Genomic investigation of two simultaneous *Candida auris* outbreaks in Southern Nevada

Presented by: Andrew Gorzalski, PhD

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Nevada State Public Health Laboratory

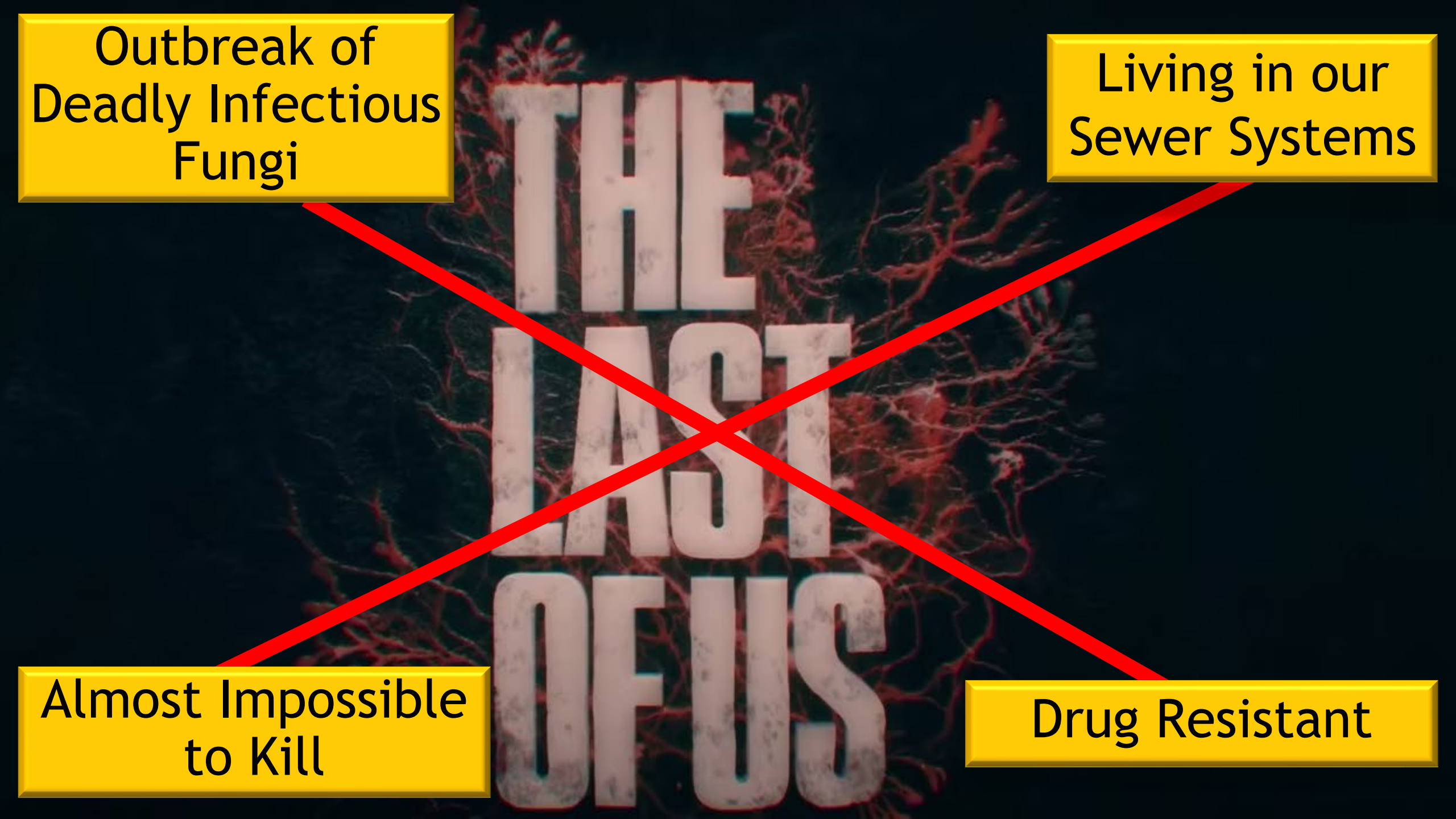
University of Nevada, Reno
School of Medicine

Outbreak of Deadly Infectious Fungi

Living in our Sewer Systems

Almost Impossible to Kill

Drug Resistant


Outbreak of Deadly Infectious Fungi
Living in our Sewer Systems
THE LAST OF US
Almost Impossible to Kill
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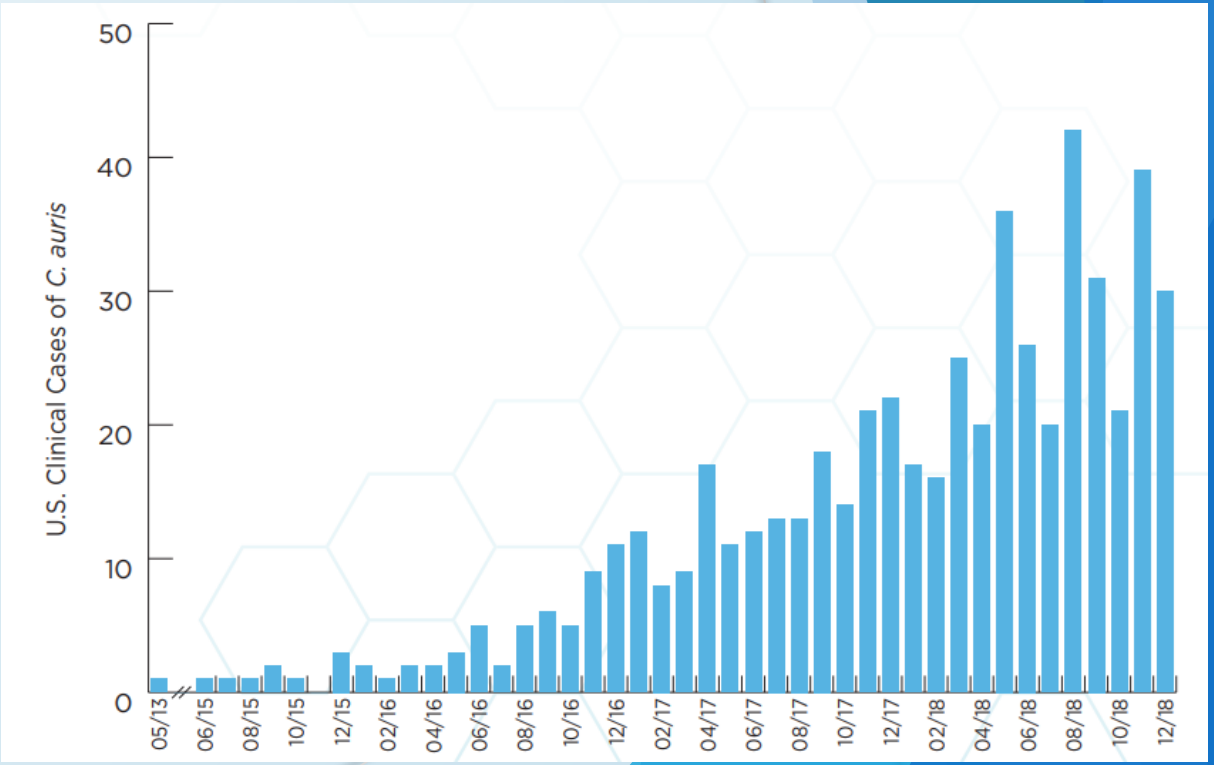
Candida auris is an emerging pathogen in the United States

- Discovered in 2009 in Asia. Started spreading in US in 2015

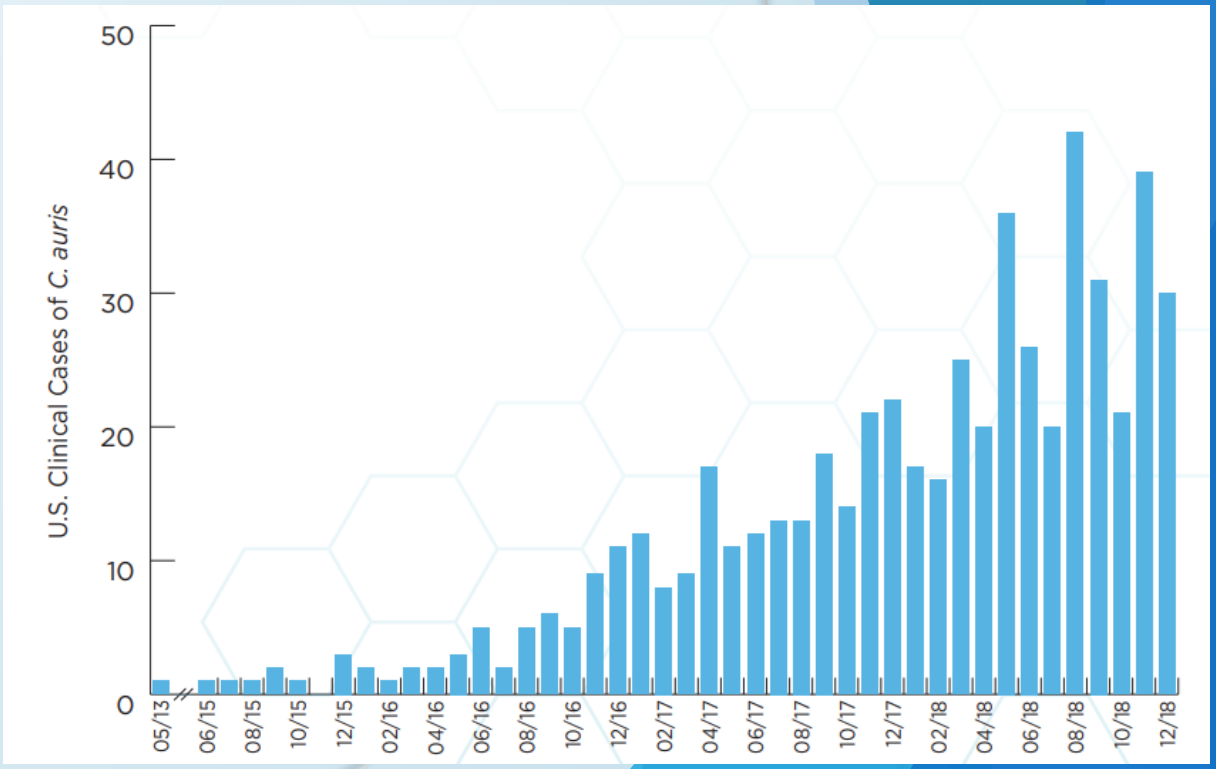
- Difficult to identify by culture or biochemicals



Source: CDC.gov

Candida auris is an emerging pathogen in the United States

- 30% of patients with *C. auris* cultured from blood are deceased in 30 days
- Environmentally stable
- Naturally resistant to azoles



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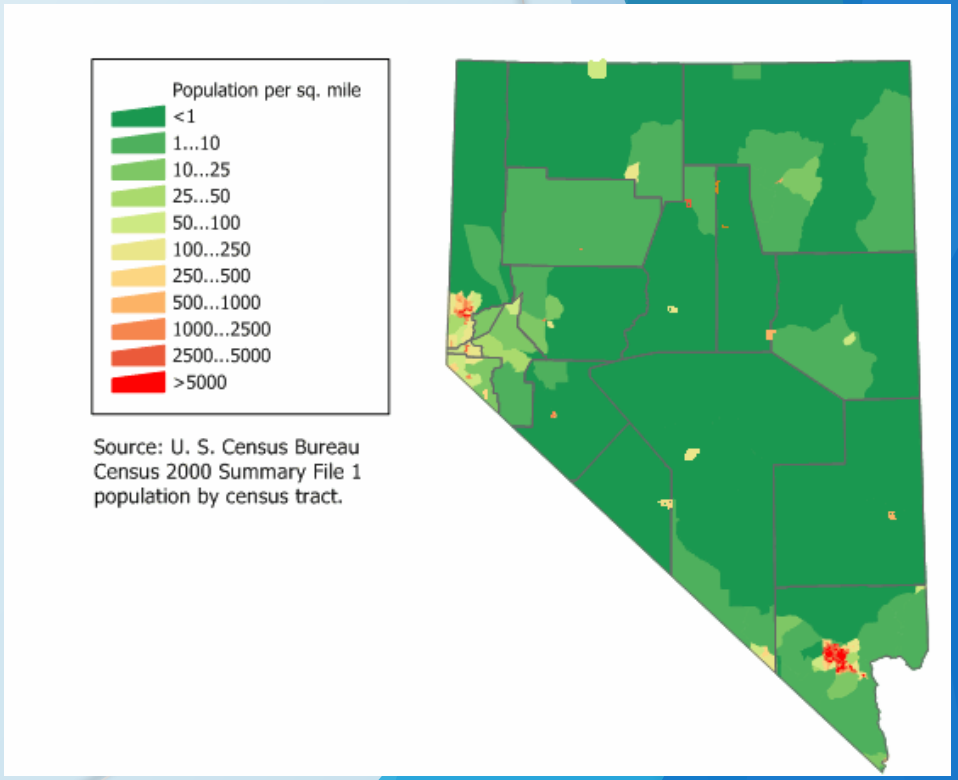
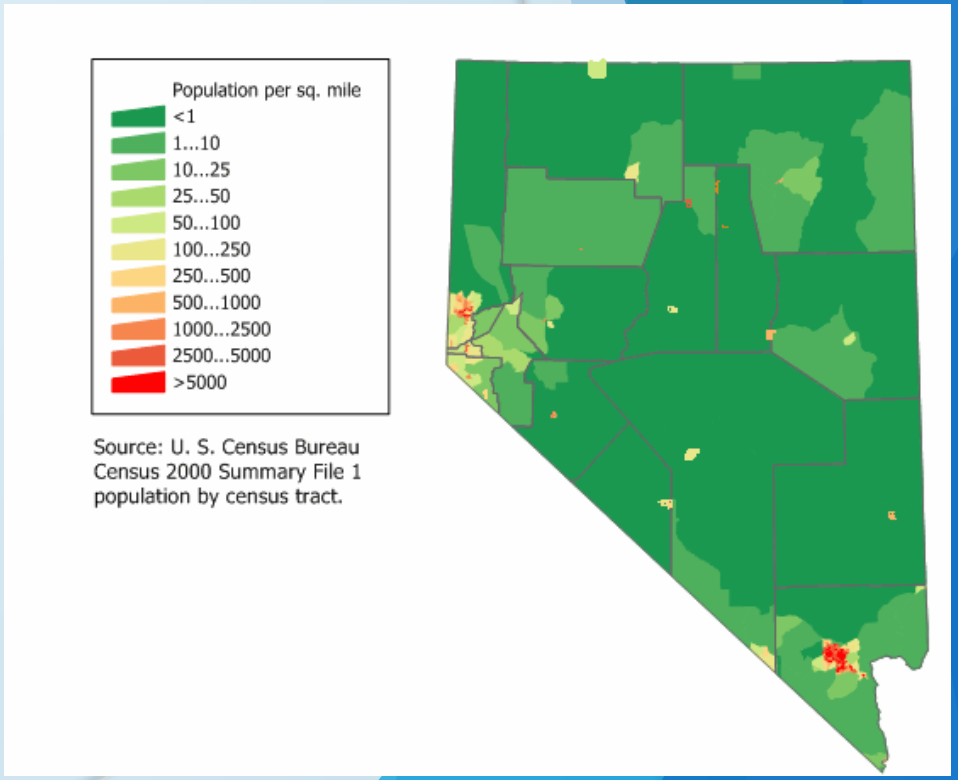
Candida auris in Nevada

- Nevada has 2 major population centers: Reno/Carson City (Northern) and Las Vegas (Southern)
  - 400+ miles of rural desert between populated regions
  - Significant tourism industry



Candida auris in Nevada

- **First** *C. auris* case was detected in Northern Nevada in January 2020
  - Patient had travel history to India



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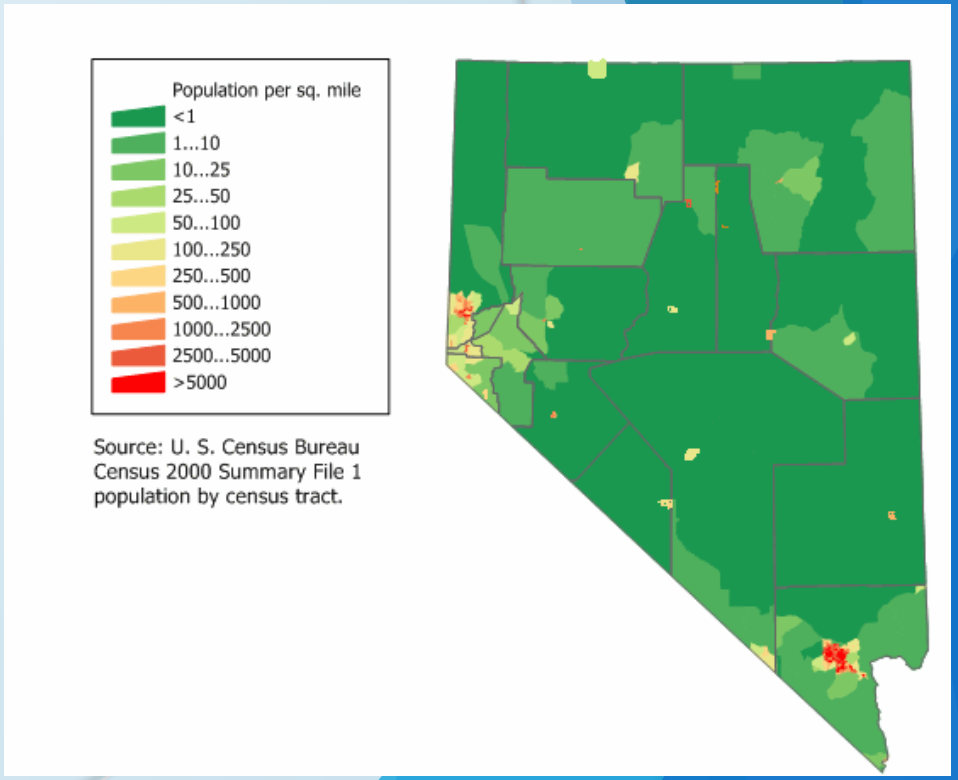

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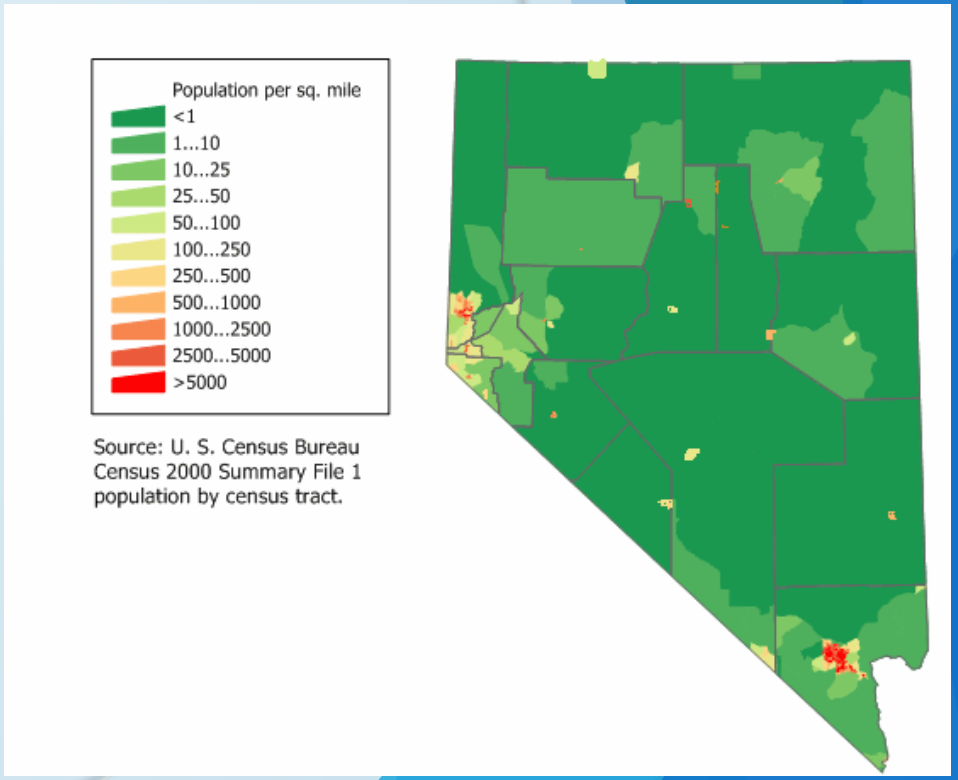
Enter the COVID-19 Pandemic!



COVID-19 pandemic allowed *C. auris* to spread throughout the U.S.

- *C. auris* had been detected in 14 states prior to the pandemic
- Screening and isolation measures for *C. auris* were in place

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- MMWR from Florida facility in 2019 had the following findings:
  - Patients were screened at admission for *C. auris* colonization
    - Infected patients were placed in *C. auris* unit

Prestel C, Anderson E, Forsberg K, et al. *Candida auris* Outbreak in a COVID-19 Specialty Care Unit — Florida, July–August 2020. MMWR Morb Mortal Wkly Rep 2021;70:56–57.

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  - Weeks later, patients admitted for COVID were now colonized with *C. auris*.

