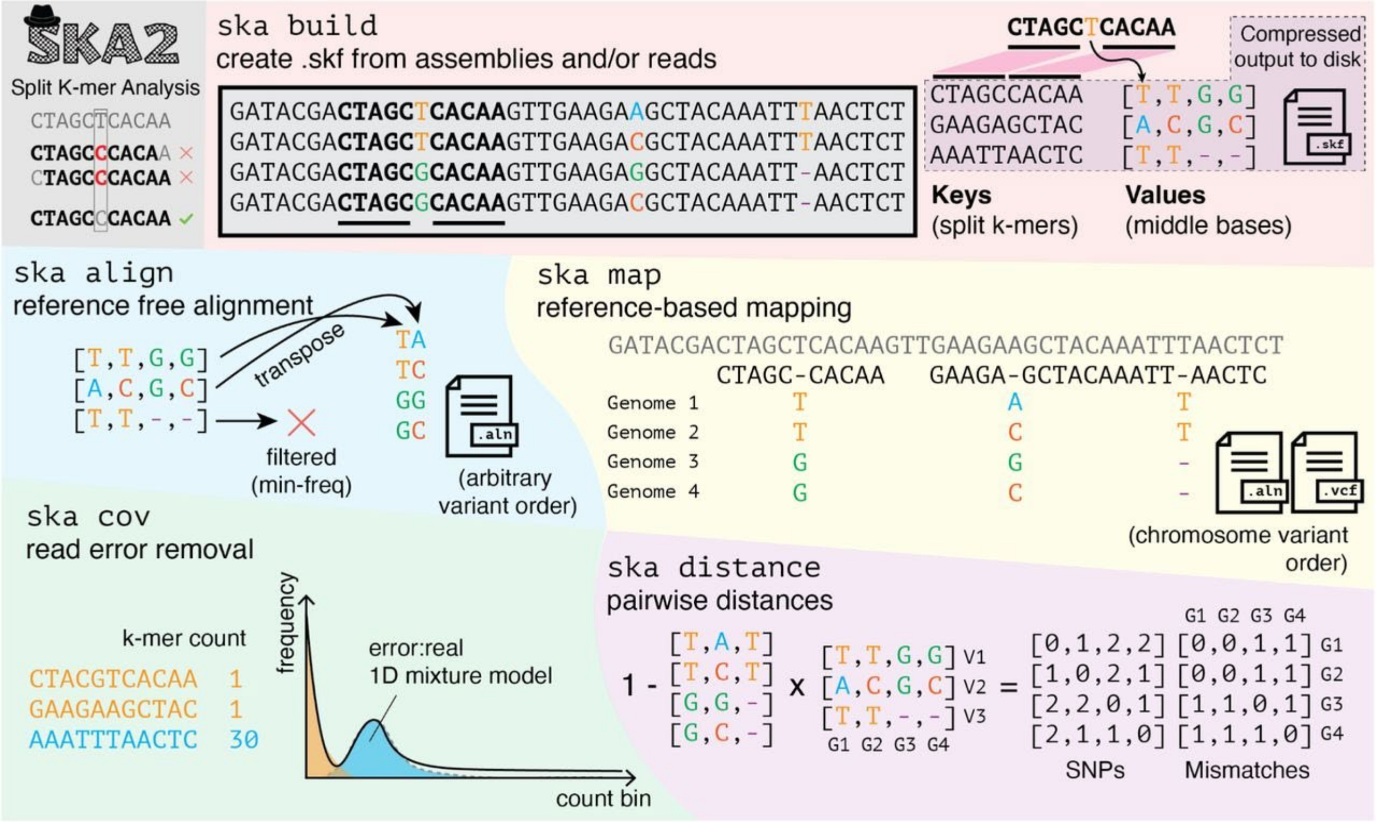
Investigation Findings

- Identical sequence for the two patients with ICU overlap
- Sequence was very similar to that of a circulating CPO in the LTCF Patient A came from
  - Unlikely that patient B had picked up that same CPO with no history of LTCF admissions and previous negative culture anywhere other than at the shared ICU with Patient A -> **likely transmission**
- Hospital and ICU had strong infection control practices, but being able to show relatedness between the two patients' organisms encouraged them to work on practices further to reduce transmission

Alameda County Case study

SKA deeper dive (if time/interest)

- SKA (www.biorxiv.org/content/10.1101/2024.03.25.586631v1.full) - genomic epidemiology analysis based on method recommended by Dan Lu at Chan Zuckerberg Biohub at UCSF
  - Split K-mer Analysis (SKA)
  - Used to identify single nucleotide polymorphism (SNP) differences
  - No reference genome needed
  - Not as computationally heavy



Thank you! Questions?

For more information, please reach out to HAIProgram@cdph.ca.gov

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California Department of
Public Health