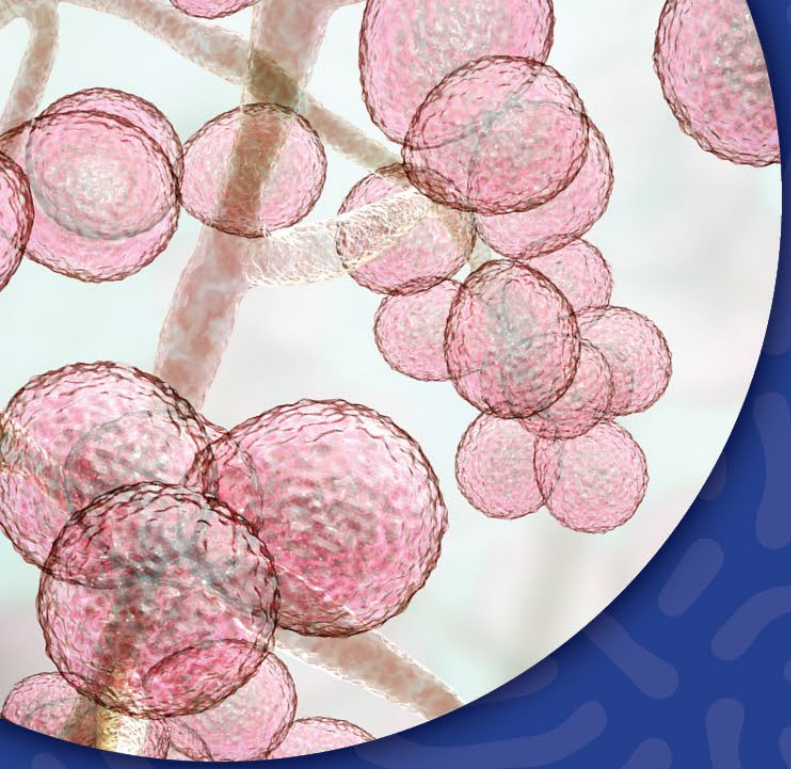
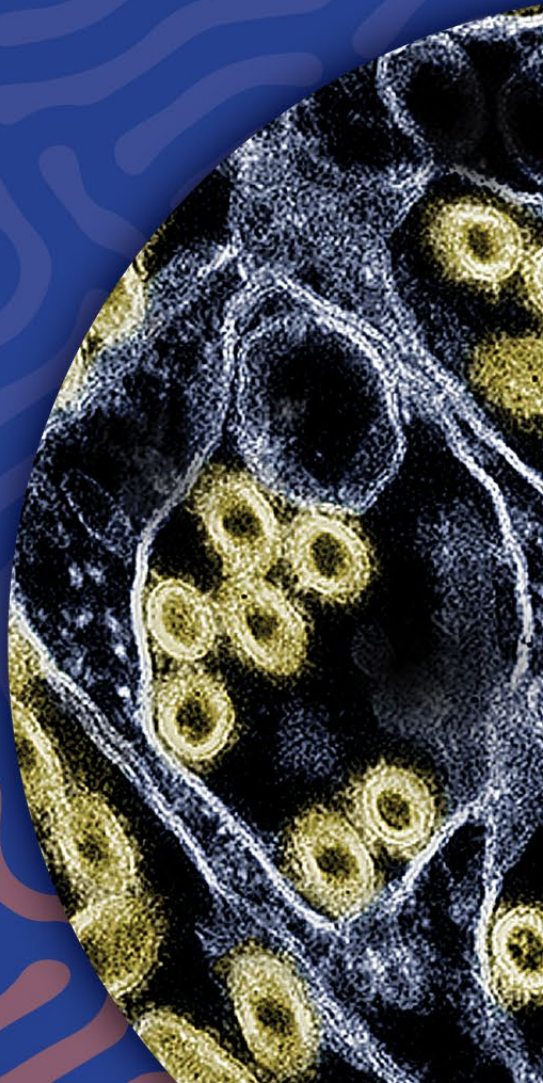


ID LAB CON

APHL

2025

#IDLabCon



The Use of WGS Surveillance of *C. auris* Reveals the Evolution of Novel Amphotericin B Non-susceptibility and a Potential Pan-resistant Isolate