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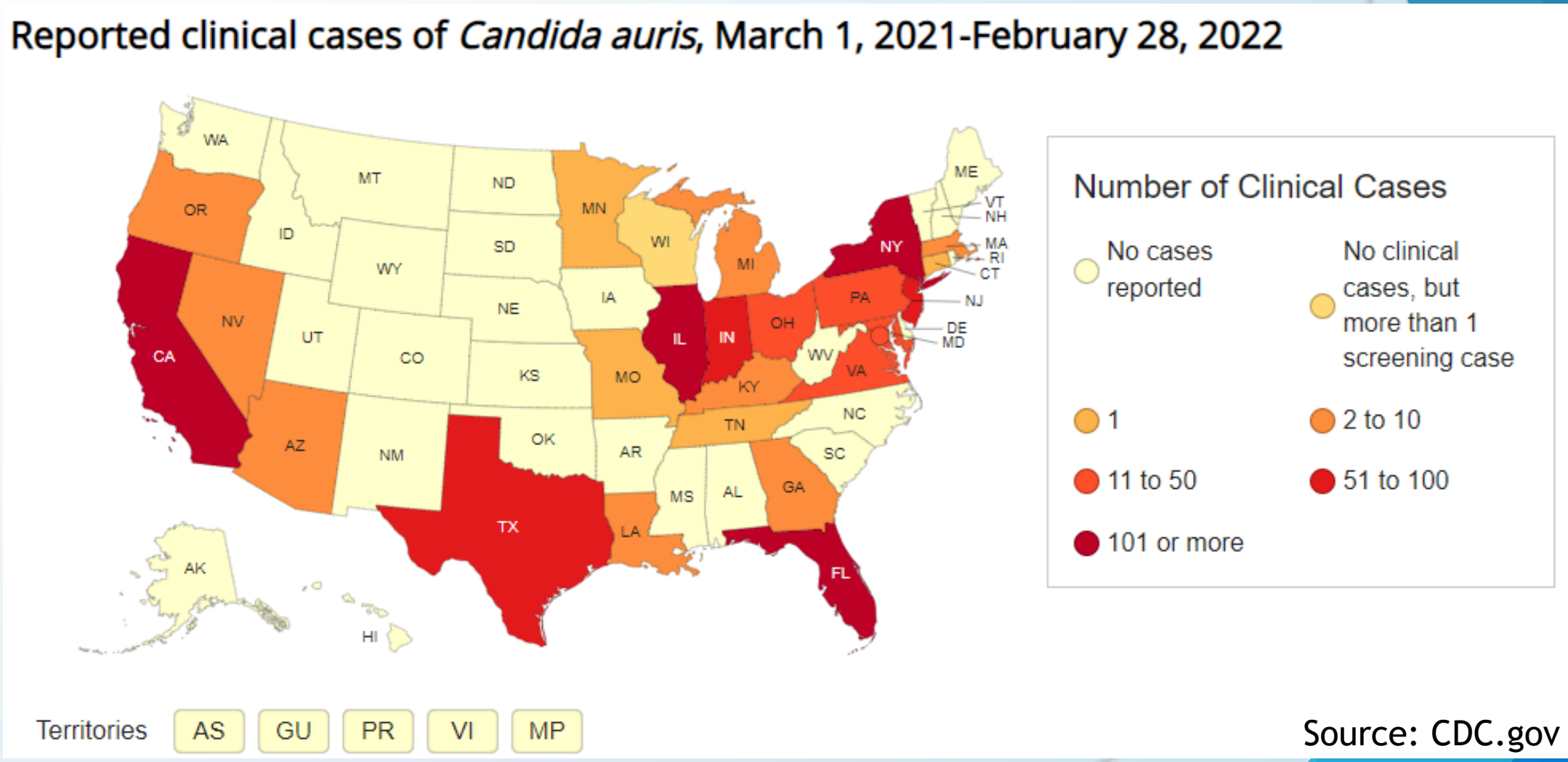
People may be colonized with no symptoms

By 2022, over half of all U.S. states had identified cases



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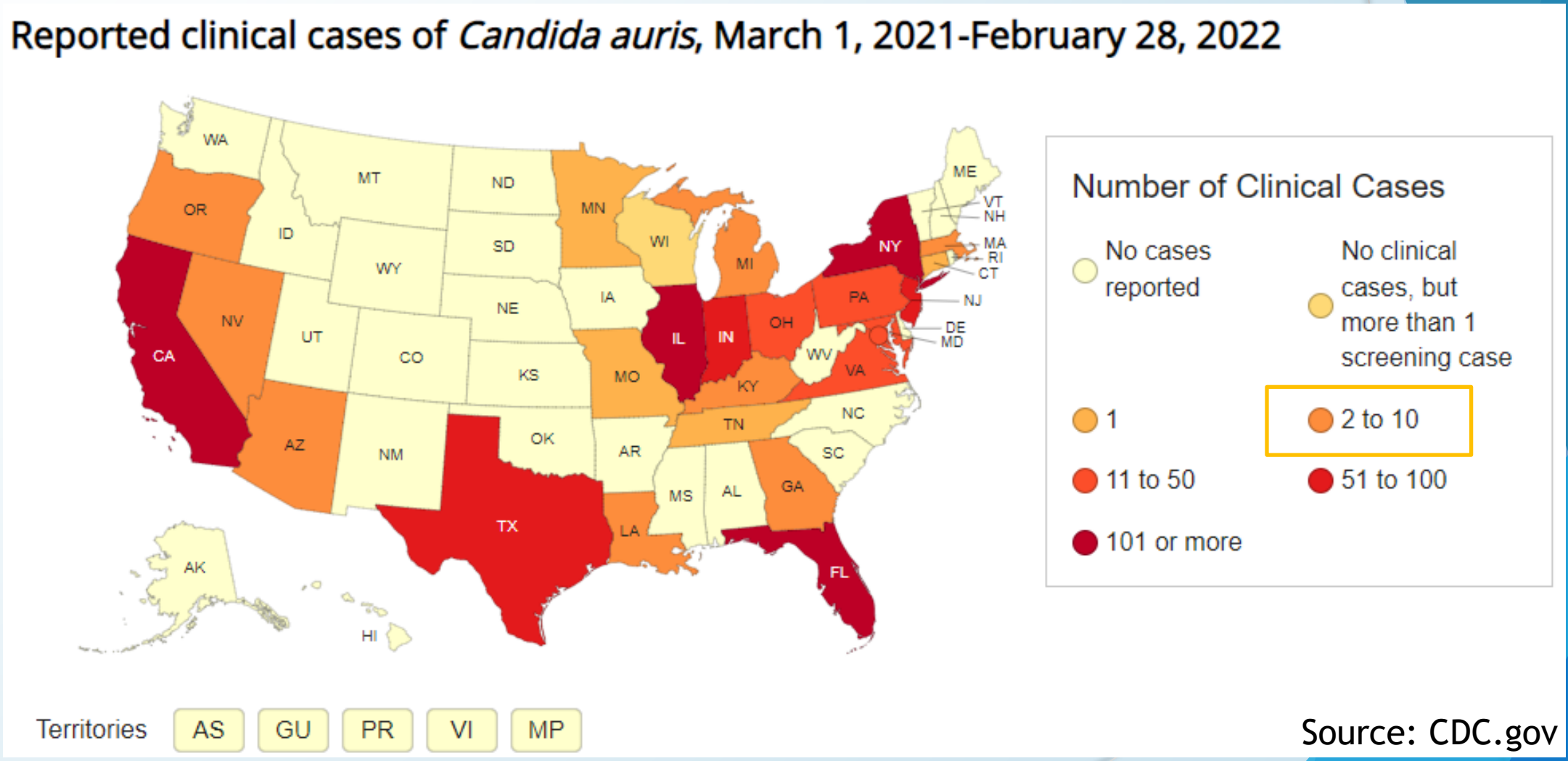

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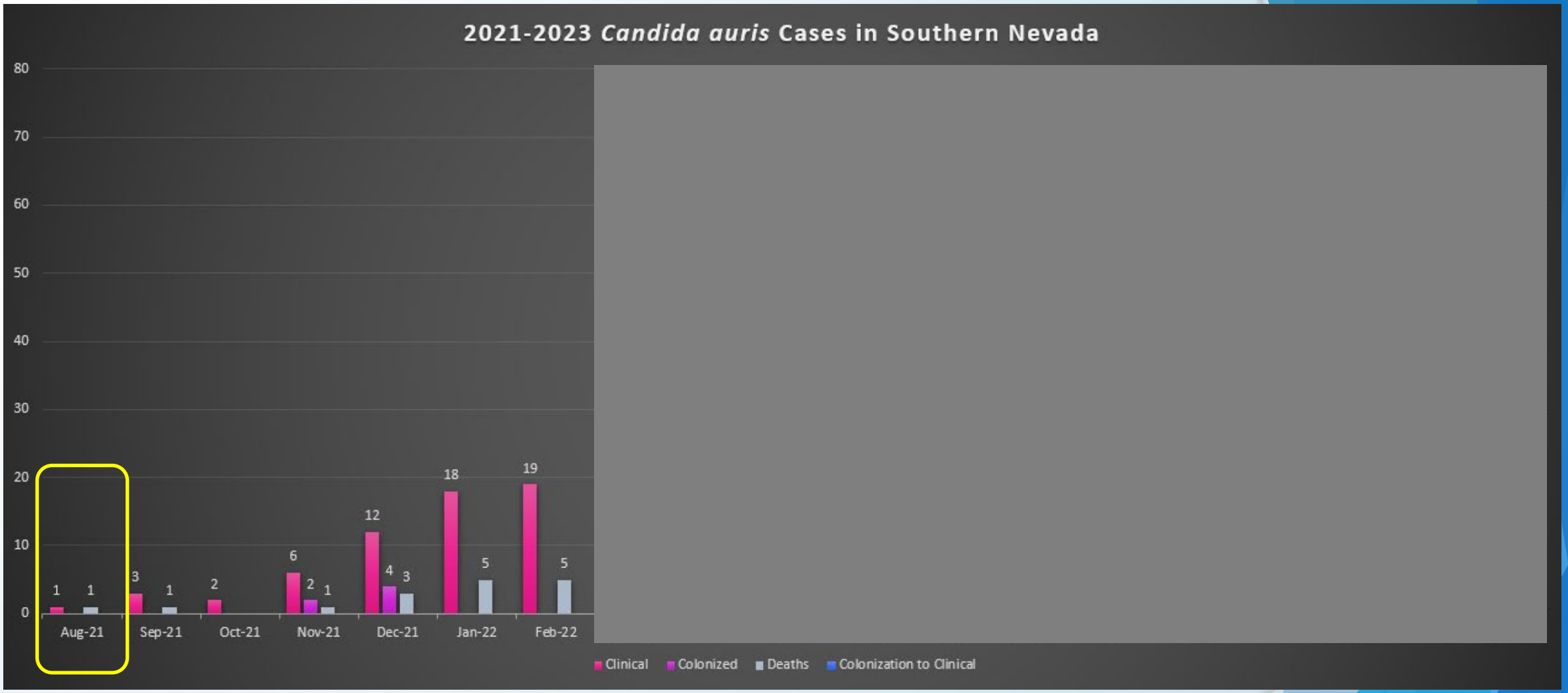
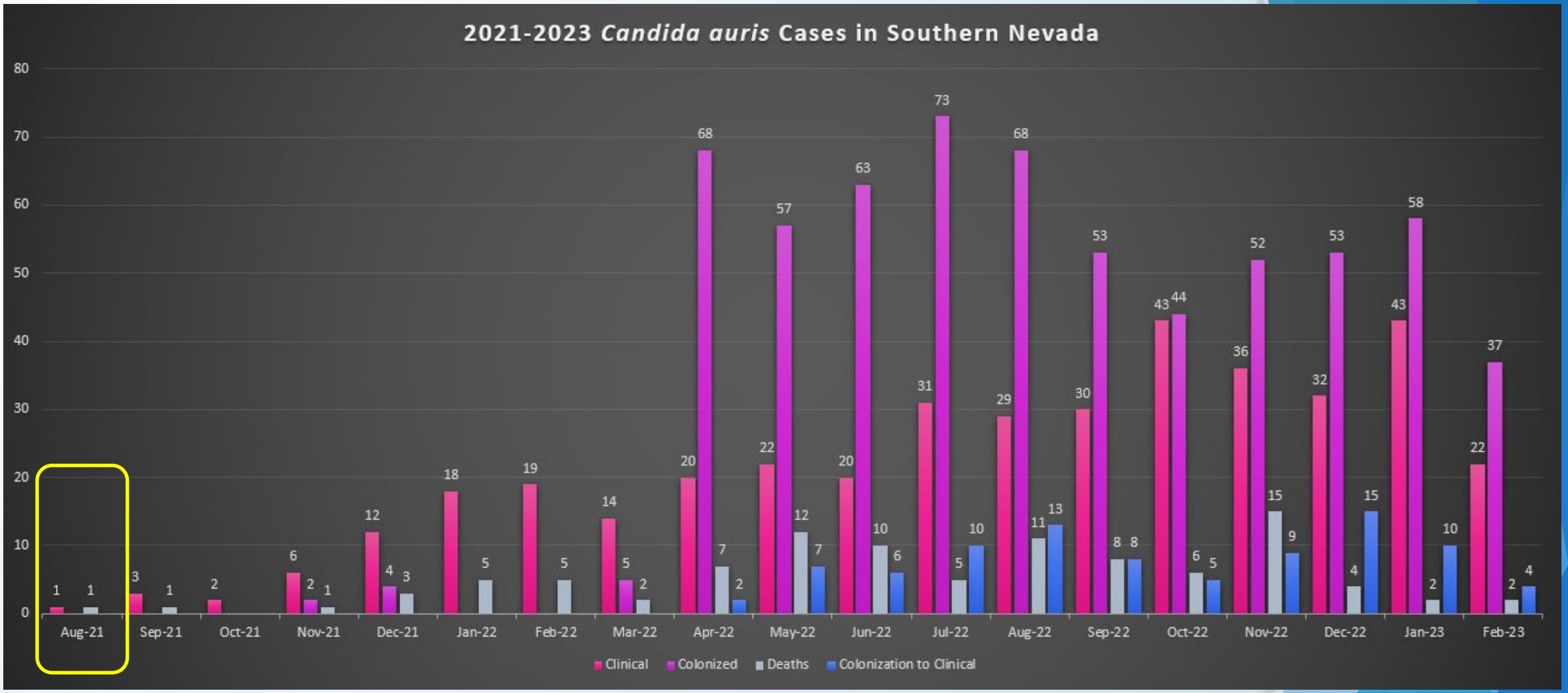
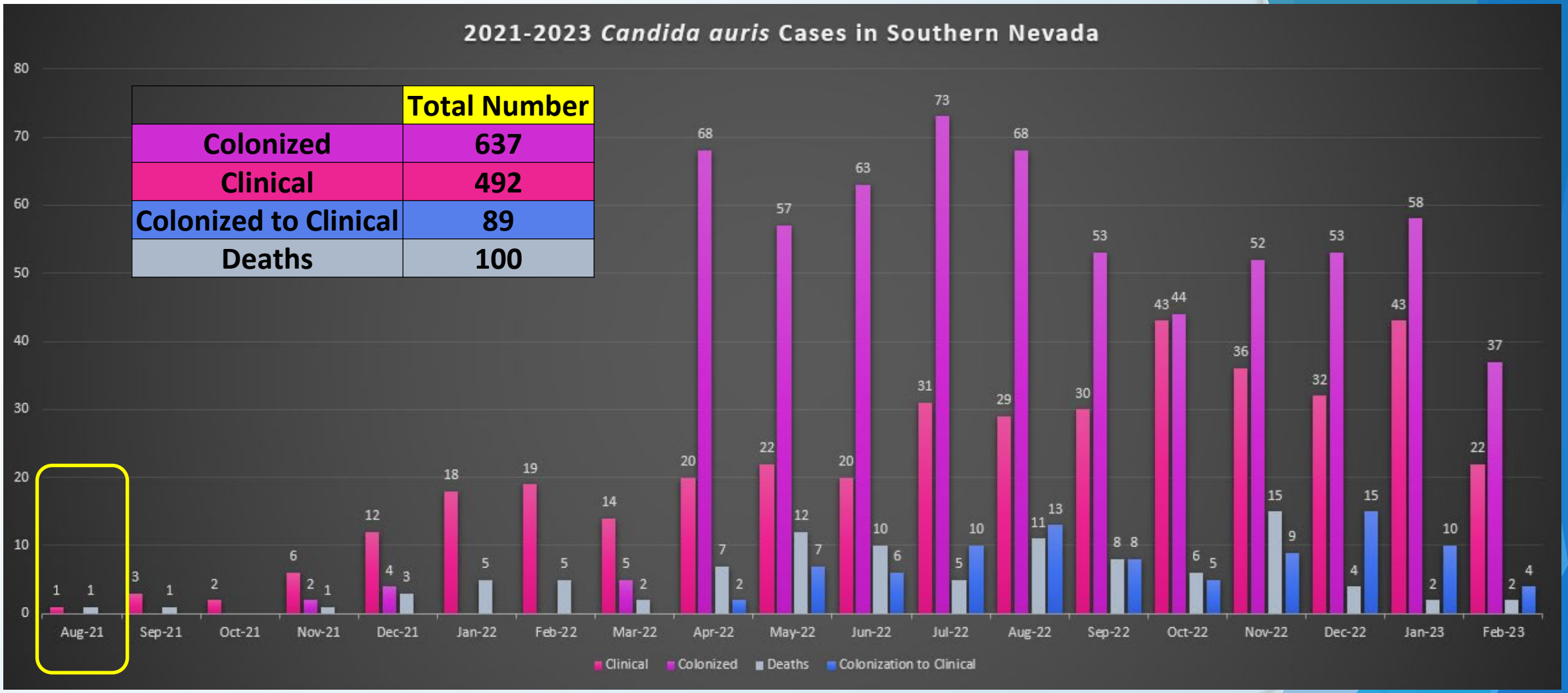


First detected case in Southern Nevada occurred August 2021



Outbreak is still ongoing



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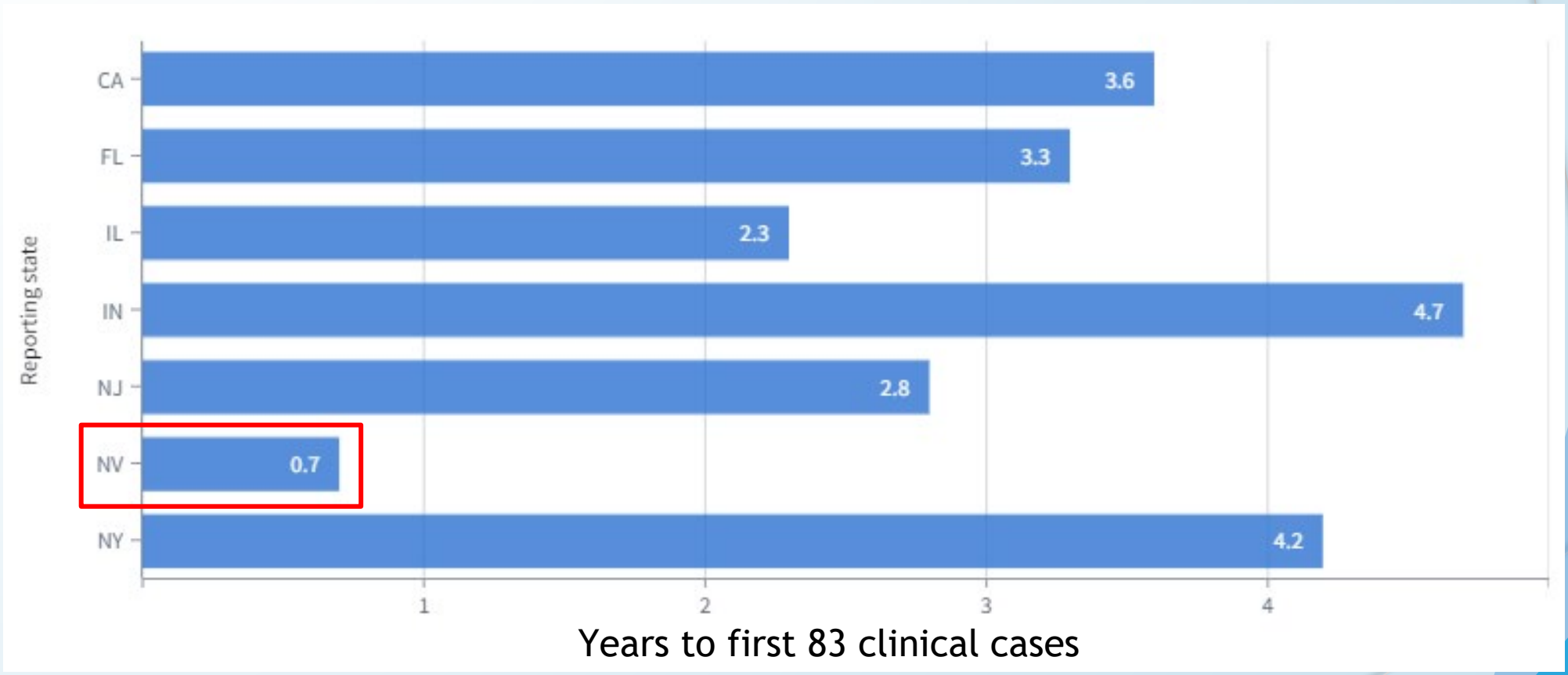
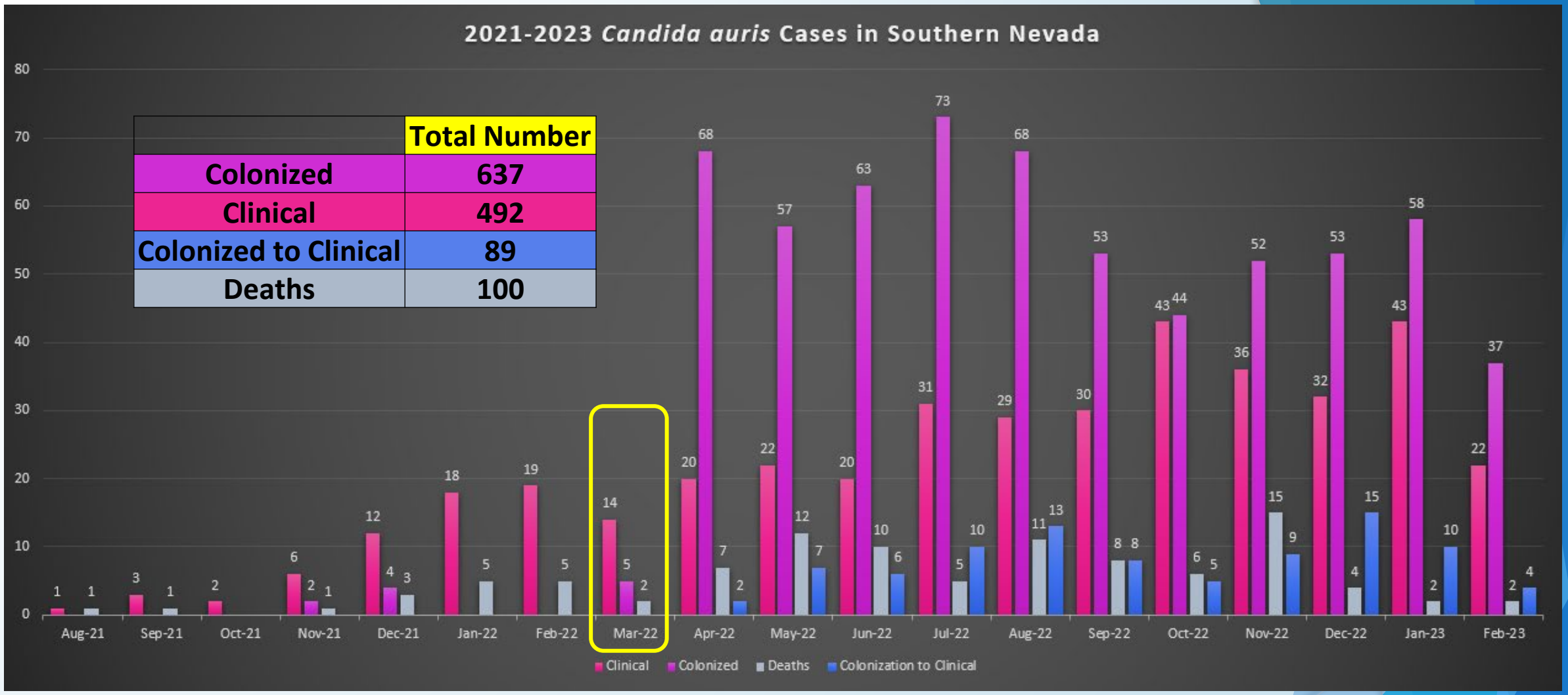
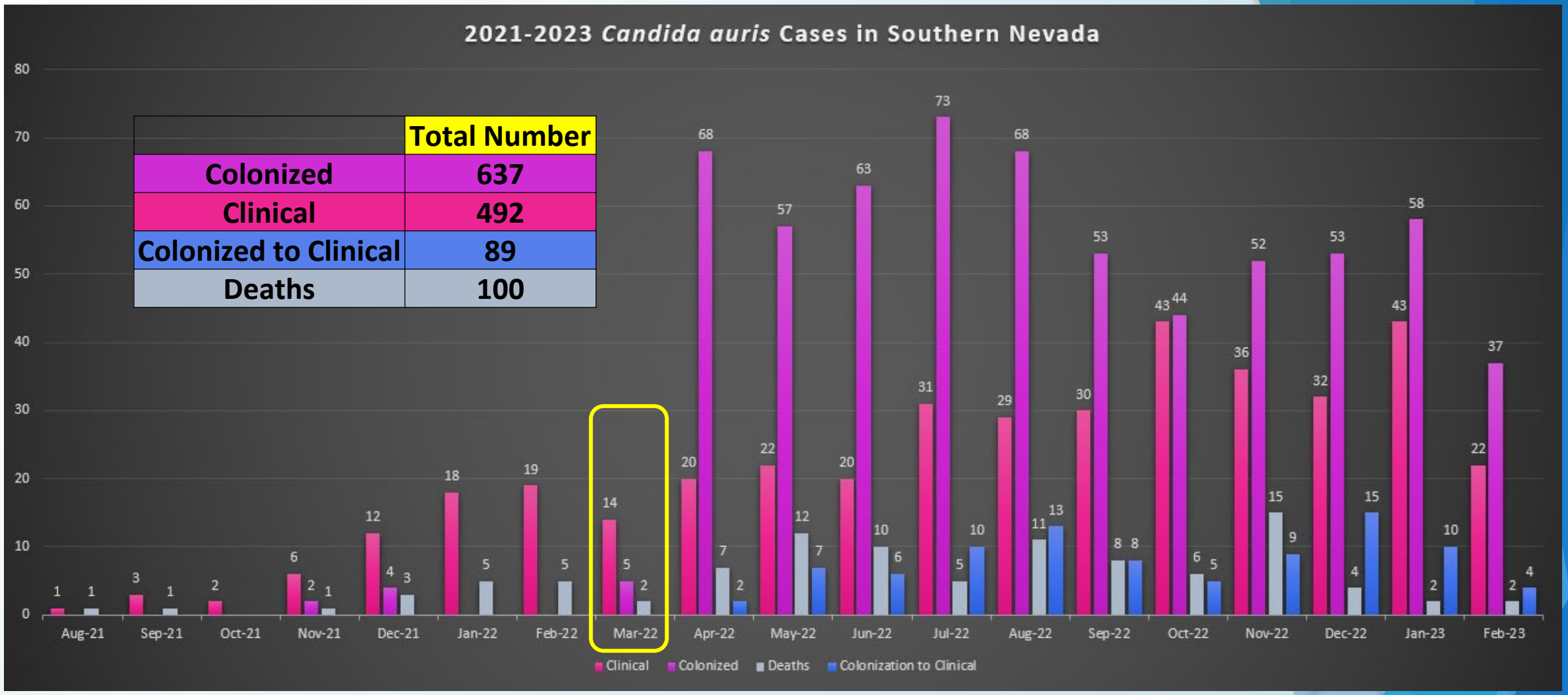
Nevada identified 83 clinical cases in much less time than other states





March 2022 – State PHL notified of *C. auris* cases in Southern Nevada

Unlike other infectious diseases, *C. auris* was not reportable in Nevada



March 2022 – Validated *C. auris* PCR screening assay.

