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

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Outbreak of Deadly Infectious Fungi

Almost Impossible to Kill

Living in our Sewer Systems

Drug Resistant
Outbreak of Deadly Infectious Fungi

Almost Impossible to Kill

Drug Resistant

Living in our Sewer Systems
*Candida auris* is an emerging pathogen in the United States

- 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

Source: CDC.gov
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
**Candida auris** in Nevada

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- **No other cases or transmission observed**
Candida auris in Nevada

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

- 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

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  - Patients were screened at admission for *C. auris* colonization
    - Infected patients were placed in *C. auris* unit
  - The pandemic necessitated placing COVID-positive patients in *C. auris* unit
  - Weeks later, patients admitted for COVID were now colonized with *C. auris*.

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

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

Why?

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

Why?

- Increased Exposure
- Decreased Screening
COVID-19 pandemic allowed \textit{C. auris} to spread throughout the U.S.

Why?

- Increased Exposure
- Ineffective Decontamination
- Decreased Screening
COVID-19 pandemic allowed *C. auris* to spread throughout the U.S.

Why?

- Increased Exposure
- Decreased Screening
- Ineffective Decontamination
- People may be colonized with no symptoms
By 2022, over half of all U.S. states had identified cases of *Candida auris*. 

Source: CDC.gov
By 2022, over half of all U.S. states had identified cases of Candida auris.
First detected case in Southern Nevada occurred August 2021
Outbreak is still ongoing
Outbreak is still ongoing
Nevada identified 83 clinical cases in much less time than other states
March 2022 - State PHL notified of \textit{C. auris} cases in Southern Nevada

Unlike other infectious diseases, \textit{C. auris} was \textbf{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
C. auris PCR Screening Test and Culture

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- Once validated, State Epi recommended that all long-term care facilities perform screening on admits
  - Perform over 200 tests per week
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
- 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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- Chromosomes
- Introns
- More Nucleotides
- Mitochondrial DNA
- Sexual Reproduction?
- Diploid?
Eukaryotic genomics are much more complex than prokaryotic and virus

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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- C. auris is comprised of 5 genetically distinct clades
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- Clades were initially associated with geographic regions
C. **auris** genetics

- Genome around 12 Mb on 7 chromosomes
- **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
Clades became genetically distinct 5,000 to 25,000 years ago

C. auris genetics

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

All Clades are capable of infecting humans

Native environment still unknown

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

- NV PHL had already acquired a NovaSeq 6000 and validated automated library preparation
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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.
By March 2022, both Clades were present in multiple facilities

Clade 1

Clade 3

Clade 4
September 2022: Clade 1 and Clade 3 found inter-dispersed among Southern Nevada Facilities. Community spread as well as facility transmission.
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Struggling with resolution to show connections between cases/isolates
Bioinformatics of *C. auris*: Problems and Solutions

- Initially, we used bacterial workflows for assembly and Clade determination.
  - Not ideal
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- 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.
Prokaryotic genetic relatedness is generally determined by SNP difference
- Fewer differences = closely related
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However, this a Eukaryotic organism and the same method might not work
Shared SNPs

- Sequencing gaps and errors become more frequent
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- Isolates with Shared SNPs represent groups of related *C. auris* specimens
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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"

~Rest of the world
42 Shared SNPs unique to NV outbreak

~Rest of the world

Clade 3

Nevada
How does this work inside an outbreak?

Core of 54
Shared SNPs

Clade 1
Using Shared SNPs to increase resolution in an outbreak
Using Shared SNPs to increase resolution in an outbreak

Group 2

Group 1

Group 3

Clade 1

Three isolates from same patient

CDC Reference
Using Shared SNPs to increase resolution in an outbreak

Clade 1

Group 2

Newborns in CICU

Group 1

Group 3

CDC Reference
Clade 1 contains 37 Shared SNP clusters
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Contains 87 isolates
Clade 1 contains 37 Shared SNP clusters

Epi are notified of all shared SNP clusters that have been collected in the past 60 days.
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They look at facilities for new introductions.
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They look at facilities for new introductions

Look at facilities where Shared SNP clusters continue to grow
Able to identify when new *C. auris* genetics are introduced into Southern Nevada.
Two new clusters lacking the core Shared SNPs

Able to identify when new *C. auris* genetics are introduced into Southern Nevada
What are we learning from all this data?

1129 Total Cases of C. auris

1019 Sequenced
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1 Clade4

788 Clade3

230 Clade1
What are we learning from all this data?

Identified 4 genetically distinct outbreaks
Three Clade 1
One Clade 3
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What are we learning from all this data?

Aid Epi investigations

1129 Total Cases of *C. auris*

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1 Clade4

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230 Clade1
What can we learn about drug resistance?
Three classes of anti-fungal drugs in use

- Azoles
- Echinocandins
- Polyenes (Amphotericin B)
Three classes of anti-fungal drugs in use

~80% *C. auris* are naturally resistant

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Three classes of anti-fungal drugs in use

- **Azoles**: ~30% of *C. auris* are resistant.
  - *C. auris* are naturally resistant.
  - Drug can be difficult on patients.

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- **Polyenes (Amphotericin B)**: ~30% of *C. auris* are resistant.
Three classes of anti-fungal drugs in use

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

- **Polyenes (Amphotericin B)**: ~30% of *C. auris* are resistant. Drug can be difficult on patients.

- **Azoles**: ~80% *C. auris* are naturally resistant.
Pathogens become drug resistant over time

Predicted MIC thresholds for resistance:
- Anidula $>= 4$
- Caspo $>= 2$
- Mica $>= 4$
In *C. auris*, mutations of the gene *FKS1* has been correlated to echinocandin resistance

- Involved in cell wall synthesis and maintenance
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- Numerous publications detail specific mutations
  - Mutational “Hot Spot” around a.a. 635-645 where majority of mutations are identified with echinocandin resistance
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- Involved in cell wall synthesis and maintenance
- Numerous publications detail specific mutations
  - 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

![Cumulative Total of Observed FKS1 mutations](image.png)
Frequency of *FKS1* mutations is increasing

These are not introductions. These mutations are happening in Southern Nevada right now.
What have we learned so far?
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Southern Nevada has multiple ongoing outbreaks of *C. auris*
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New genetics are still being introduced, and NV’s Core SNPs have been identified in neighboring states.
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Observing echinocandin resistance evolve in real-time.
What have we learned so far?

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

100 patients have died, and more are infected every day

New genetics are still being introduced, and NV’s Core SNPs have been identified in neighboring states

Observing echinocandin resistance evolve in real-time
How is all the sequencing helping?

- A *C. auris* outbreak of this scale has never happened nor been sequenced
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- Developed wet-bench protocols and open-source bioinformatic tools
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- Monitor *FKS1* mutations in real-time and potentially identify novel mutations
How is all the sequencing helping?

- A *C. auris* outbreak of this scale has never happened nor been sequenced

- 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

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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

Most mutations in “Hot Spot” area

<table>
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<tr>
<th>clade_type</th>
<th>Variants Detected</th>
<th>Anidulafungin</th>
<th>Caspofungin</th>
<th>Micafungin</th>
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<td>FKS1: Leu640Val</td>
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Predicted MIC thresholds for resistance:
Anidula $>= 4$
Caso $>= 2$
Mica $>= 4$

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

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)