#IDLabCon

Dr. David Hess
Chief Genomics Officer
NV State Public Health Lab

Today's Agenda

Part I: *Candida* background & shape of the outbreak in Southern Nevada

Part II: Vignettes on Antifungal resistant

a. Amphotericin B resistance

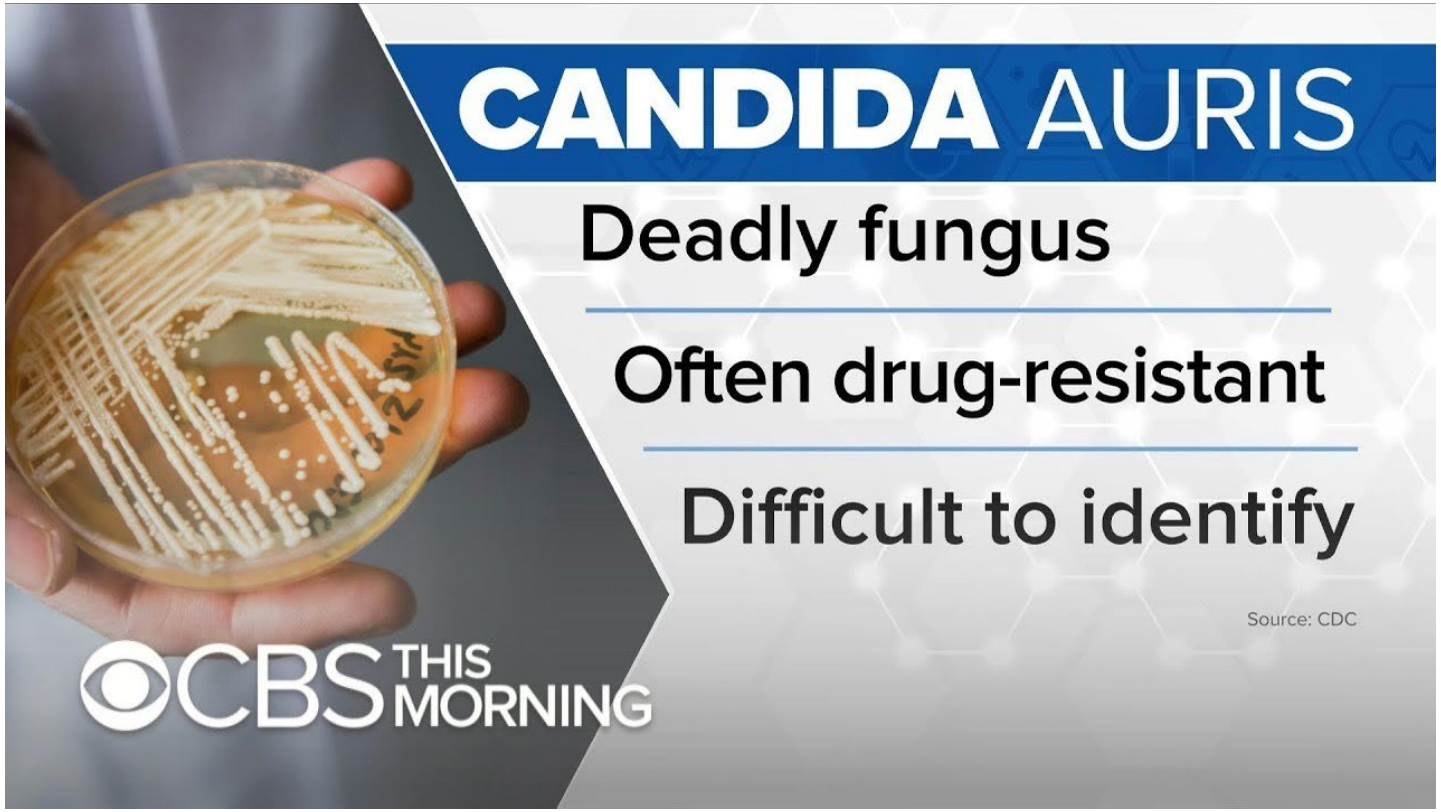
b. Pan resistance case

Summary and Questions

Candida auris is an emerging pathogen in the United States

C. auris traits

- Resistant to several drugs
- Can be deadly
- Environmentally stable
- Often associated with healthcare facilities
- Outbreaks have been reported in the majority of states in the US