- Allowed colonization screening testing at State PHL.

Validated *C. auris* culture protocol in microbiology.

C. auris PCR Screening Test and Culture

- Combined automated extraction with real-time PCR based on CDC's published protocol
  - Diagnostic test validated to CLIA standards

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  - Perform over 200 tests per week

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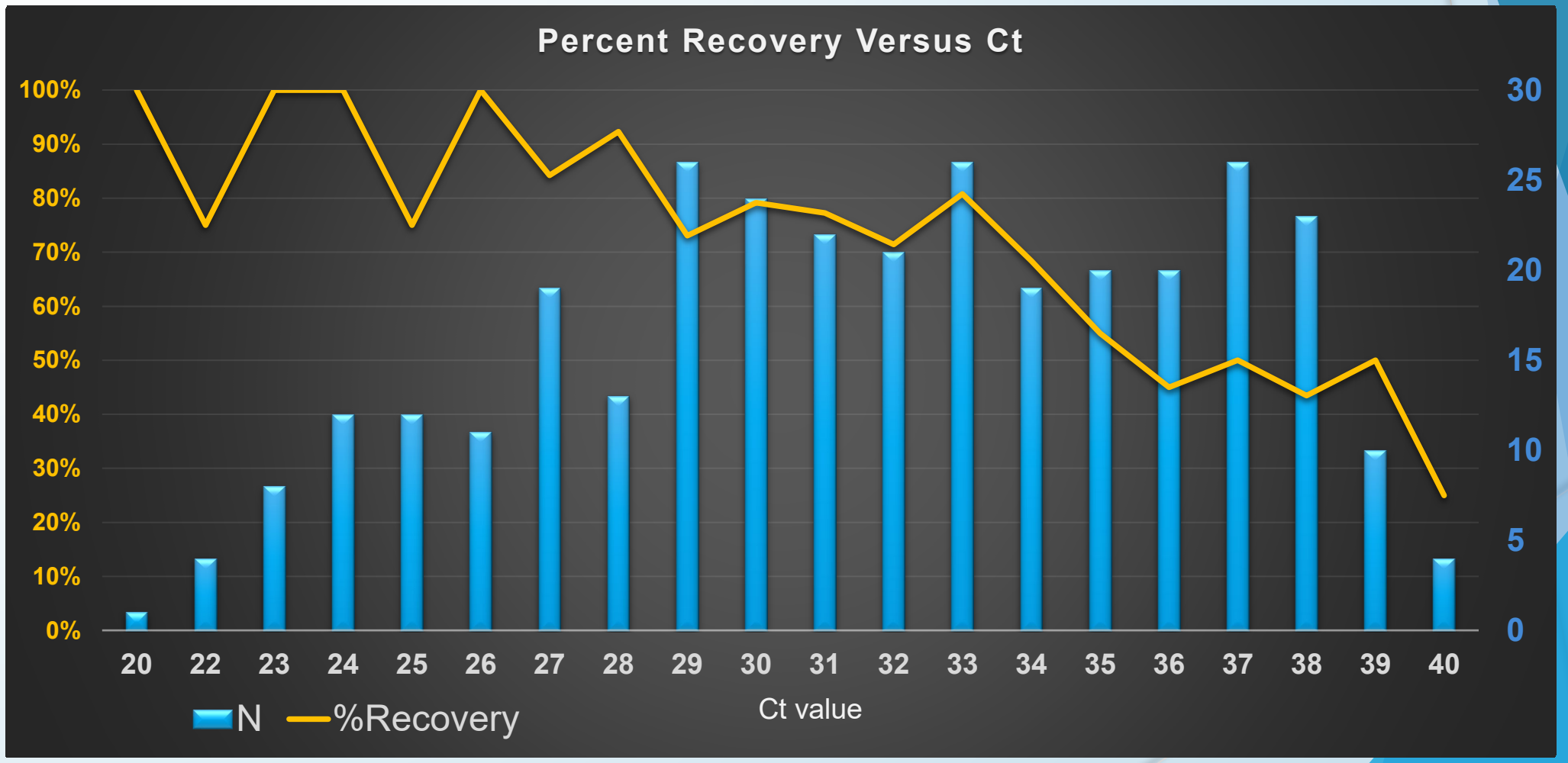
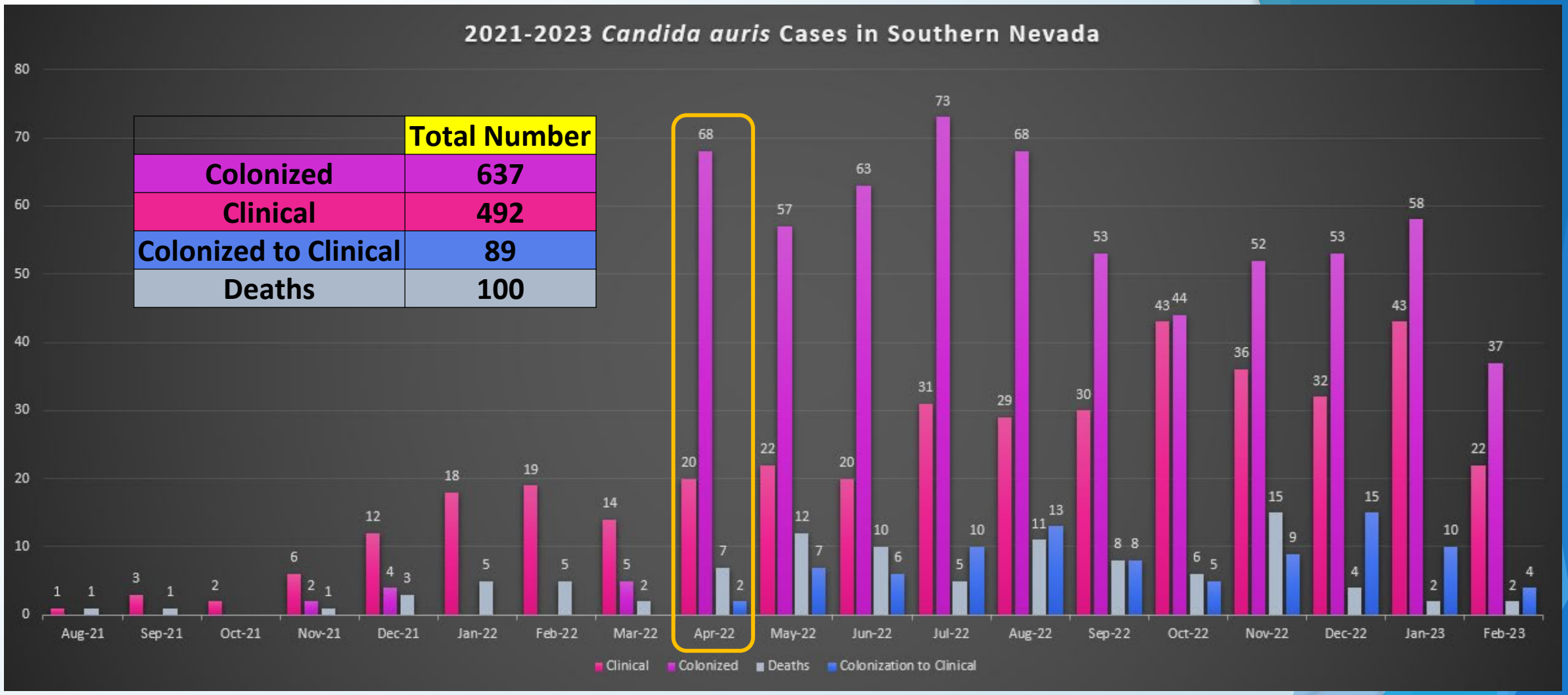
- Combined automated extraction with real-time PCR based on CDC's published protocol
  - Diagnostic test validated to CLIA standards
- Once validated, State Epi recommended that all long-term care facilities perform screening on admits
  - Perform over 200 tests per week
- All positive PCRs are sent to micro department for culture and isolation
  - All isolates obtained are sequenced

Likelihood of recovering *C. auris* isolate from a PCR-positive specimen





April 2022 – Started receiving *C. auris* isolates from WA PHL and started sequencing all isolates received.

Eukaryotic genomics are much more complex than prokaryotic and virus

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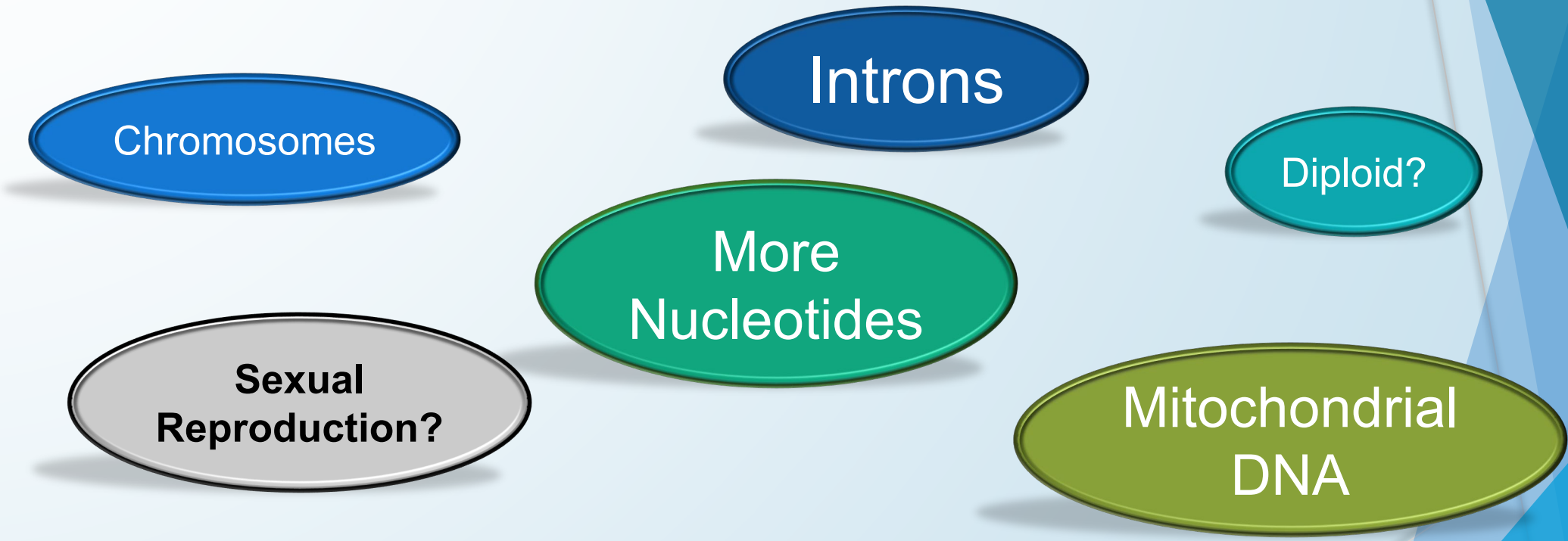

Eukaryotic genomics are much more complex than prokaryotic and virus

Chromosomes

Introns

More Nucleotides

Sexual Reproduction

Mitoc
D

By a lucky coincidence, Dr. David Hess, a yeast expert started working for the lab April 1, 2022!

C. auris genetics

- Genome around 12 Mb on 7 chromosomes

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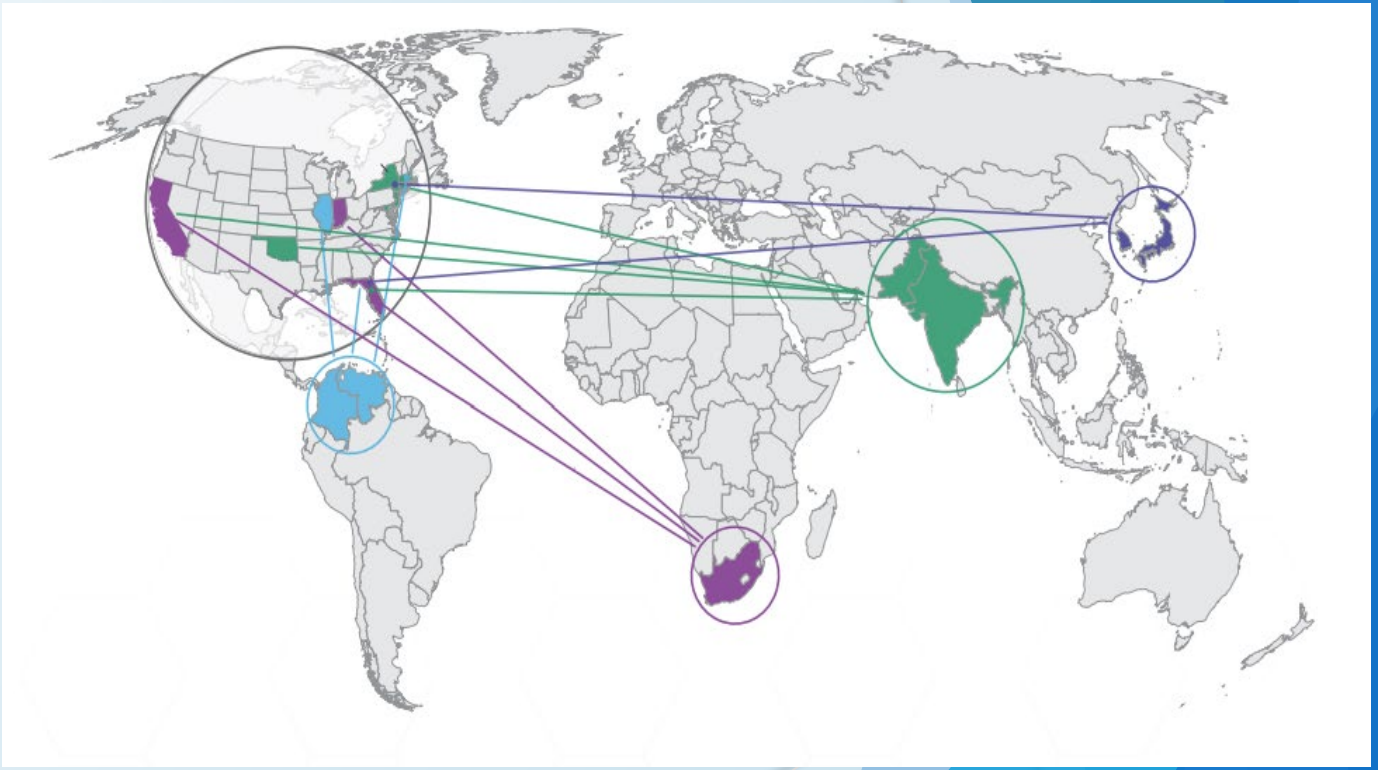

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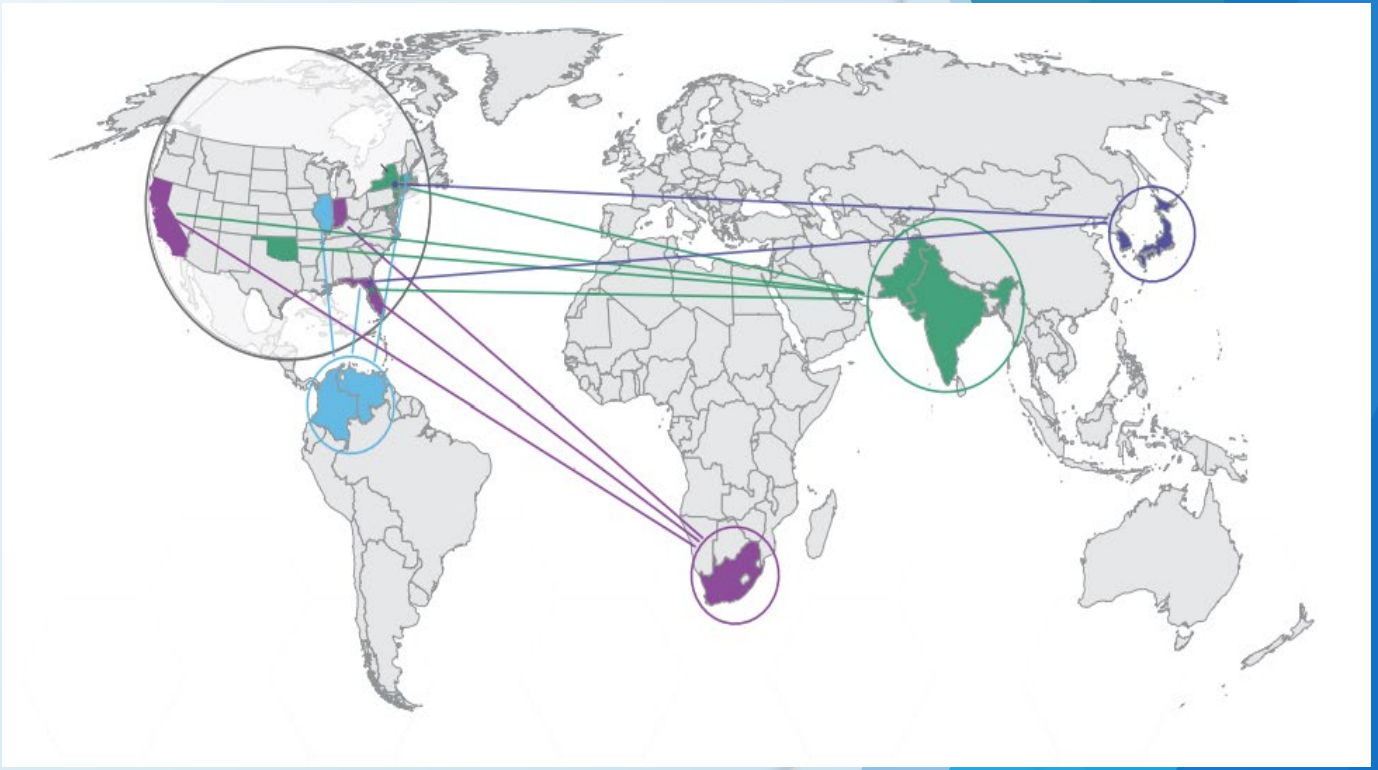
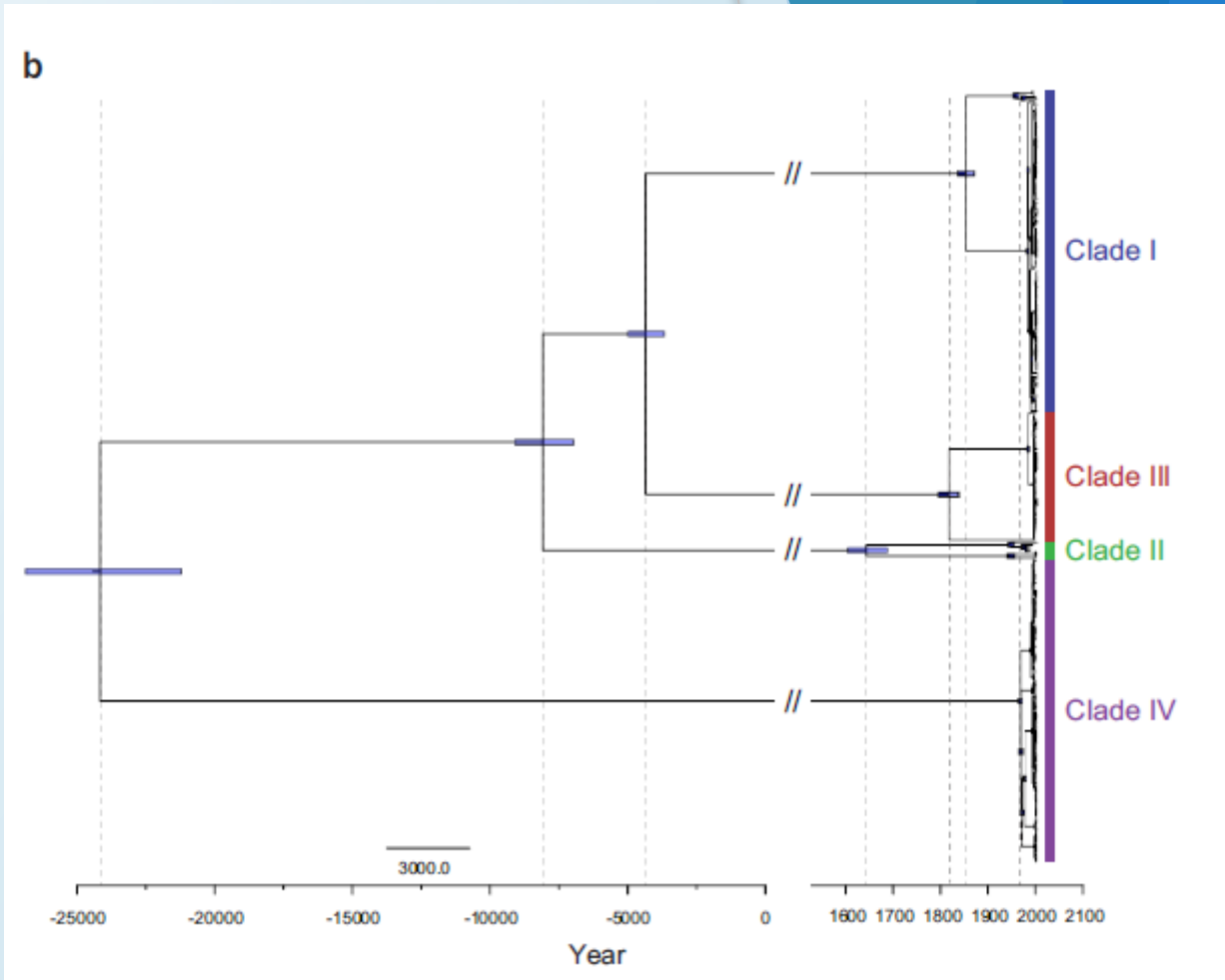
- *C. auris* is comprised of 5 genetically distinct clades
- Clades were initially associated with geographic regions
  - 98.7% nucleotide identity
- Presently all clades are seen throughout the globe



C. auris genetics

- Clades became genetically distinct 5,000 to 25,000 years ago



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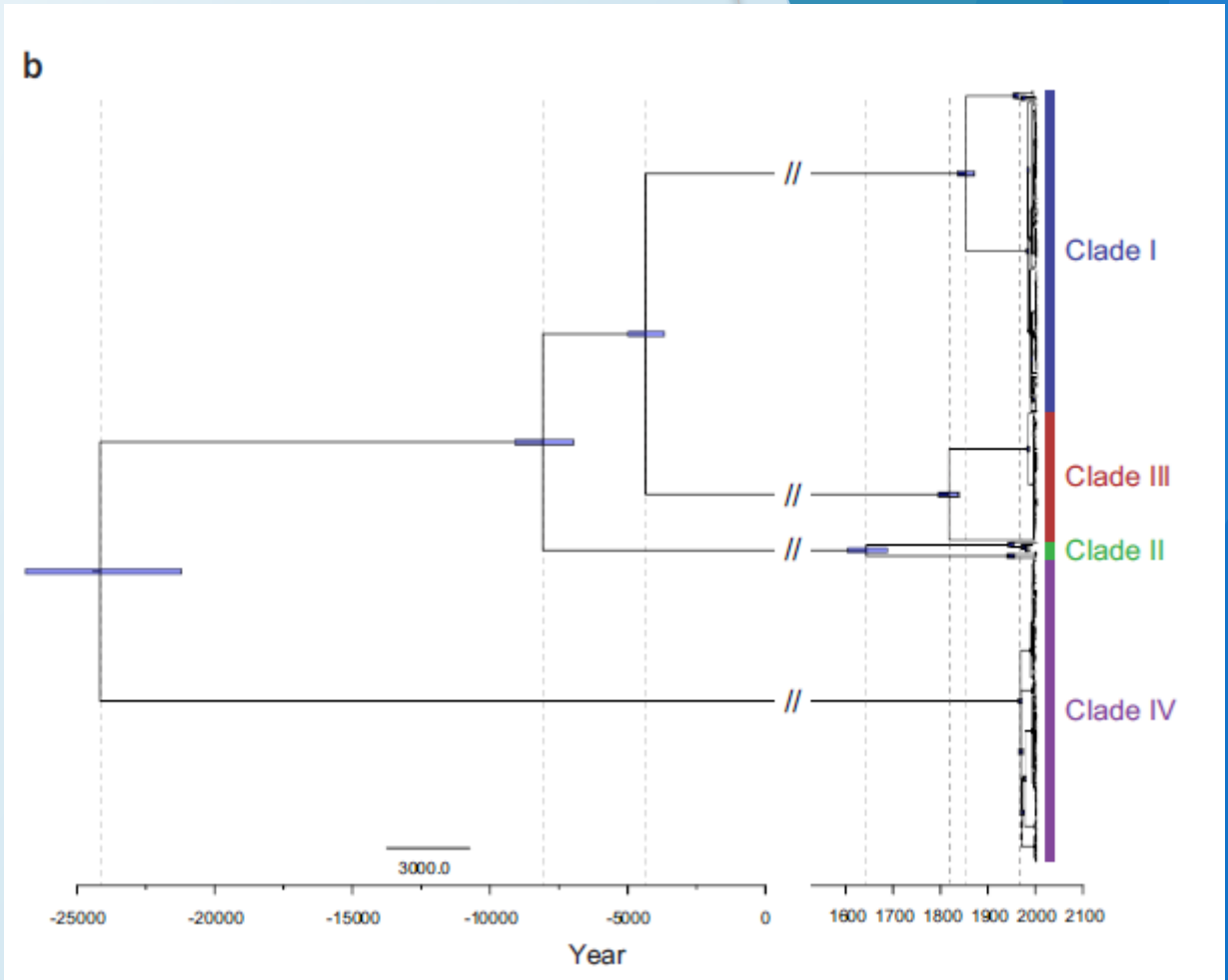

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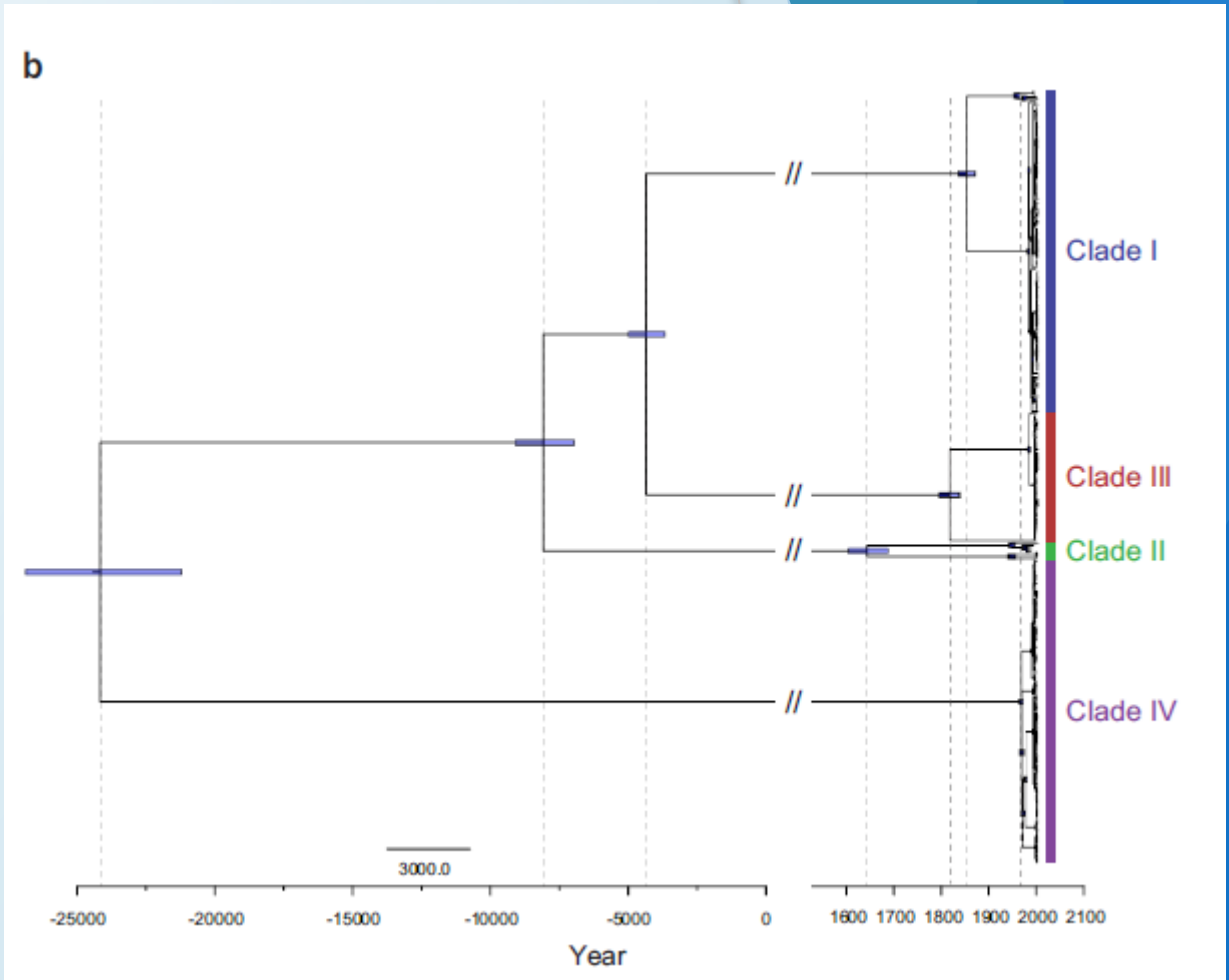
C. auris genetics

- Clades became genetically distinct 5,000 to 25,000 years ago
- All Clades are capable of infecting humans
- Native environment still unknown



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NSPHL began whole genome sequencing *C. auris* isolates in April 2022

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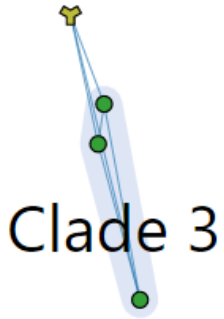
- NV PHL had already acquired a NovaSeq 6000 and validated automated library preparation
- Early analysis of the first few dozen isolates revealed something more complex was occurring
- Southern Nevada isolates were found from Clade I, Clade III and Clade IV
  - **Evidence of multiple outbreaks**

In November 2021, Clade 1 and Clade 3 were sequestered to specific facilities

Node Deidentified Provider	Count	Color
(Empty)	3	
Ach1	3	
Washoe	1	
Snf6	1	
Ach3	1	

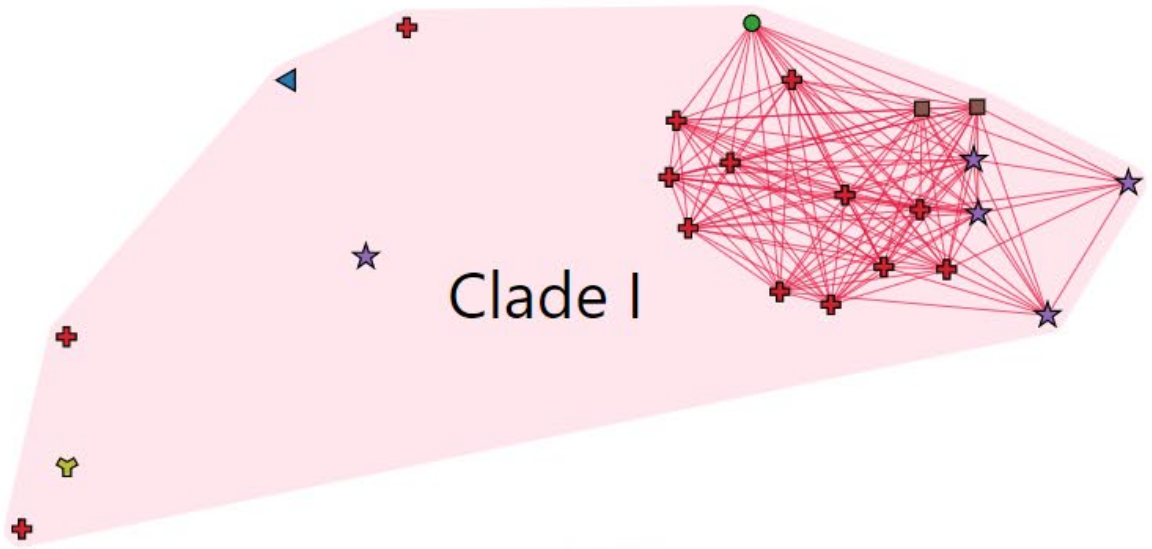


Collection Date

Nov 2021

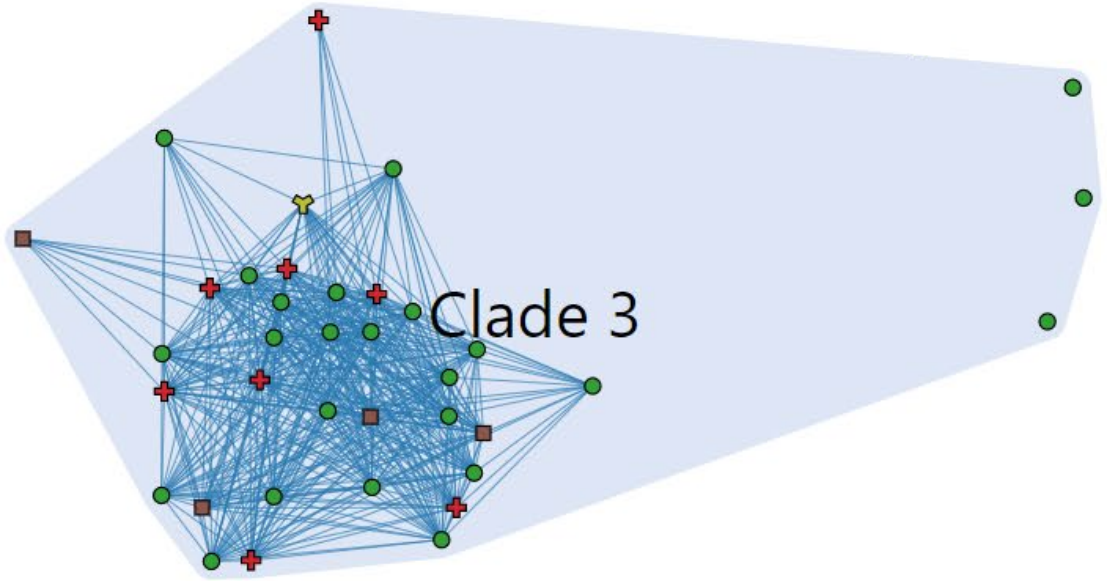
Oct 20 Jan 21 Apr 21 Jul 21 Oct 21 Jan 22 Apr 22 Jul 22 Oct 22

9 (0) Nodes (Selected)
6 Links
1 Clusters
5 Singletons



By March 2022, both Clades were present in multiple facilities

Clade 4



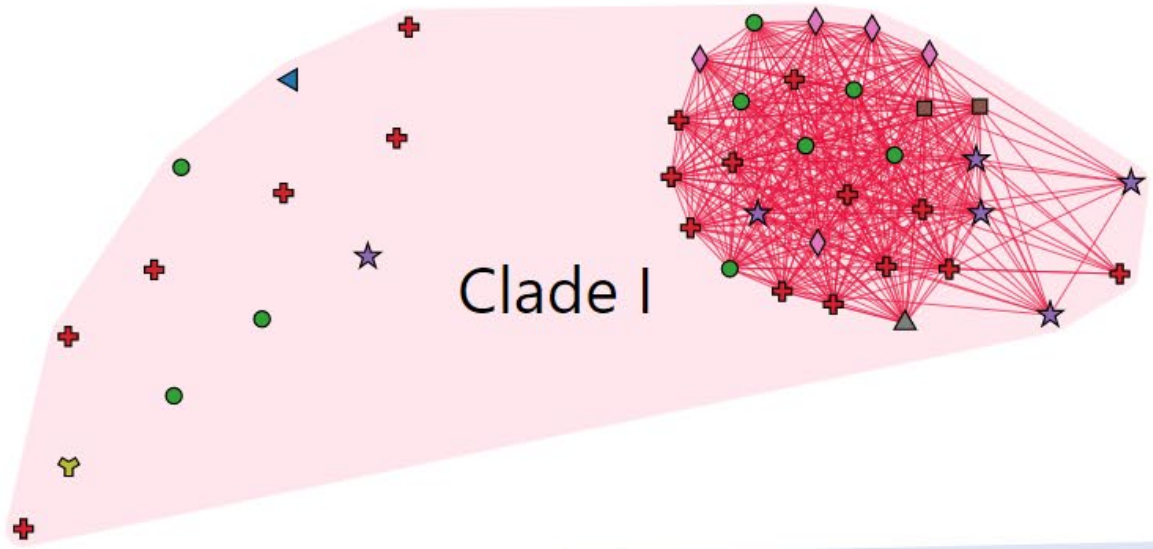
Node Deidentified Provider	Count	Color
Ach1	26	
Ach3	22	
Ach4	6	
Snf6	5	
(Empty)	3	
Washoe	1	

Collection Date

Mar 2022

Oct 20 Jan 21 Apr 21 Jul 21 Oct 21 Jan 22 Apr 22 Jul 22 Oct 22

63 (0) Nodes (Selected)
596 Links
2 Clusters
11 Singletons



September 2022: Clade1 and Clade3 found inter-dispersed among Southern Nevada Facilities. Community spread as well as facility transmission.

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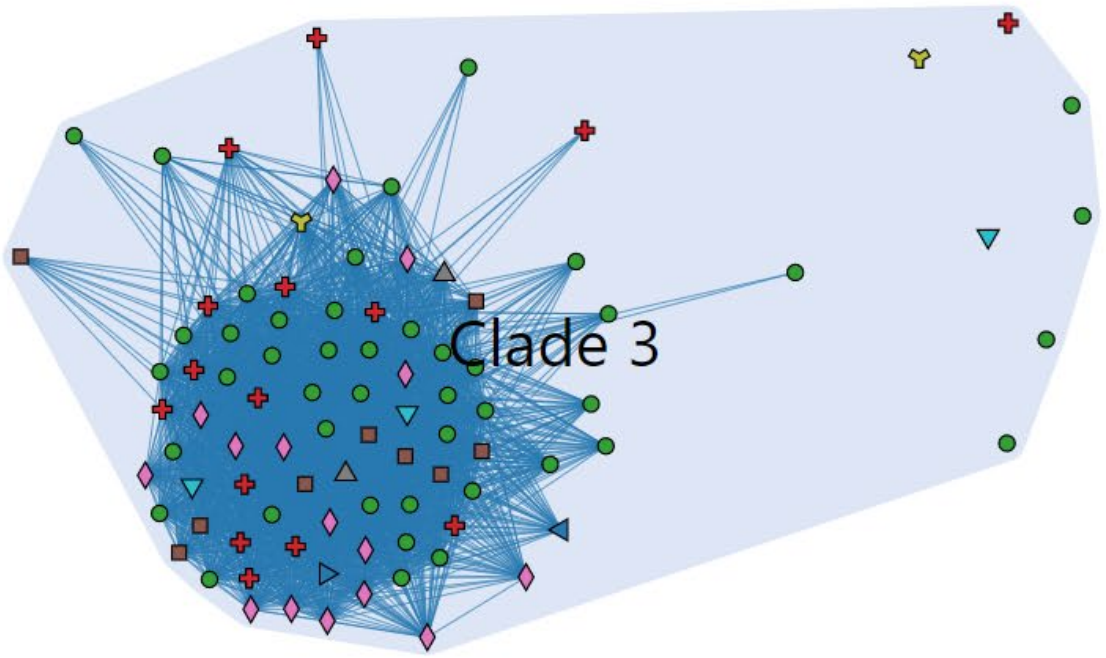
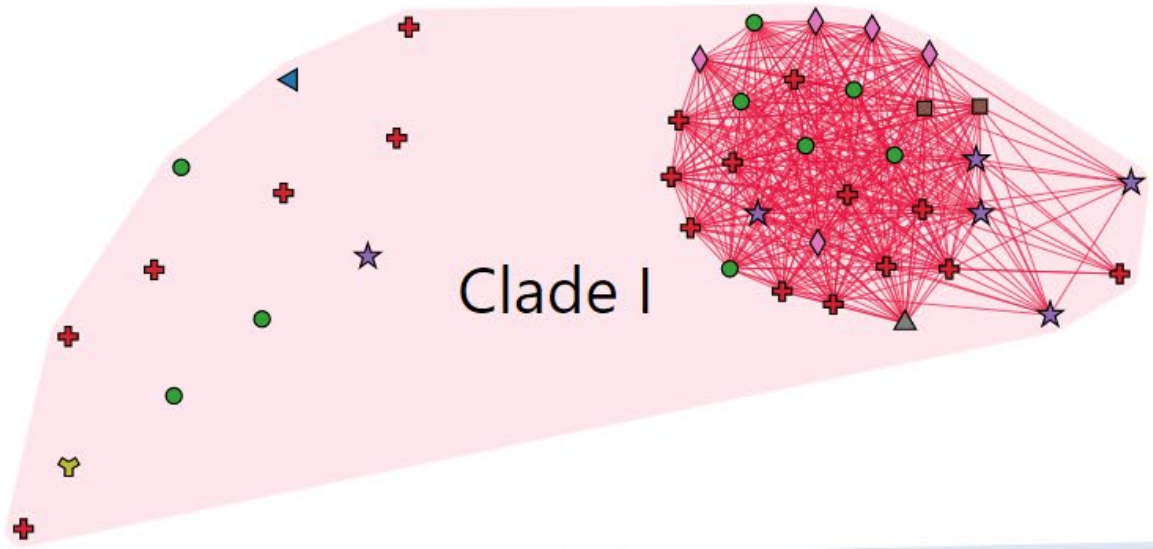
Ach1	54	
Ach3	33	
Snphl	20	
Ach4	11	
Snf6	6	
(Empty)	3	
Unknown	3	
Cdc	3	
Washoe	2	
Ach5	1	



Collection Date

Oct 20 Jan 21 Apr 21 Jul 21 Oct 21 Jan 22 Apr 22 Jul 22 Sep 2022 Oct 22

136 (0) Nodes (Selected)
2,977 Links
2 Clusters
20 Singletons



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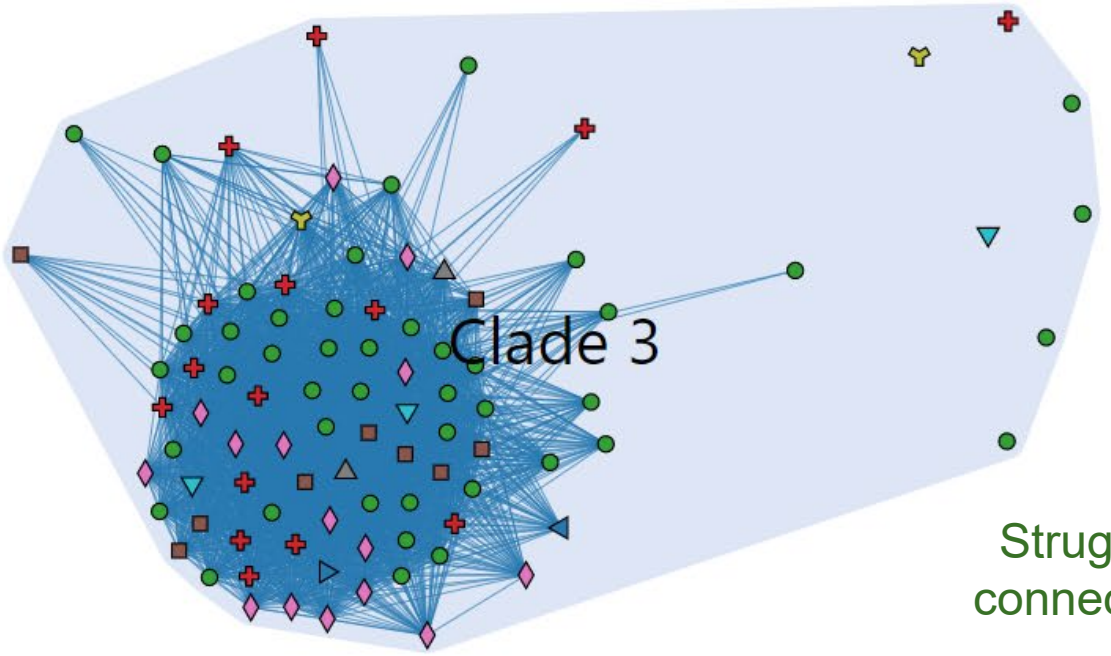

Struggling with resolution to show connections between cases/isolates

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Bioinformatics of *C. auris*: Problems and Solutions

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  - Not ideal

- Contracted Theiagen Genomics to develop *C. auris* bioinformatic workflows using Terra
- All analysis is performed in the cloud with no command line interface

How do we translate our genomic data into information Epis can use?