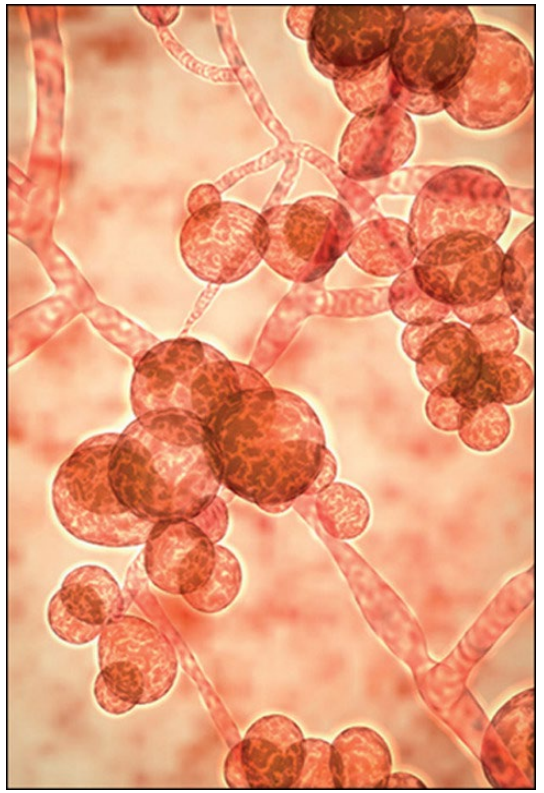


C. auris outbreak in Southern Nevada

- Outbreak began in August 2021 and is ongoing
- Cases seen almost exclusively in Southern Nevada
- CDC determined this outbreak was the fastest to 100 cases in the US
- Over 5000 cases have been isolated and sequenced
- Isolates recovered from over 20 facilities including:

  Acute Care Hospitals (ACHs), Skilled Nursing Facilities (SNFs),

  Long Term Acute Care (LTACs)
- At least 100 associated patient deaths



Increasing Threat of Spread of Antimicrobial-resistant Fungus in Healthcare Facilities

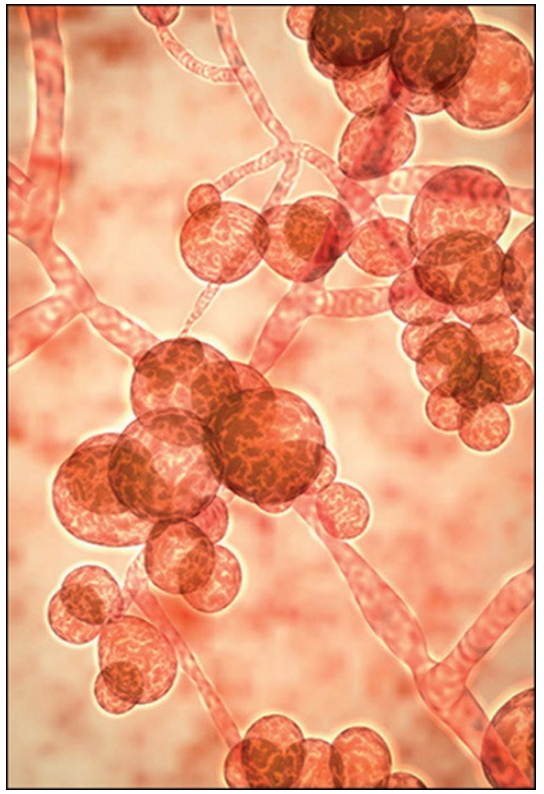
Press Release

For Immediate Release: Monday, March 20, 2023
Contact: Media Relations
(404) 639-3286

Candida auris (C. auris), an emerging fungus considered an urgent antimicrobial resistance (AR) threat, spread at an alarming rate in U.S. healthcare facilities in 2020-2021, according to data from the Centers for Disease Control and Prevention (CDC) published in the Annals of Internal Medicine. Equally concerning was a tripling in 2021 of the number of cases that were resistant to echinocandins, the antifungal medicine most recommended for treatment of *C. auris* infections. In general, *C. auris* is not a threat to healthy people. People who are very sick, have invasive medical devices, or have long or frequent stays in healthcare facilities are at increased risk for acquiring *C. auris*. CDC has deemed *C. auris* as an urgent AR threat, because it is often resistant to multiple antifungal drugs, spreads easily in healthcare facilities, and can cause severe infections with high death rates.