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Shared SNPs

Single Nucleotide Polymorphisms (**SNPs**) are genetic changes observed when comparing genomes

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  - **Fewer differences = closely related**

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However, this a Eukaryotic organism and the same method might not work

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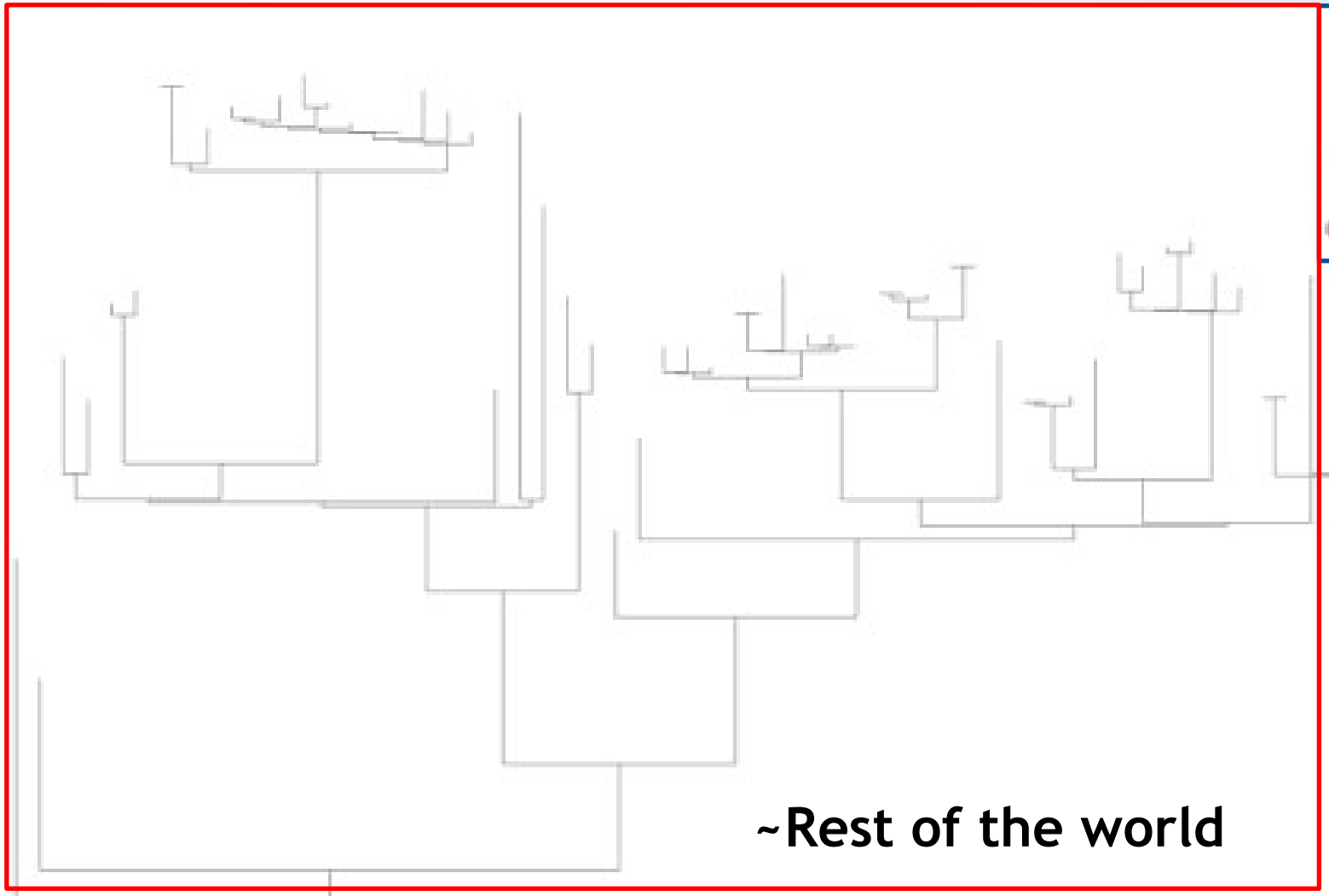
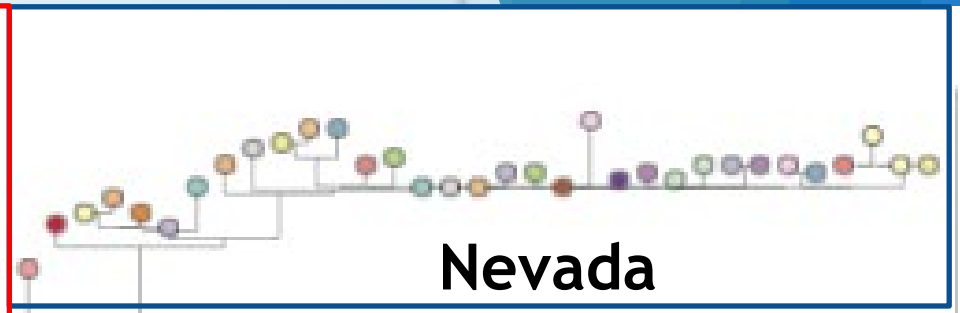
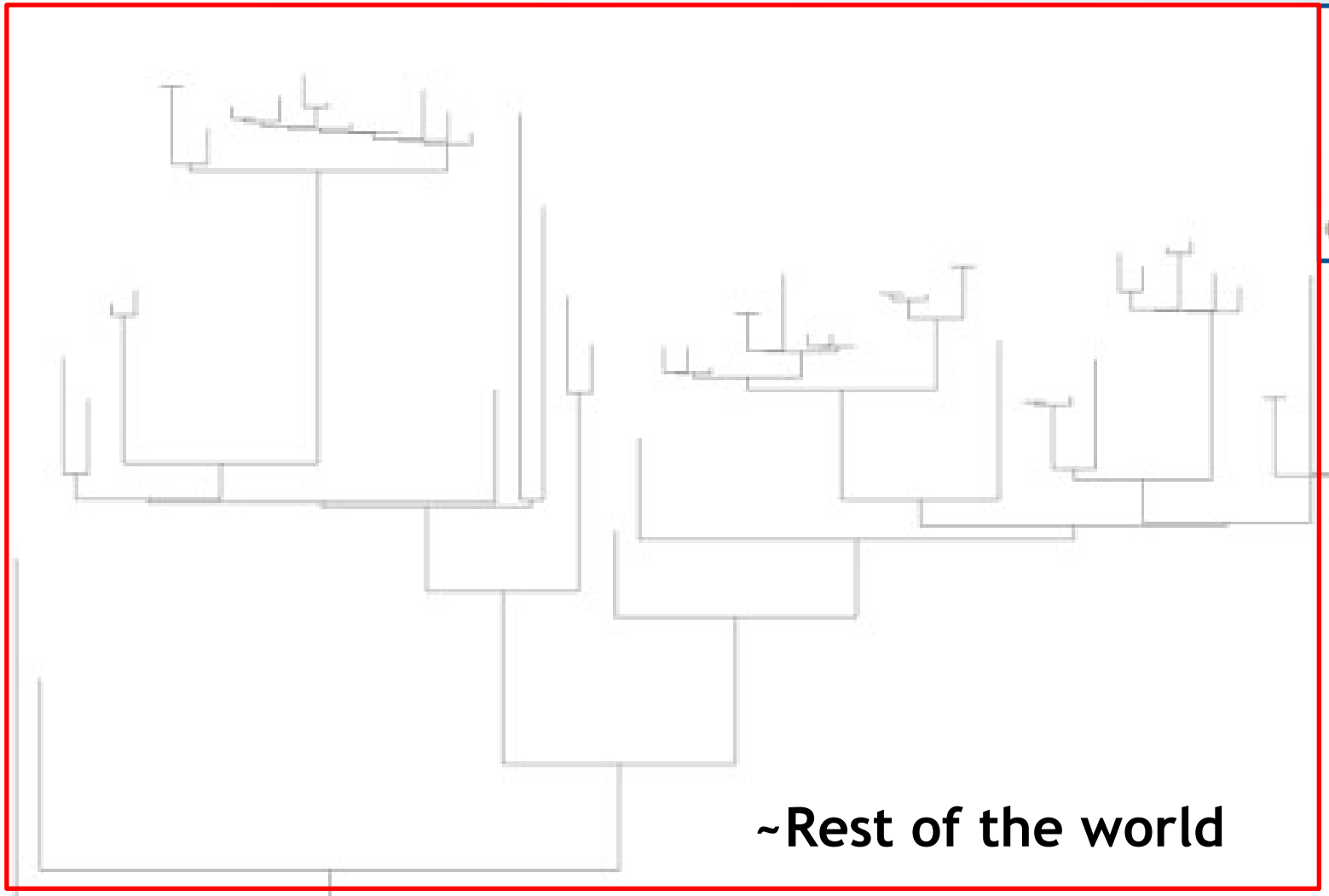
Actionable evidence for epidemiologists

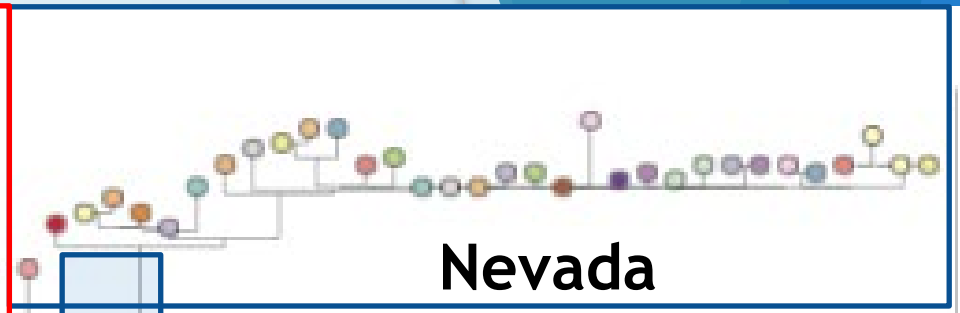
Nevada Clade 1 versus the world



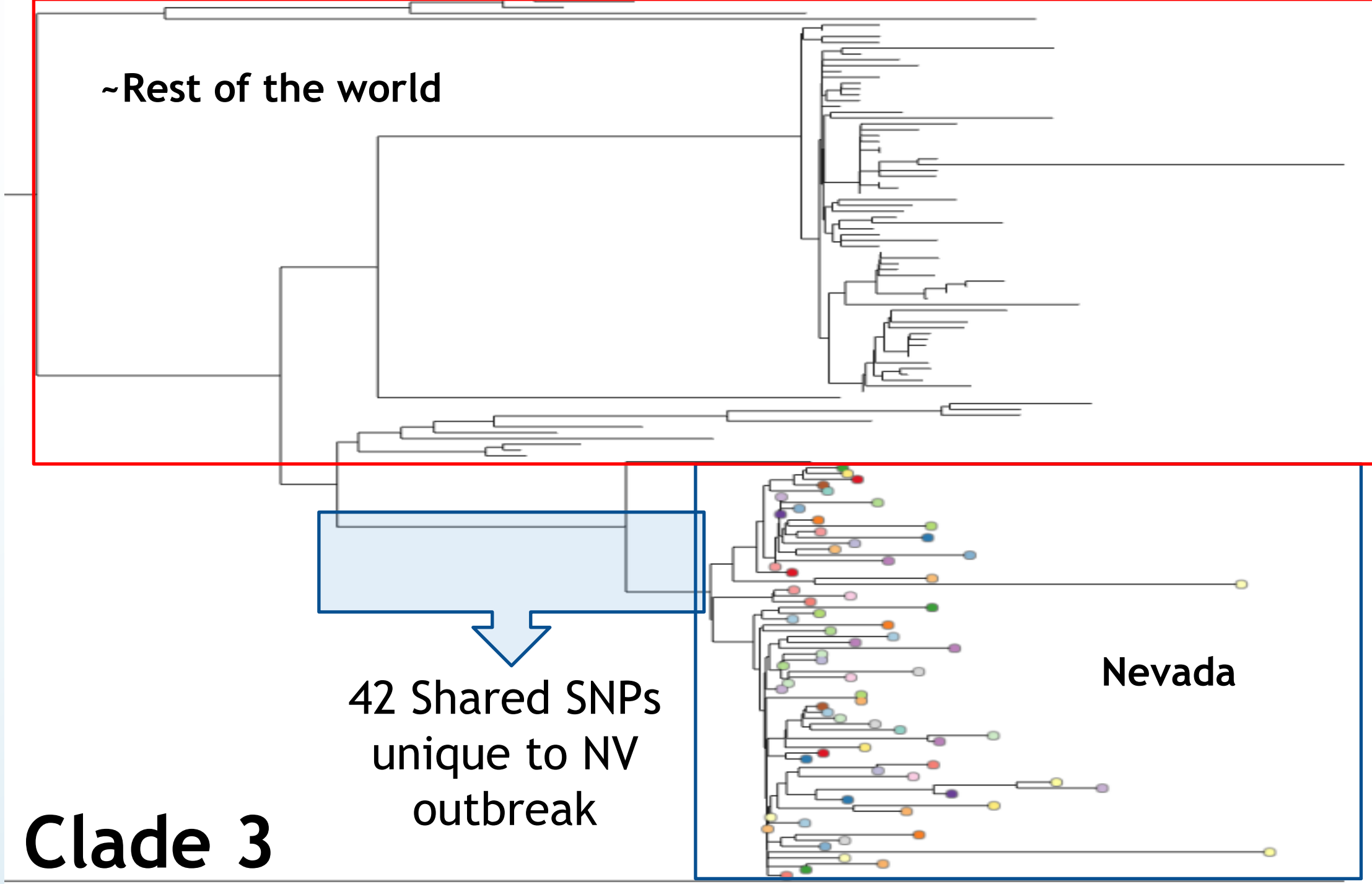


Nevada Clade 1 versus the world





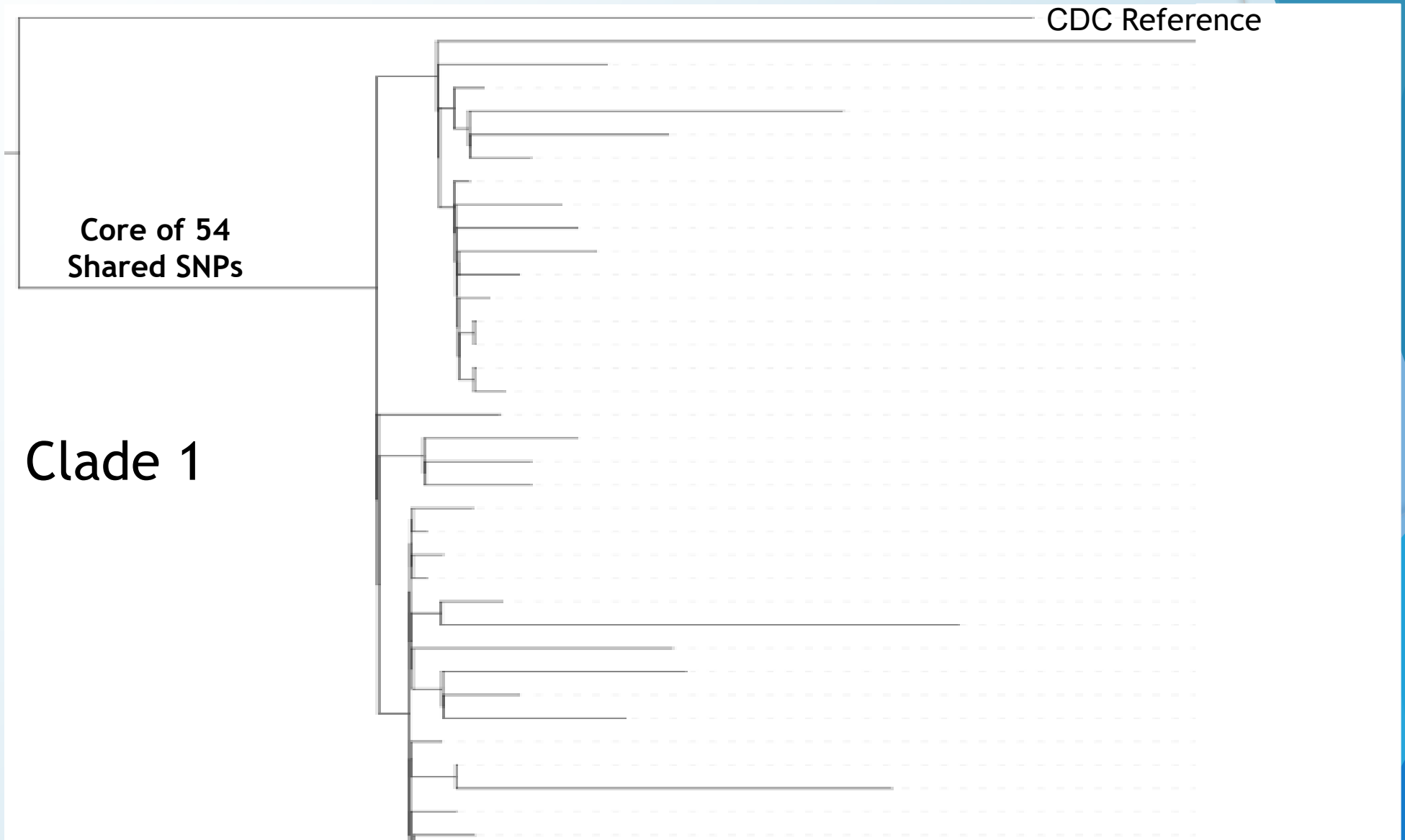
54 Shared SNPs that create a unique NV signature or “Core”



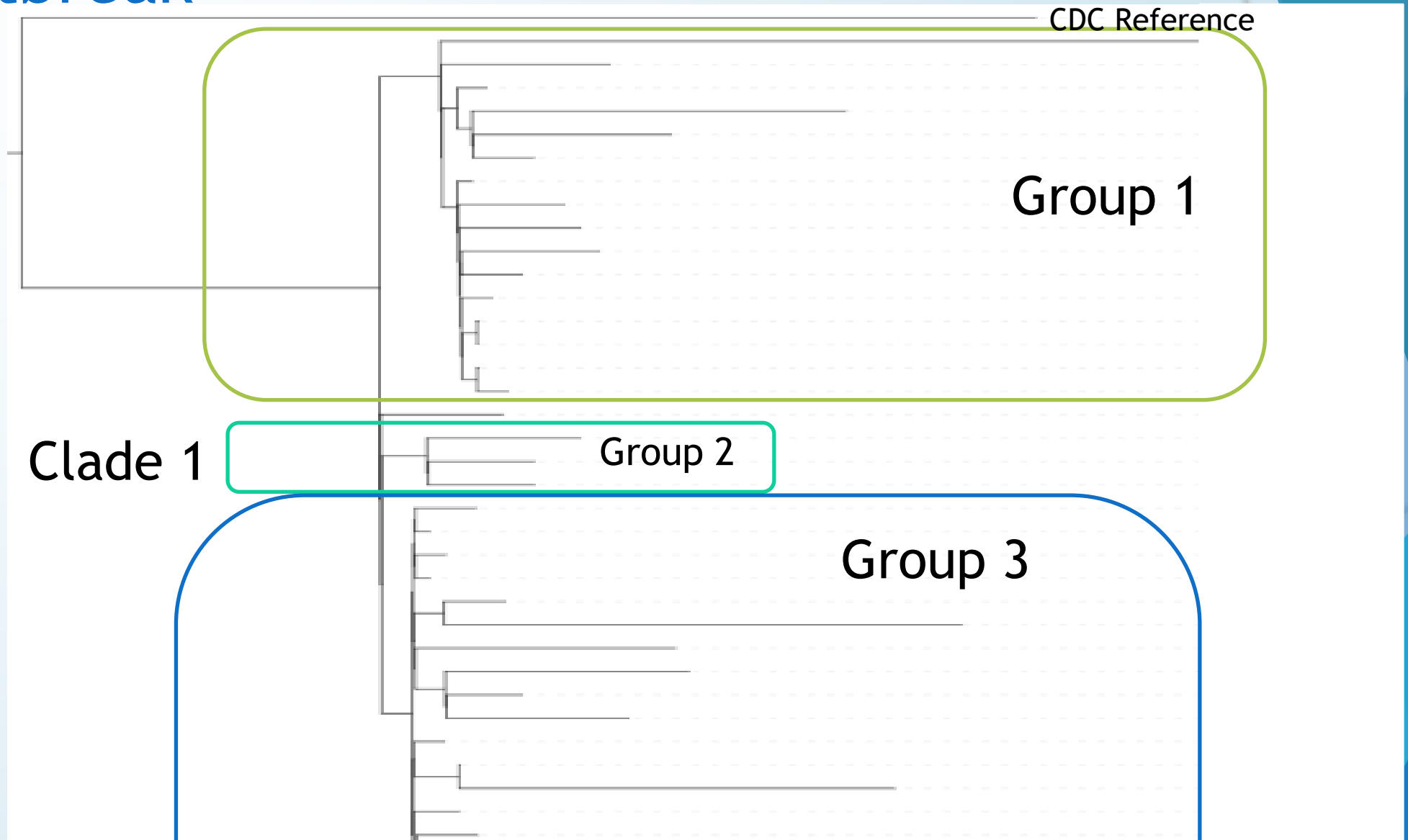
42 Shared SNPs unique to NV outbreak

Clade 3

How does this work inside an outbreak?



Using Shared SNPs to increase resolution in an outbreak



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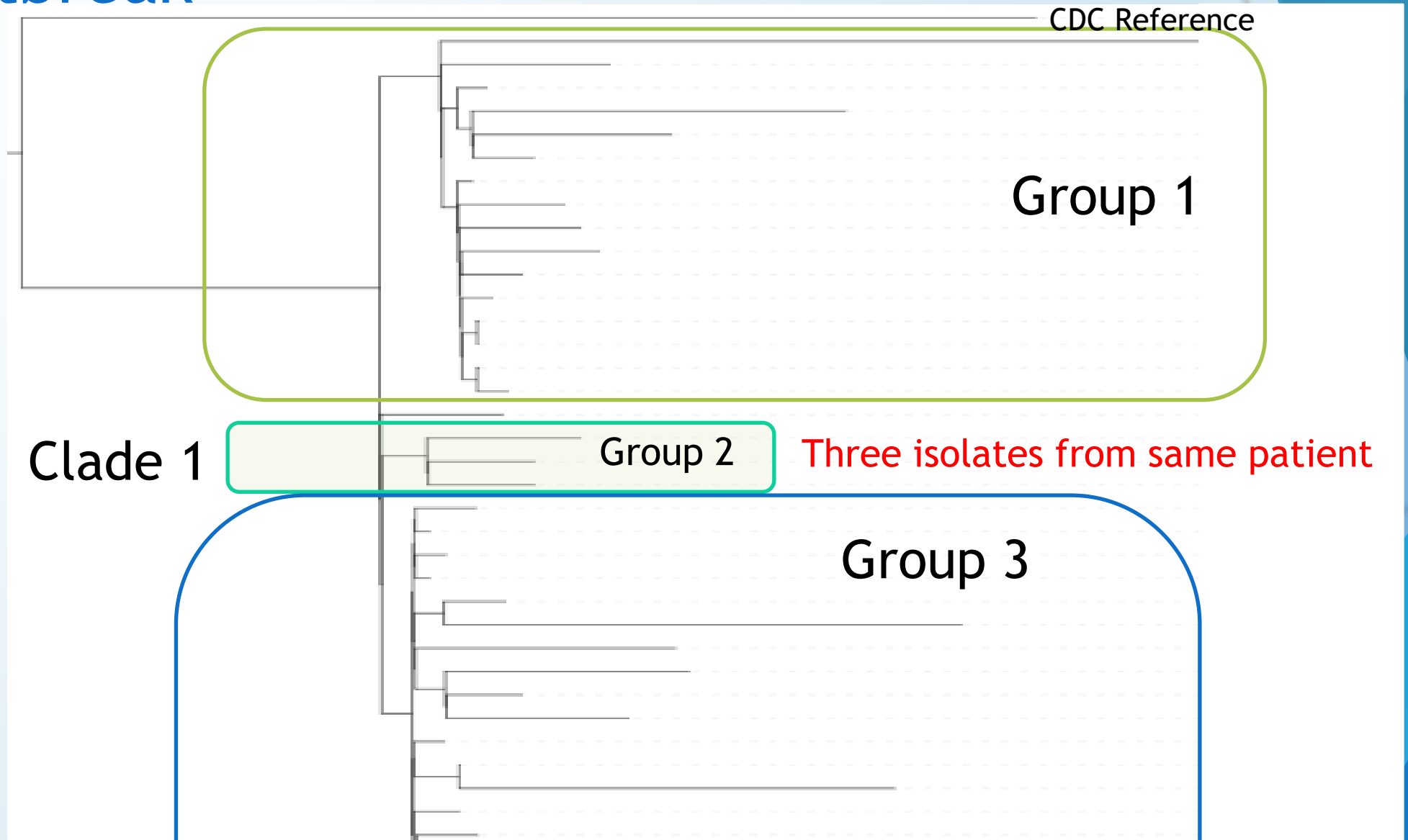

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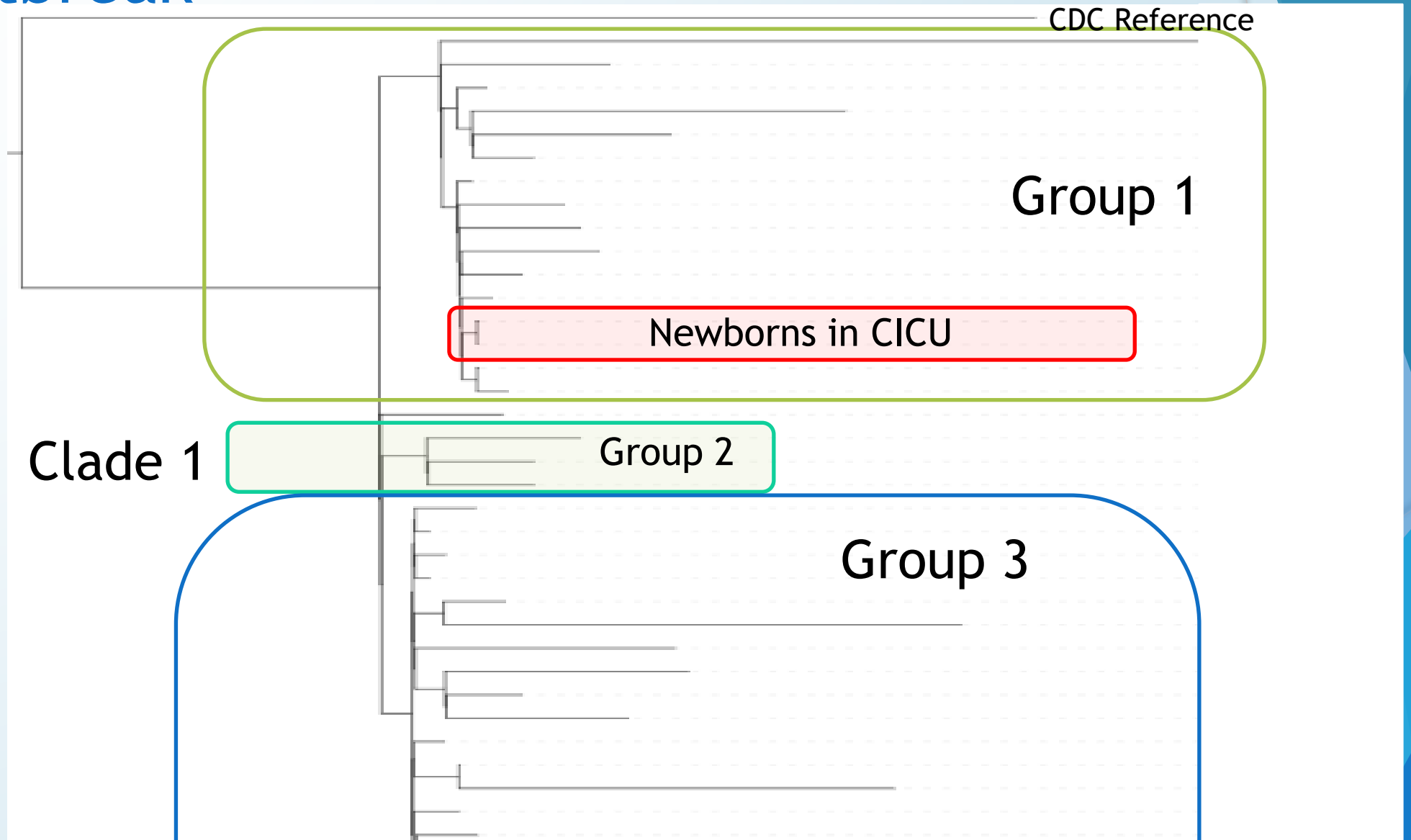

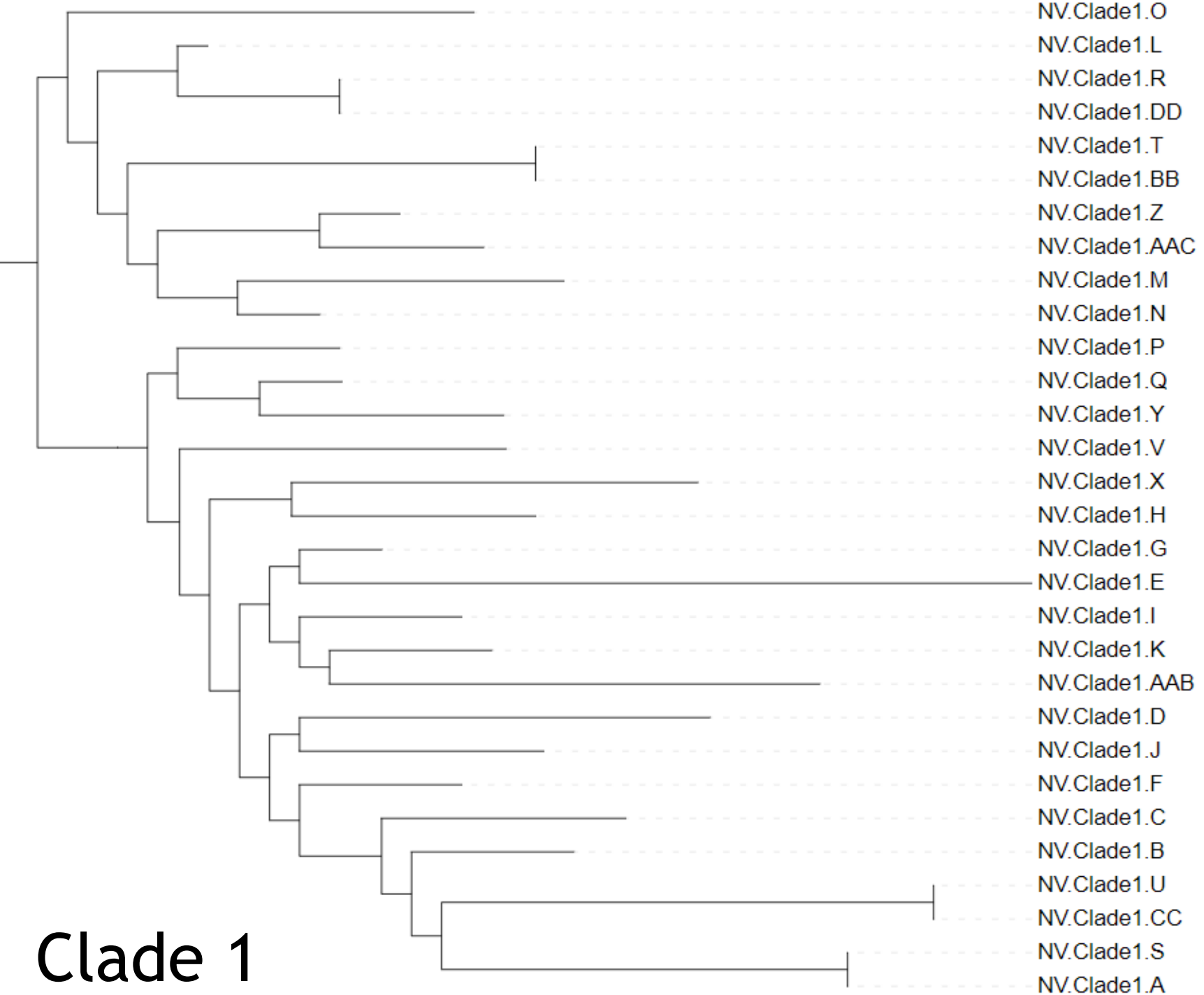

- Clade 1 contains 37 Shared SNP clusters

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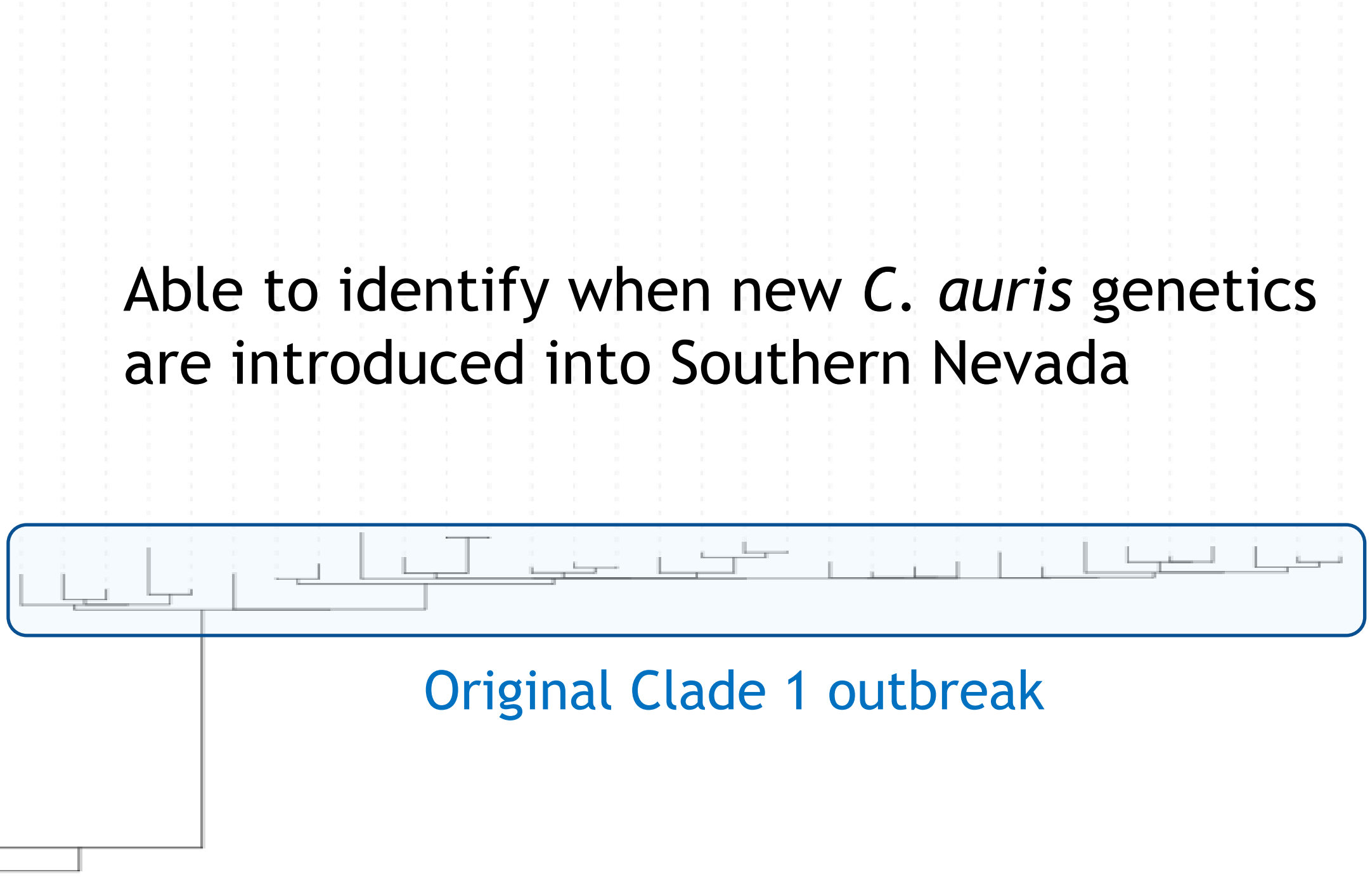
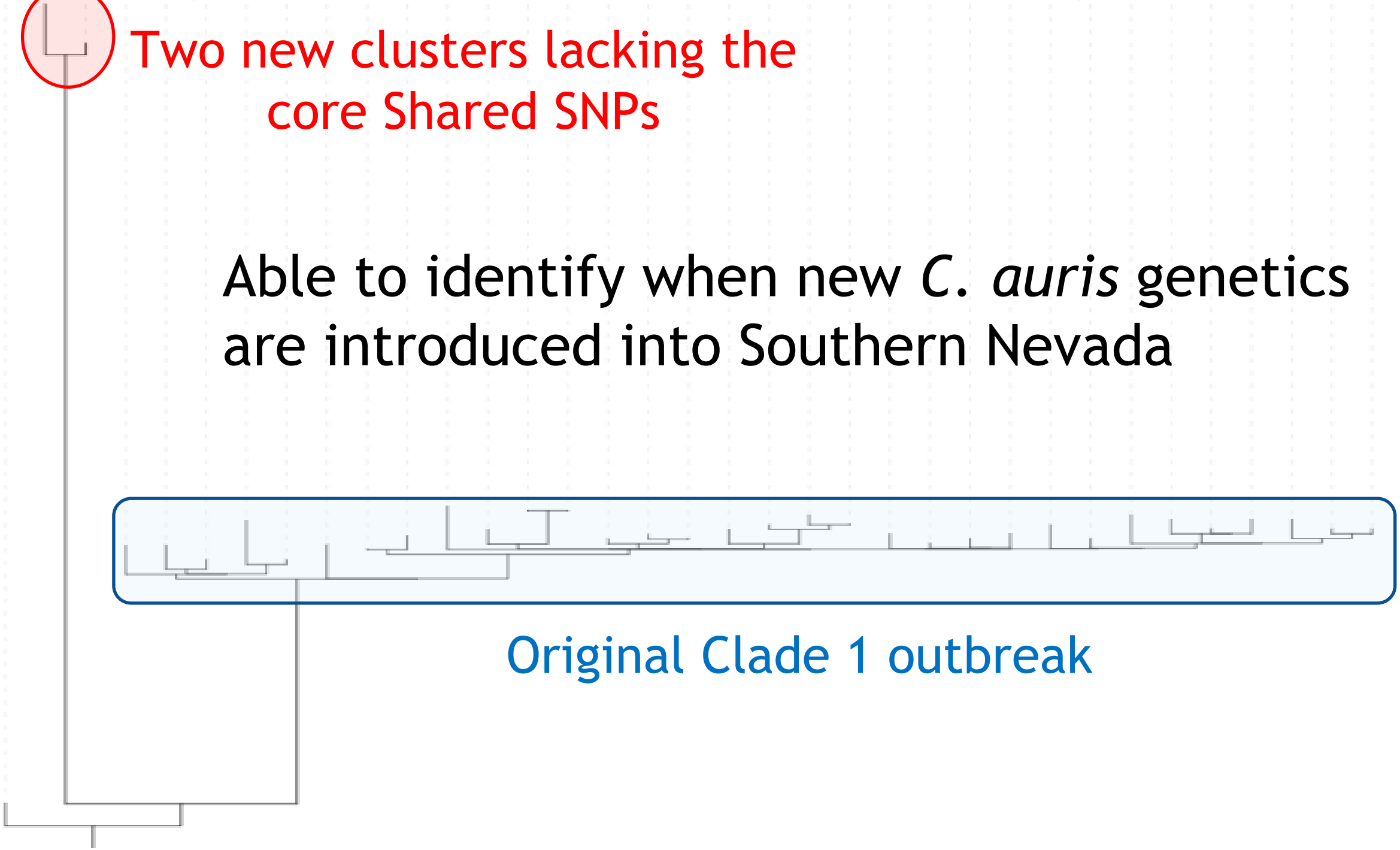
- Look at facilities where Shared SNP clusters continue to grow

Able to identify when new *C. auris* genetics are introduced into Southern Nevada



Original Clade 1 outbreak



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What are we learning from all this data?

1129 Total Cases of *C. auris*

1019 Sequenced

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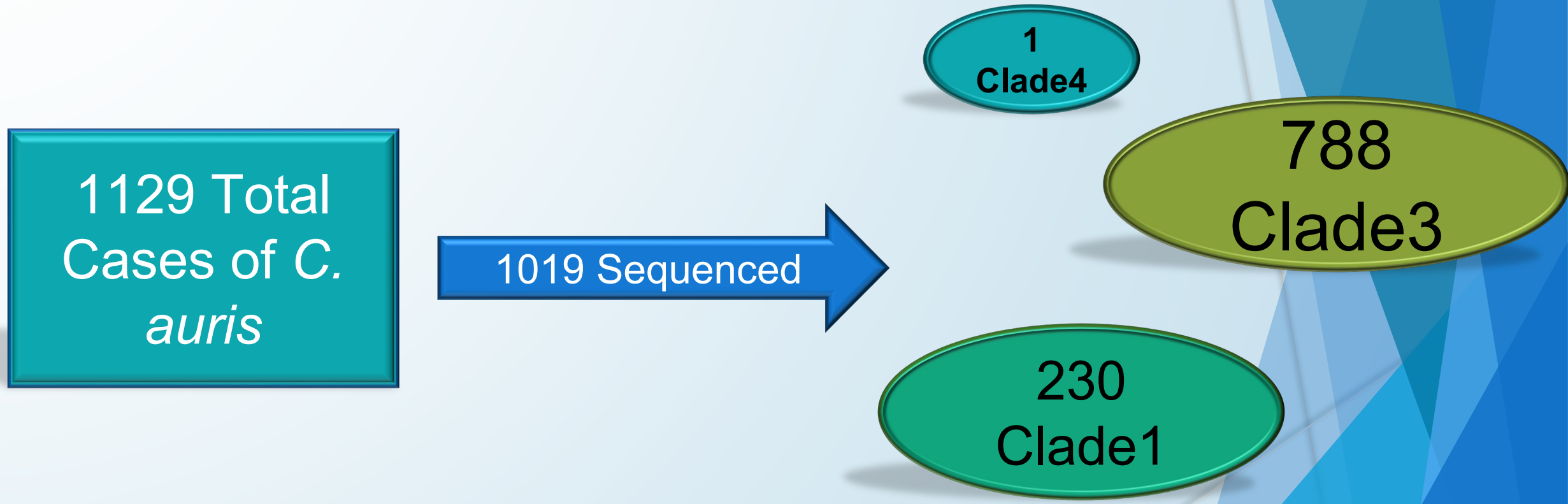

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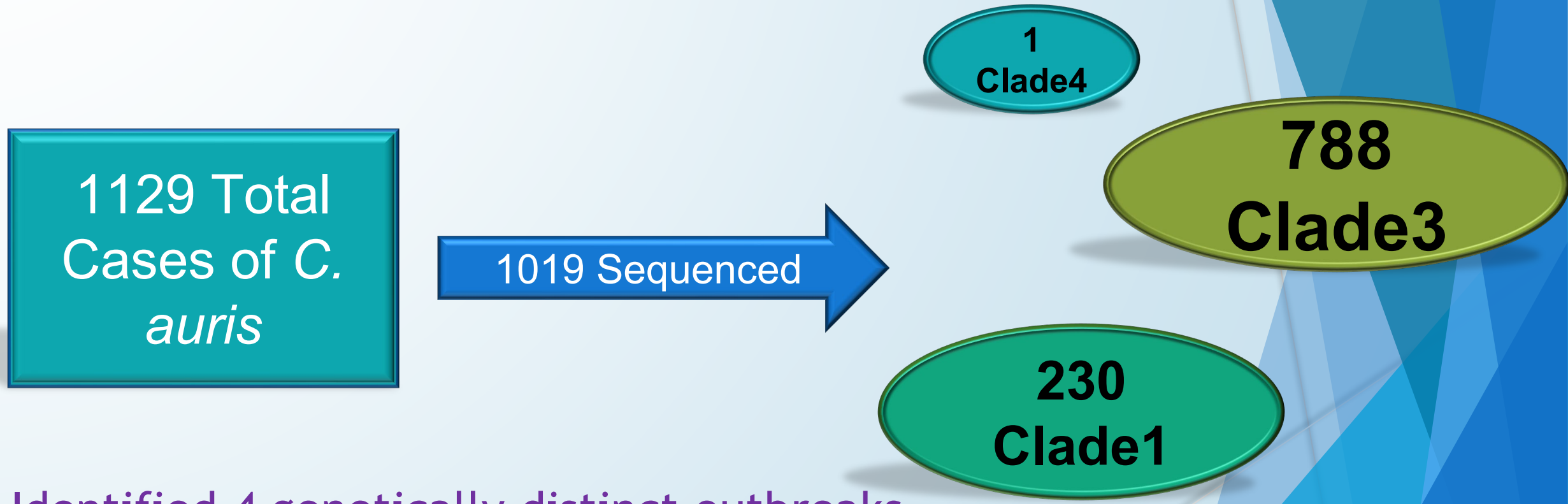

Identified 4 genetically distinct outbreaks

- Three Clade 1
- One Clade 3

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Aid Epi investigations

1129 Total Cases of *C. auris*

1019 Sequenced

1 Clade4

788 Clade3

230 Clade1

Identified 4 genetically distinct outbreaks

- Three Clade 1
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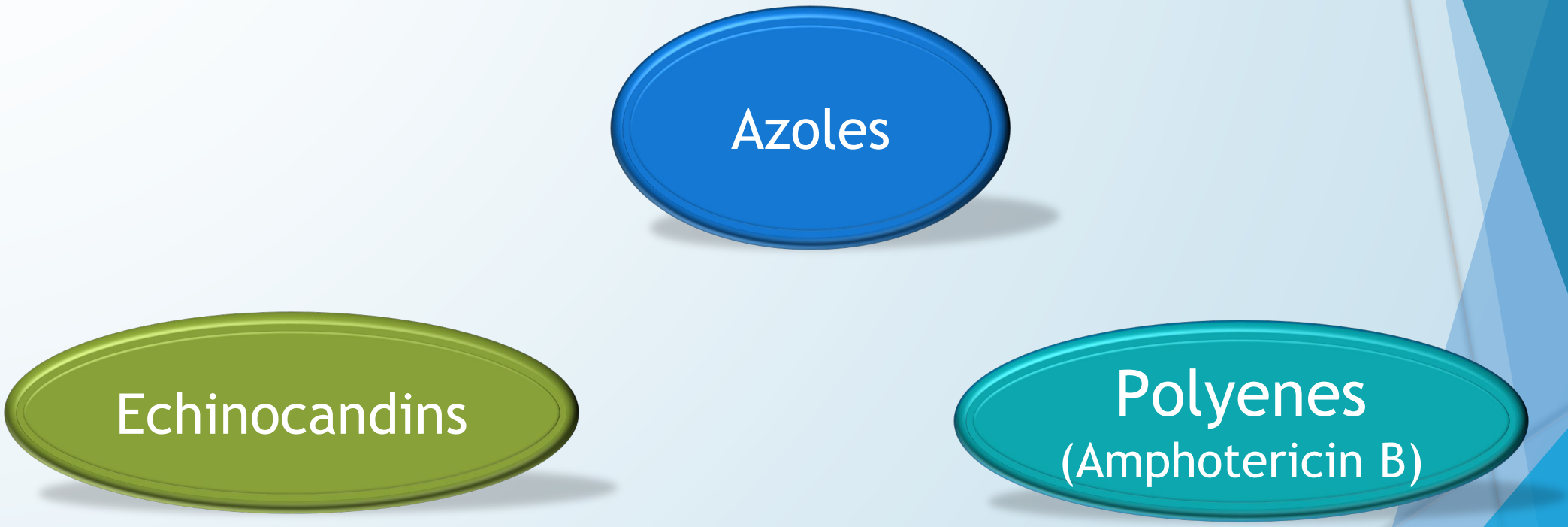
What can we learn about drug resistance?