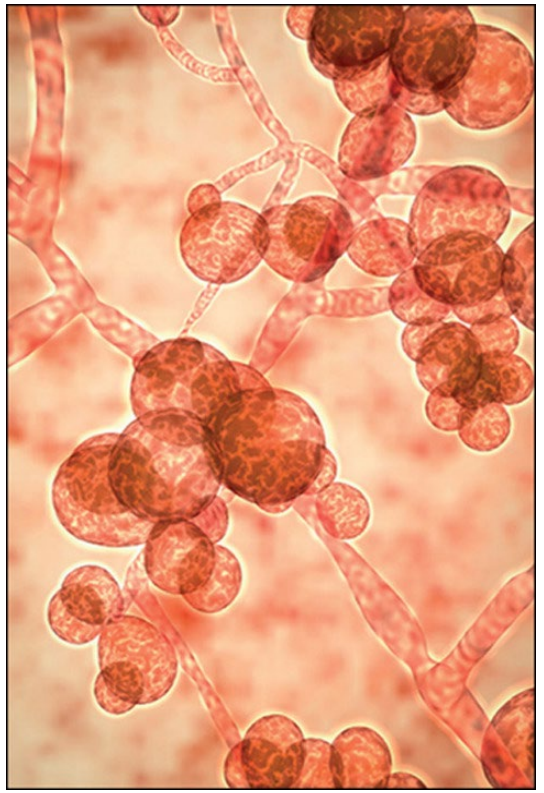
Whole genome sequencing is superior for outbreak tracking of *C. auris*

- Detailed genomic epidemiology not possible with traditional isolates
- Detailed understanding of the emergence of anti-fungal resistance



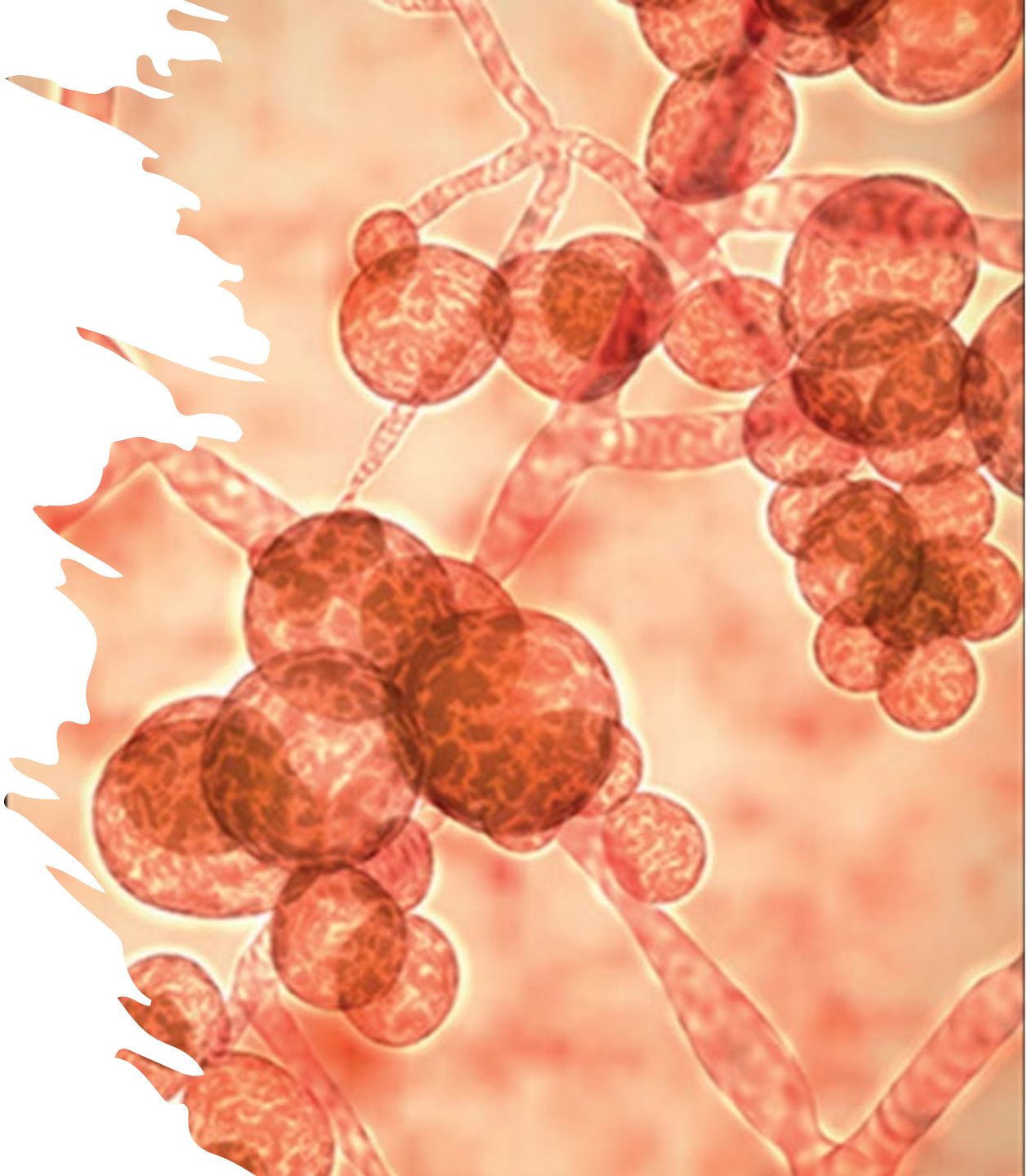
Whole genome sequencing is superior for outbreak tracking of *C. auris*