Three classes of anti-fungal drugs in use



Three classes of anti-fungal drugs in use

~80% *C. auris* are naturally resistant

Echinocandins

Polyenes
(Amphotericin B)

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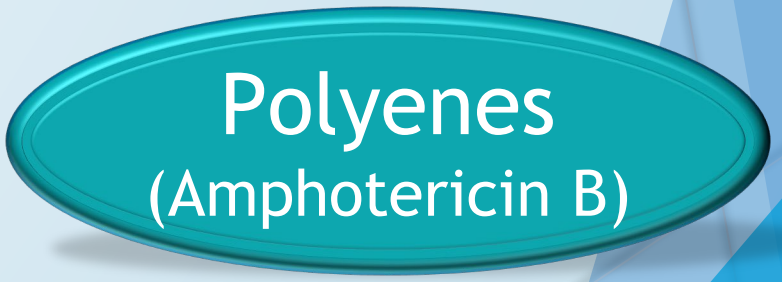
~30% of C. auris are resistant.
Drug can be difficult on patients

Three classes of anti-fungal drugs in use

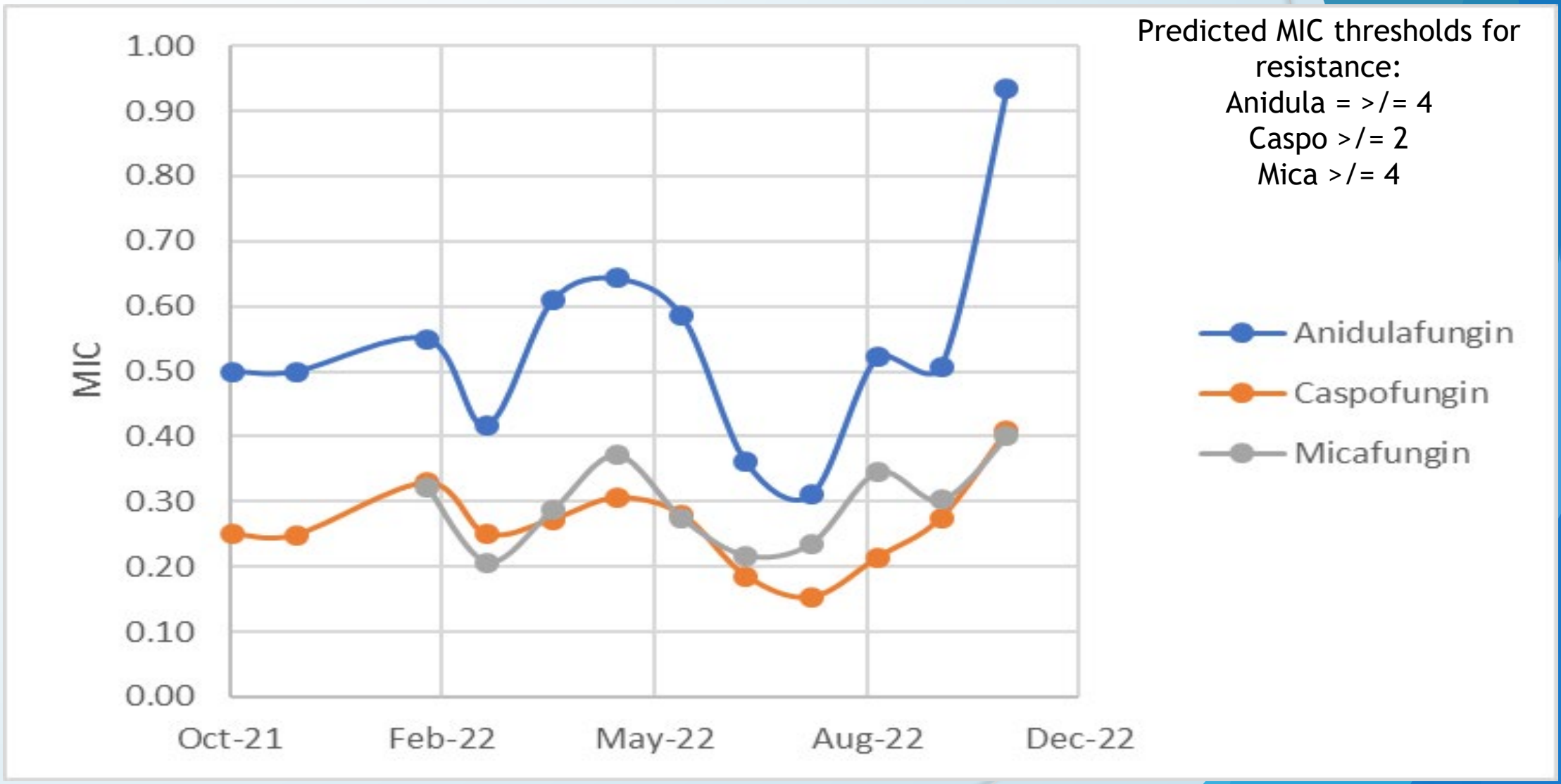
***~80% C. auris* are naturally resistant**

Current preferred treatment. Only 2-3% of *C. auris* are resistant



~30% of C. auris are resistant. Drug can be difficult on patients

Pathogens become drug resistant over time



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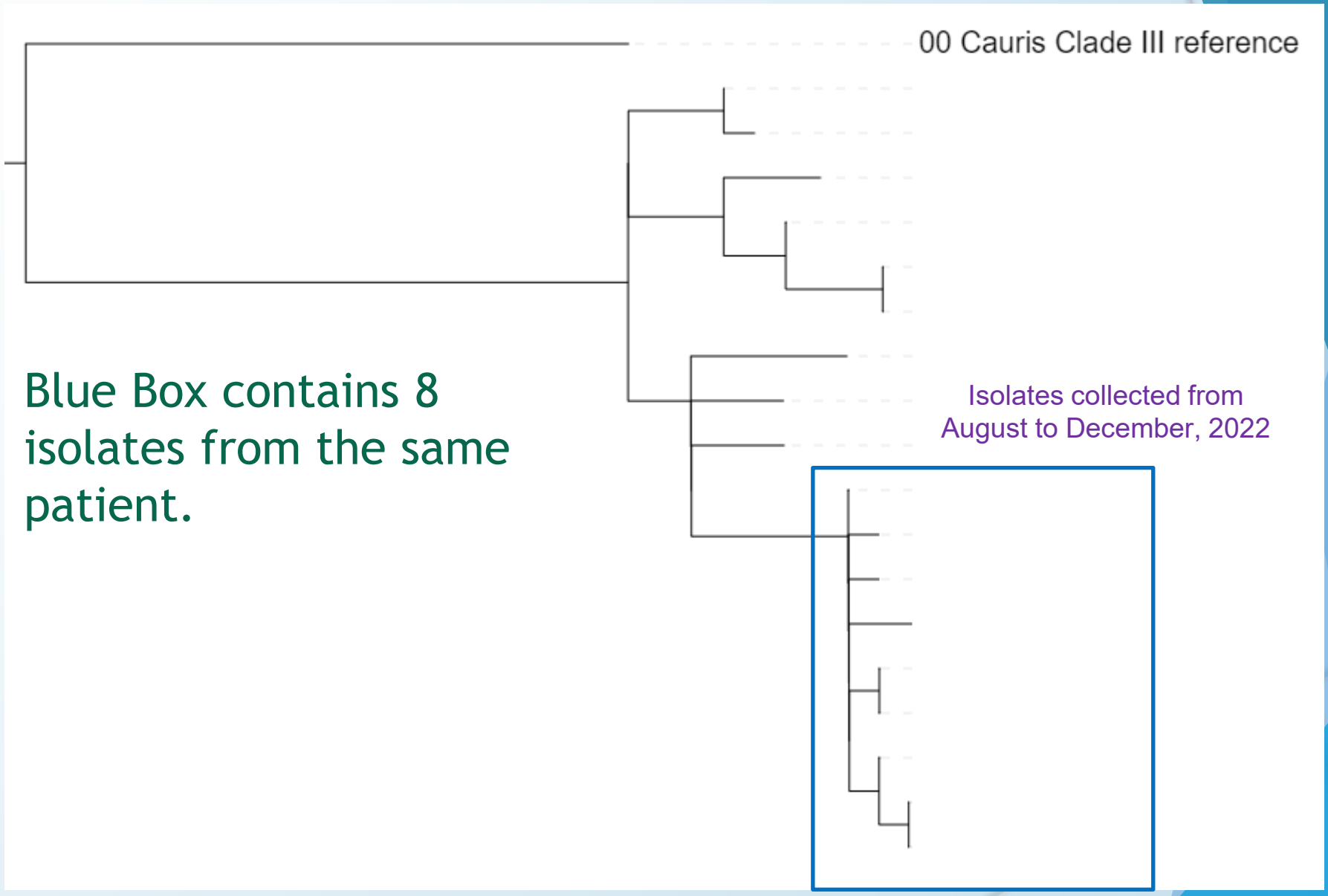
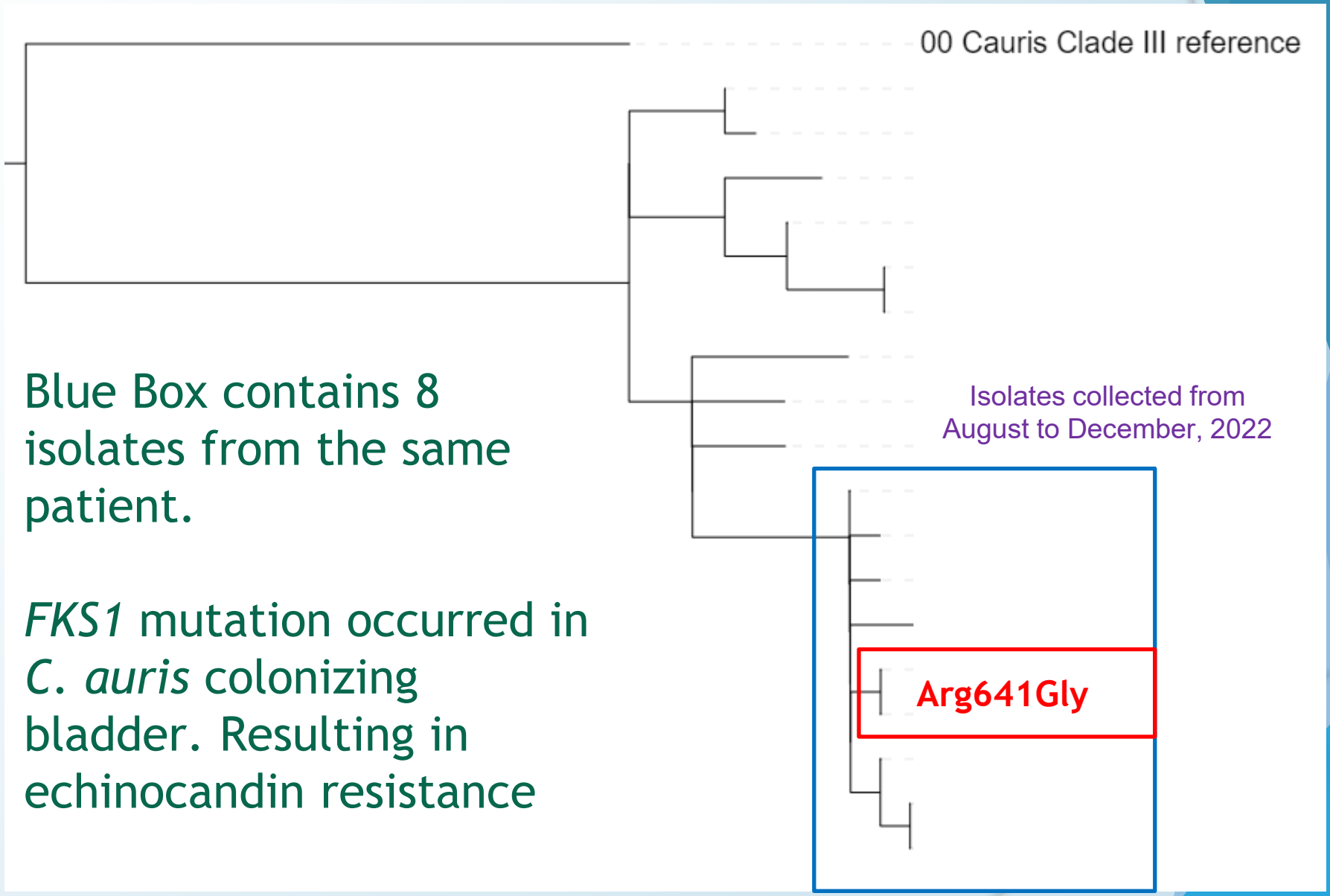
  - Mutational “Hot Spot” around a.a. 635-645 where majority of mutations are identified with echinocandin resistance
- Detection of *FKS1* mutations was built into the workflow



Blue Box contains 8 isolates from the same patient.



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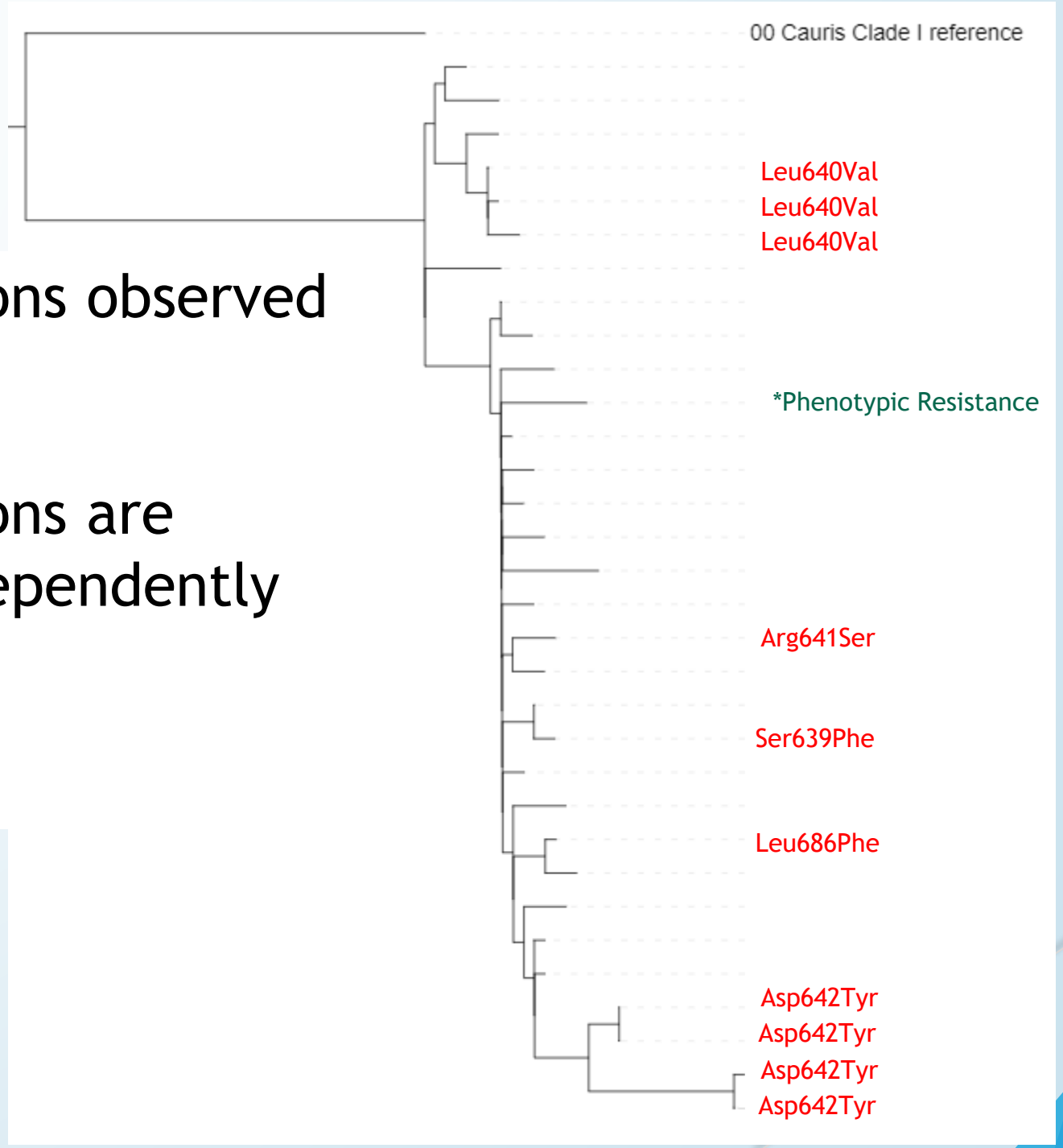
FKS1 mutation occurred in *C. auris* colonizing bladder. Resulting in echinocandin resistance

FKS1 mutations observed in Clade 1

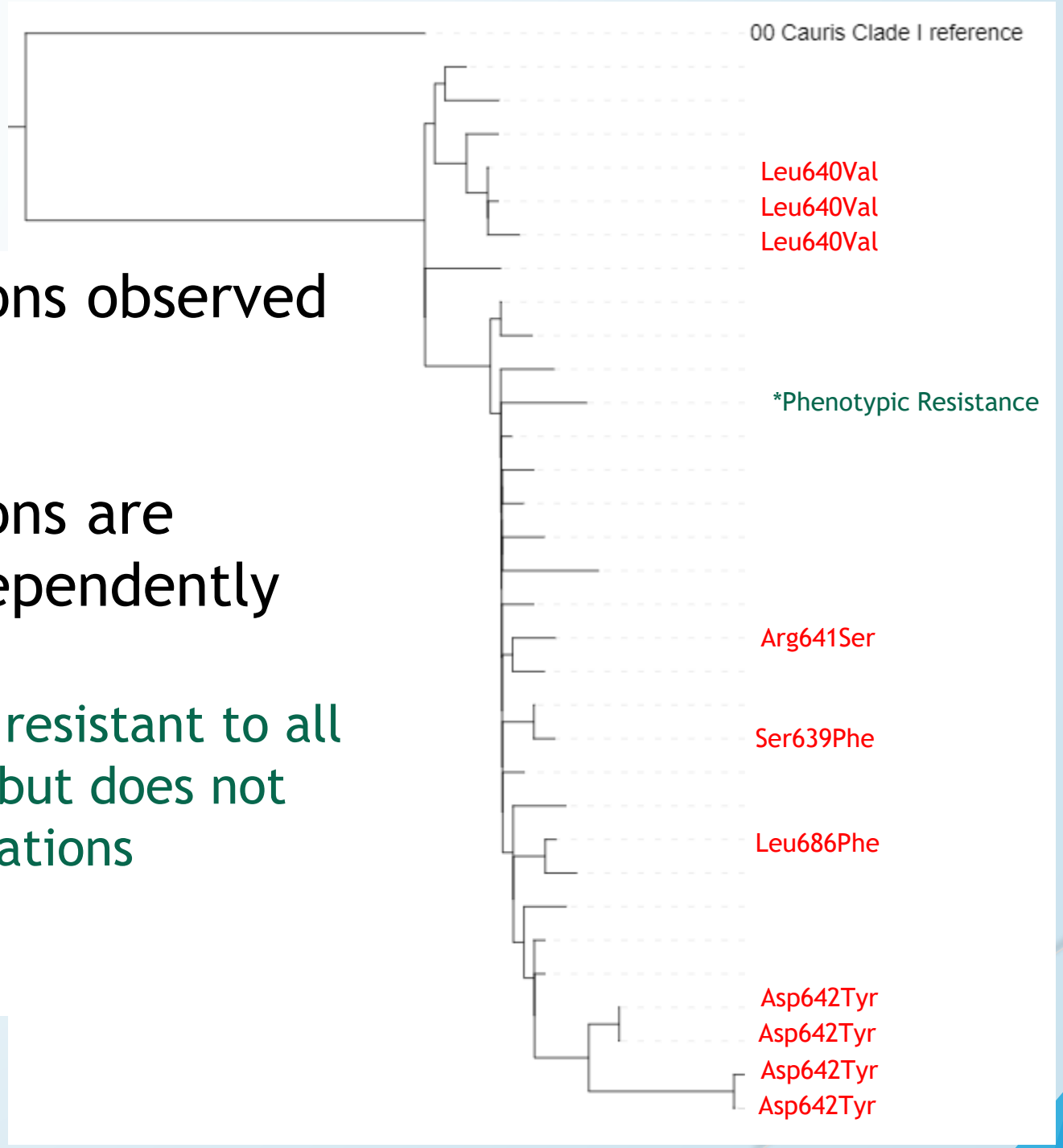
FKS1 mutations are evolving independently

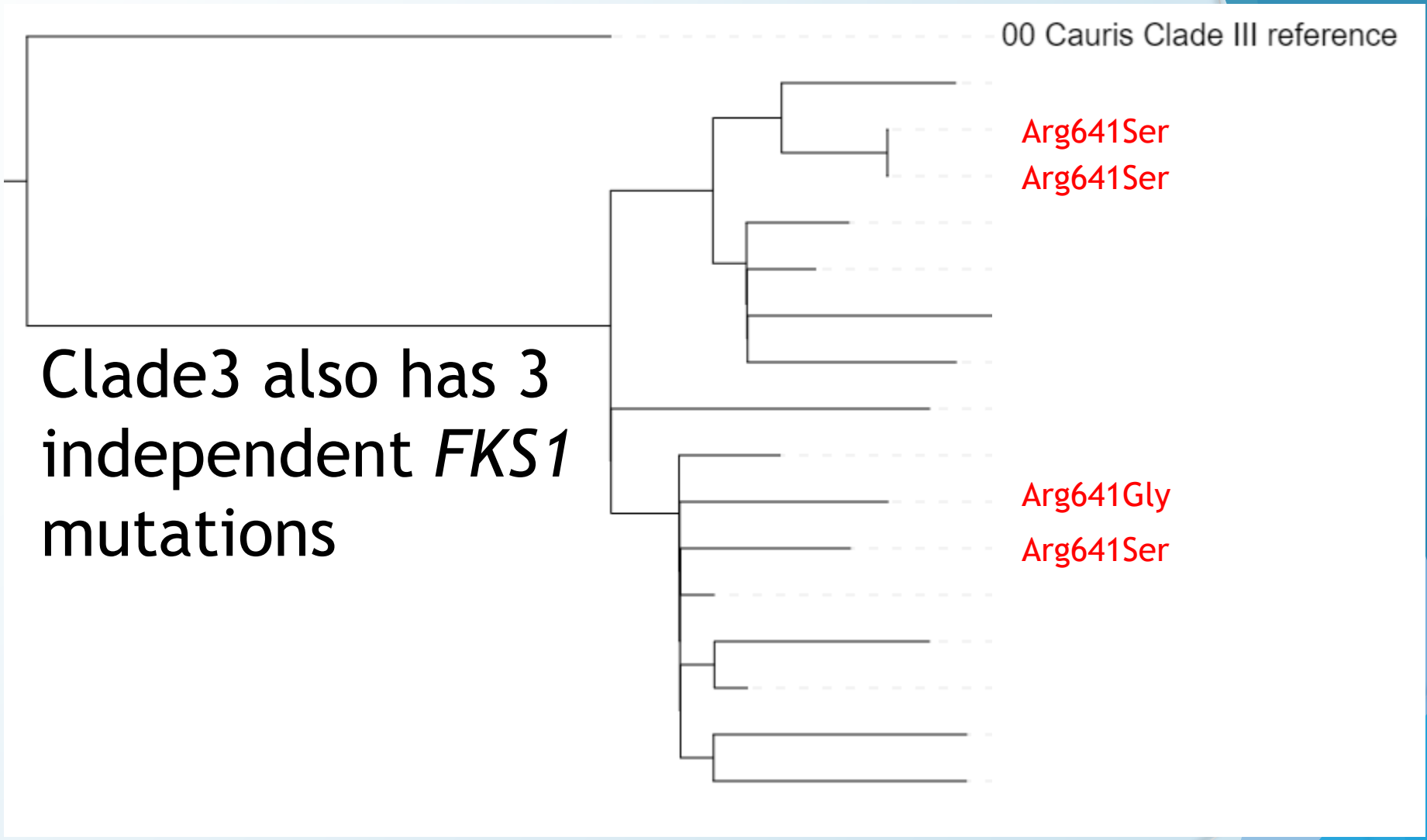


FKS1 mutations observed in Clade 1

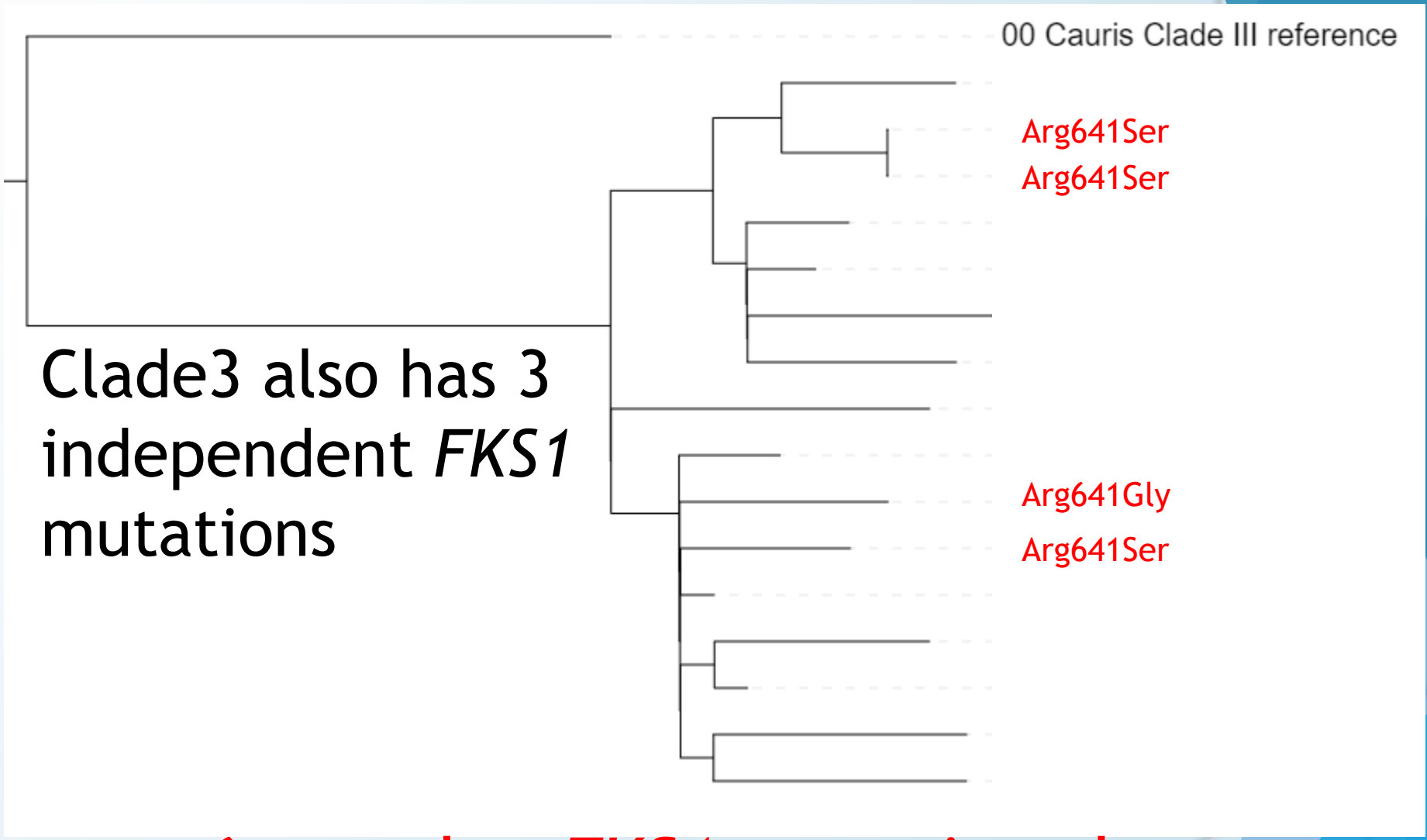
FKS1 mutations are evolving independently

*This isolate is resistant to all echinocandins but does not have FKS1 mutations



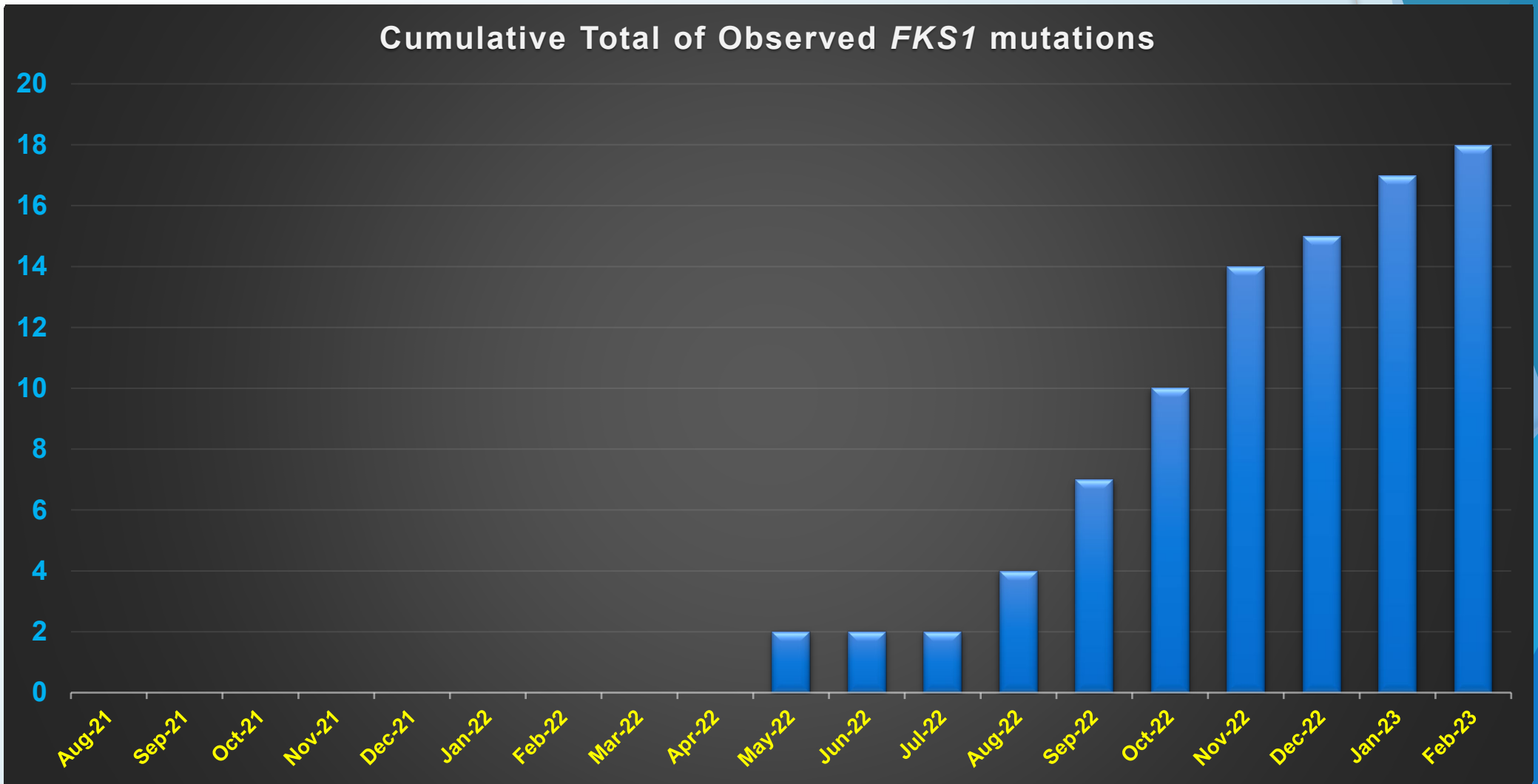


Clade3 also has 3 independent *FKS1* mutations



In the past 6 months, *FKS1* mutations have spontaneously occurred 8 times

Frequency of *FKS1* mutations is increasing



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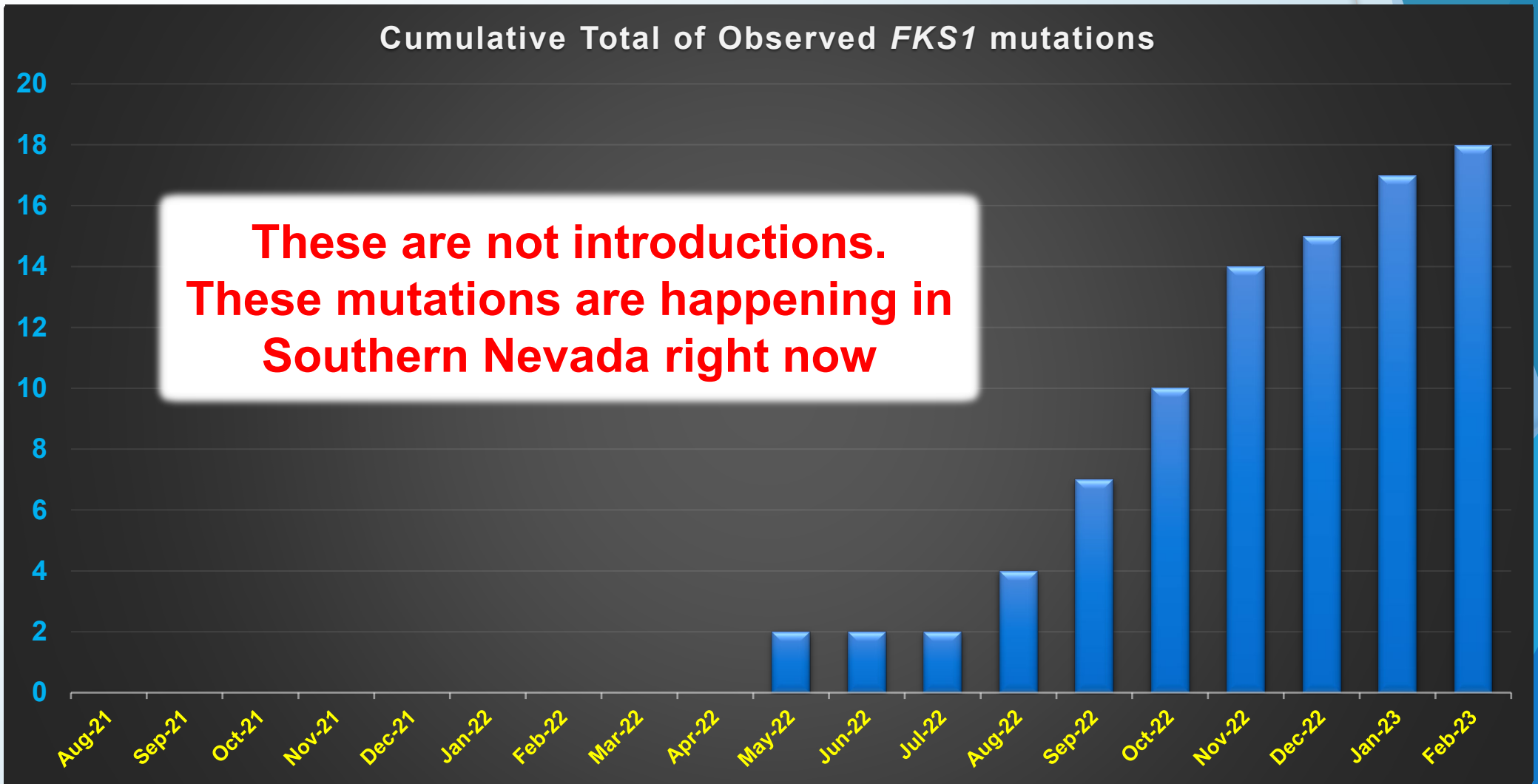

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Observing echinocandin resistance evolve in real-time

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What have we learned so far?

100 patients have died, and more are infected every day

Southern Nevada has multiple ongoing outbreaks of *C. auris*

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- Developed wet-bench protocols and open-source bioinformatic tools
- Monitor *FKS1* mutations in real-time and potentially identify novel mutations
- Create database for future targeted PCR screening assays

Acknowledgements

Team at NSPHL

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Mark Pandori

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School of Medicine

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Chidinma Njoku
Jeanne Ruff

Theiagen

Joel Sevinsky
Frank Ambrosio
Michelle Scribner
Kevin Libuit

Washington State Department of HEALTH

Washington PHL

Philip Dykema
Emily Schneider
Cchi Hua

Questions?

- Contact NV PHL
  - Dr. Andrew Gorzalski: AndrewG@unr.edu
  - Dr. David Hess: Dhess@unr.edu
  - Dr. Mark Pandori: Mpandori@unr.edu

FKS1 mutations observed in Nevada isolates with susceptibility data

clade_type	Variants Detected	Anidulafungin	Caspofungin	Micafungin
Clade1	FKS1: Leu640Val	0.5	0.25	
Clade1	FKS1: Arg641Ser	1	0.5	2
Clade1	FKS1: Asp642Tyr	2	2	
Clade1	FKS1: Asp642Tyr	2	0.5	
Clade1	FKS1: Asp642Tyr	2	0.5	1
Clade1	FKS1: Asp642Tyr	2	0.5	
Clade1	FKS1: Leu686Phe	1	1	0.25
Clade3	FKS1: Arg641Gly	4	1	2
Clade3	FKS1: Arg641Ser	0.5	0.25	
Clade3	FKS1: Arg641Ser	0.25	0.12	1

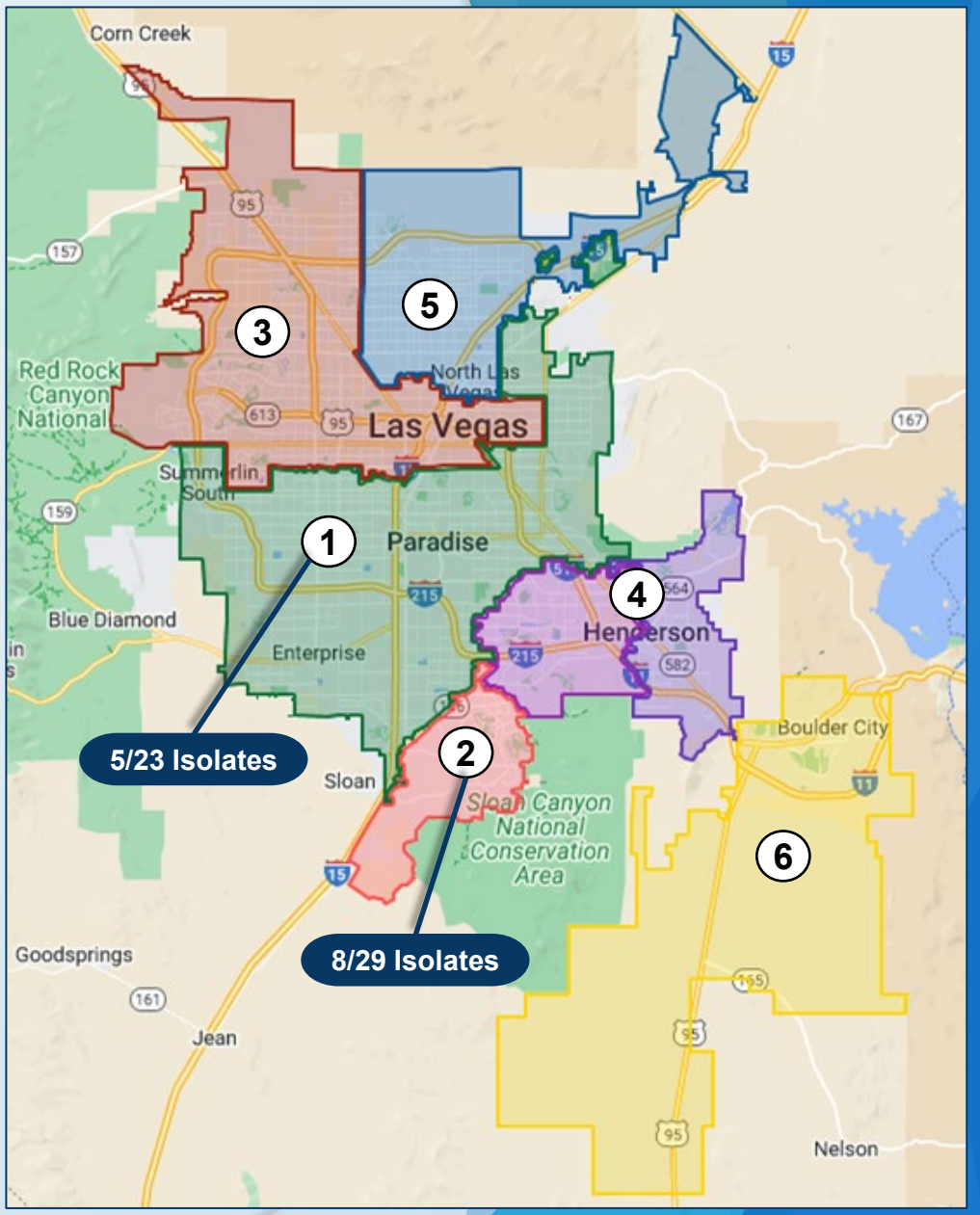
Predicted MIC thresholds for resistance:
Anidula = >/= 4
Caspo >/= 2
Mica >/= 4

- Most mutations in “Hot Spot” area

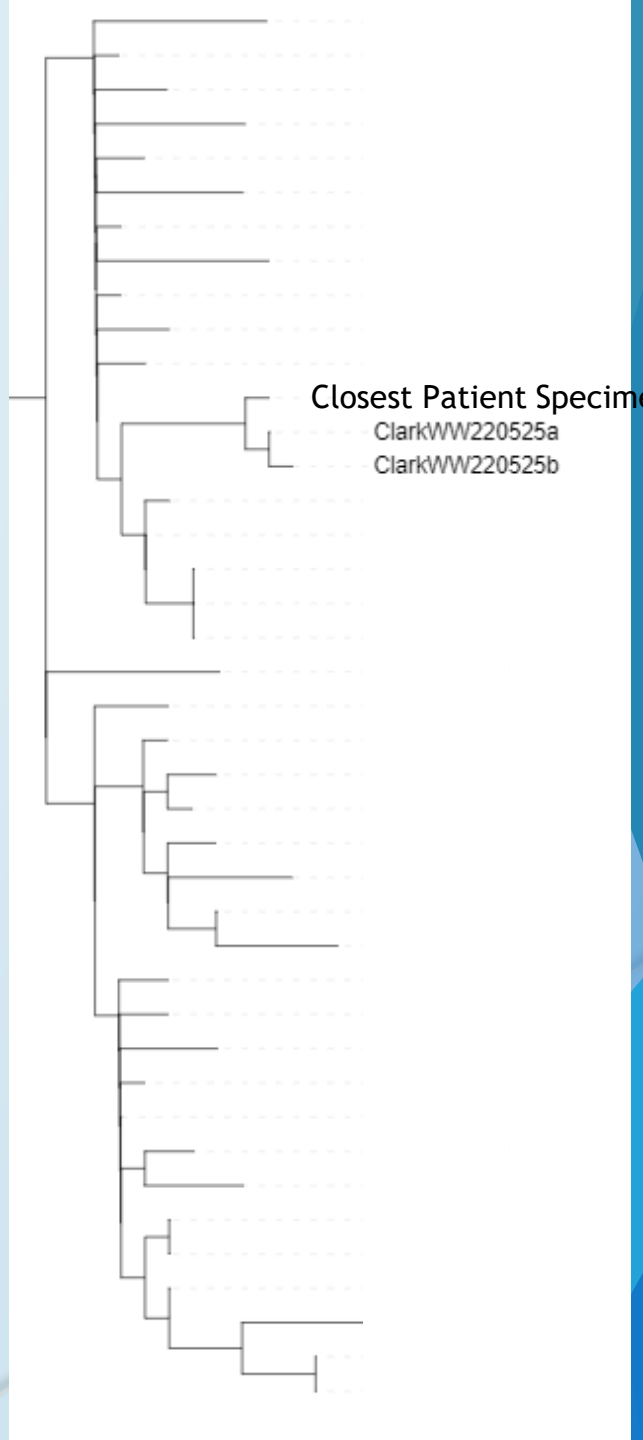
Isolation Source of isolates with FKS1 mutations
Axilla / Groin x3
Blood x3
Catheter x2
Urine x6

Most infectious diseases are spread from California instead of across the Nevada.

- 2 isolates collected May 25, 2022
  - Clade3
- 2 isolates collected August 29, 2022
  - Clade1



Clade 3 genetic diversity at one Las Vegas ACH which feeds Facility 1.



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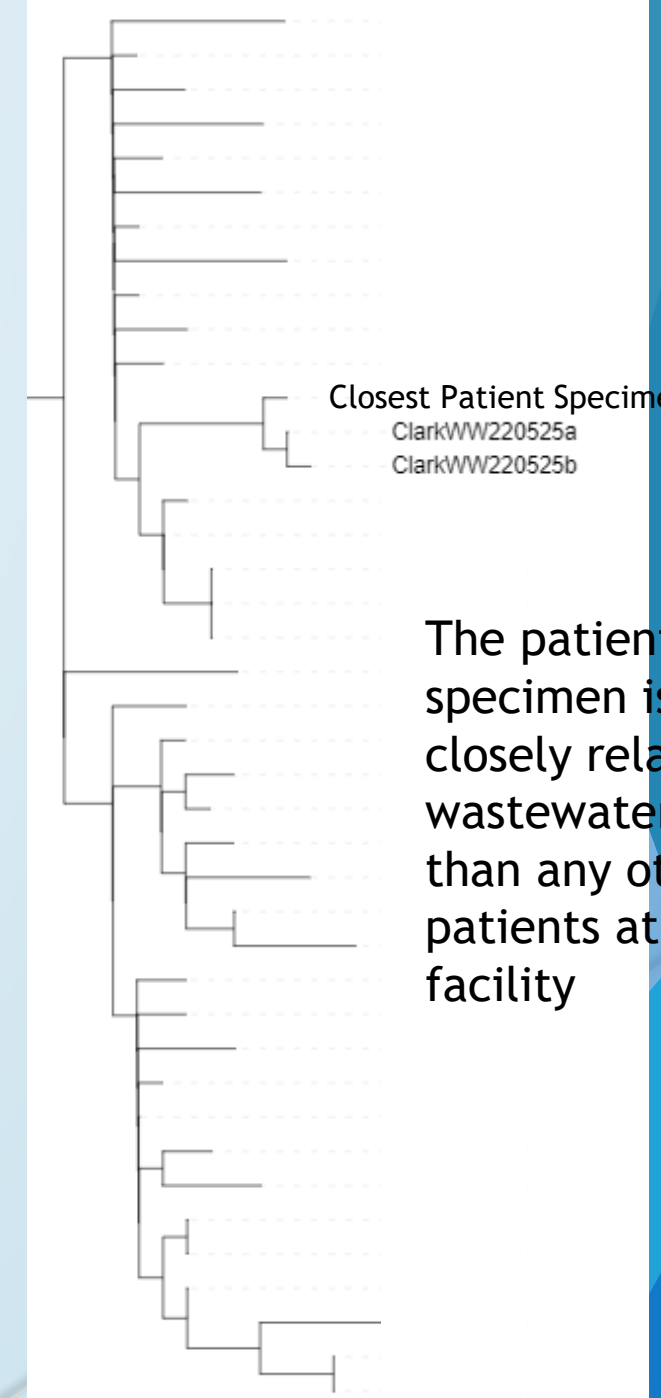
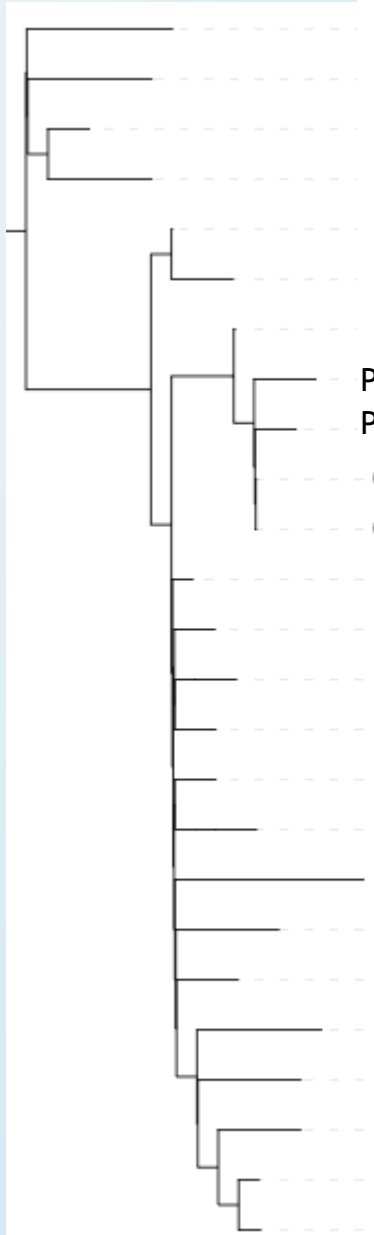


The patient specimen is more closely related to wastewater isolates than any other patients at the facility

Clade 1 genetic diversity in Clark County



Wastewater isolates are most closely related to *C. auris* isolated from patients of Henderson facilities.

Sequencing results summary

- Isolates obtained from wastewater are closely related to patient specimens.
  - Wastewater isolates show most genetic similarity to facilities in the corresponding sewer shed.
- Hypothesize that there is a substantial amount of organism shedding occurring from patients.
- Monitoring *C. auris* DNA in wastewater is an effective early warning sign in areas with little or no cases.
  - Utah was able to detect *C. auris* in wastewater shortly after a *C. auris* positive patient was transferred (St. George, UT)