- Detailed genomic epidemiology not possible with traditional isolates
- **Detailed understanding of the emergence of anti-fungal resistance**



Focus on emerging drug resistance during the outbreak

- Three drug classes
  - Azoles
  - Polyenes
  - Echinocandins

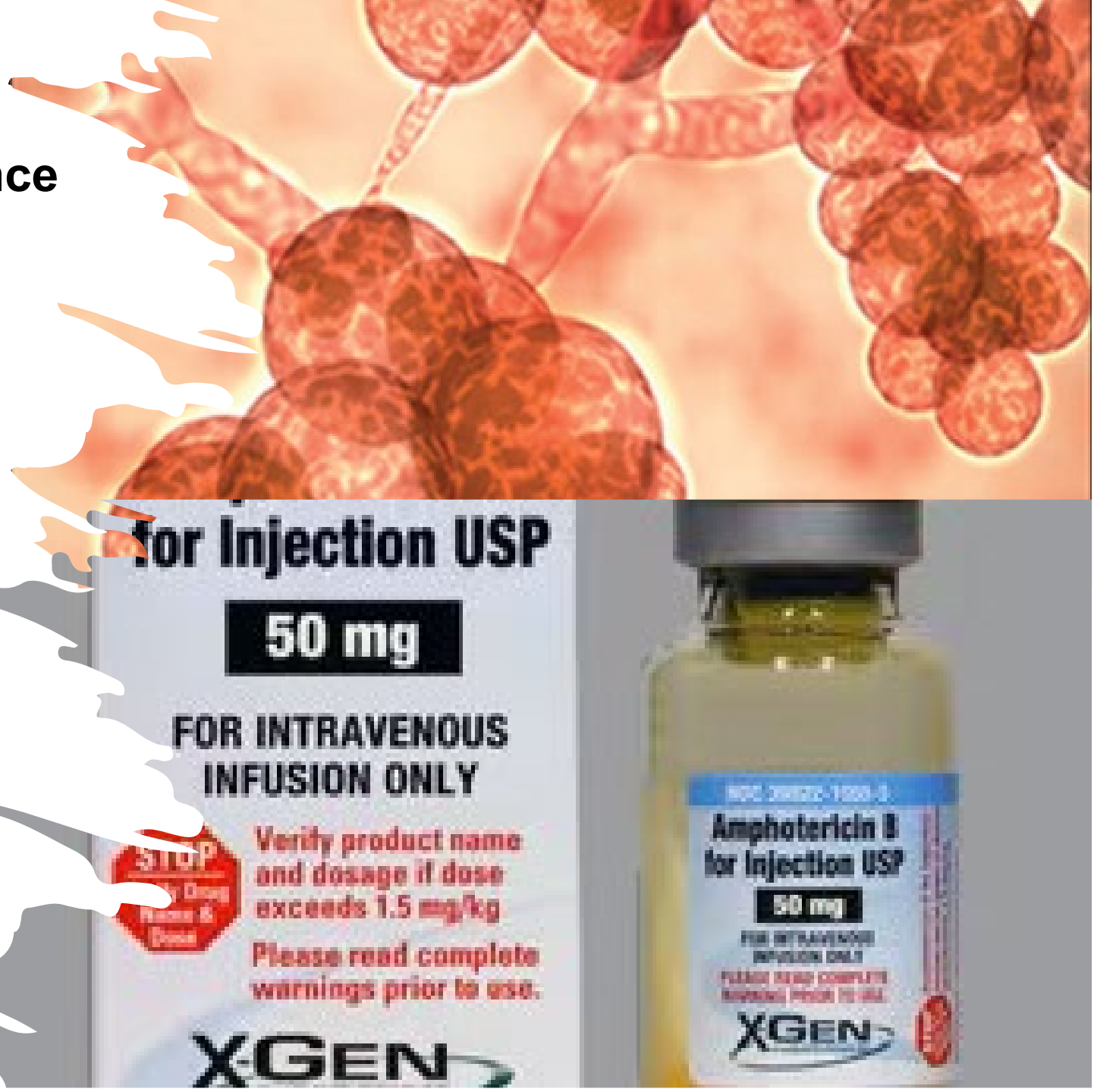


Focus on emerging drug resistance during the outbreak

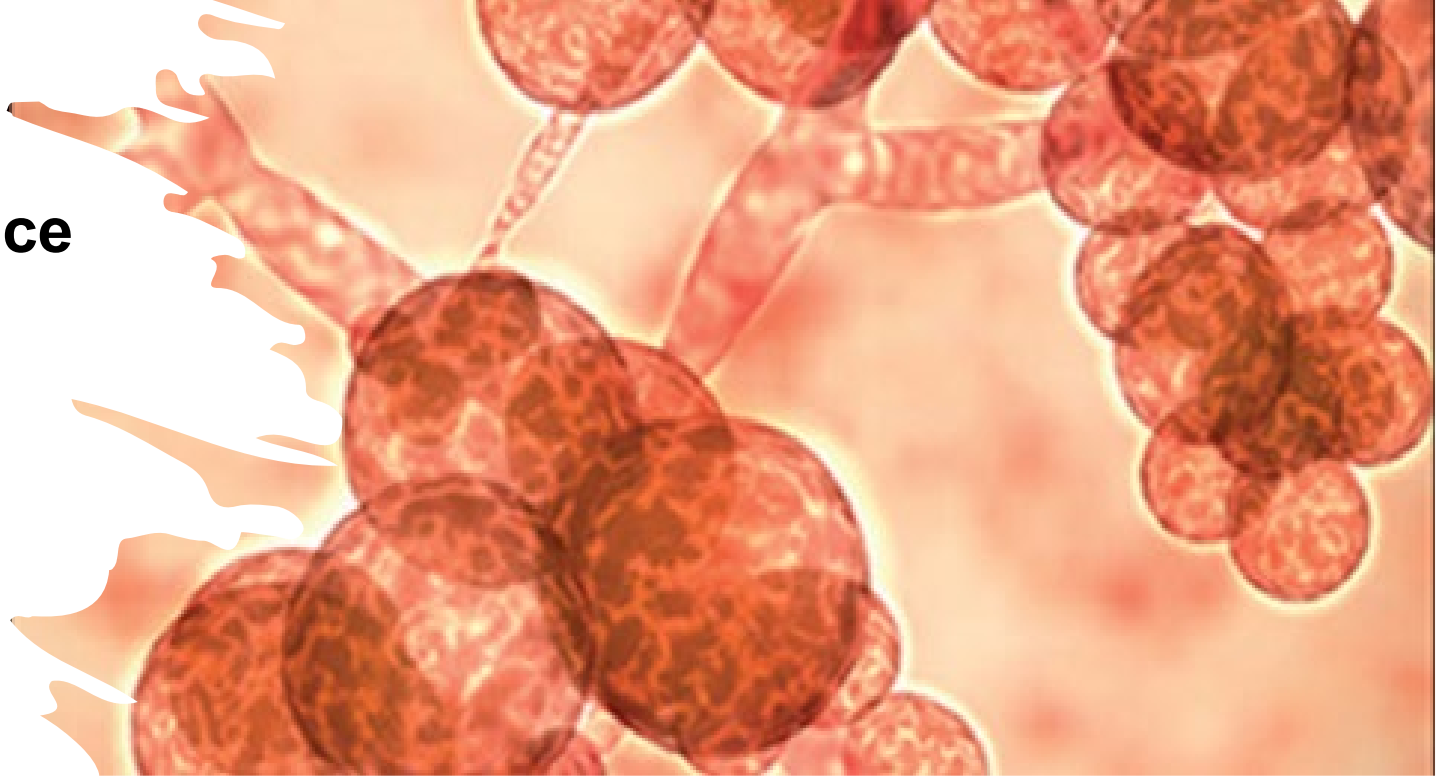
- Three drug classes
  - ~~Azoles~~
    - 80% of *C. auris* isolates are resistant to fluconazole
  - Polyenes
  - Echinocandins

Focus on emerging drug resistance during the outbreak

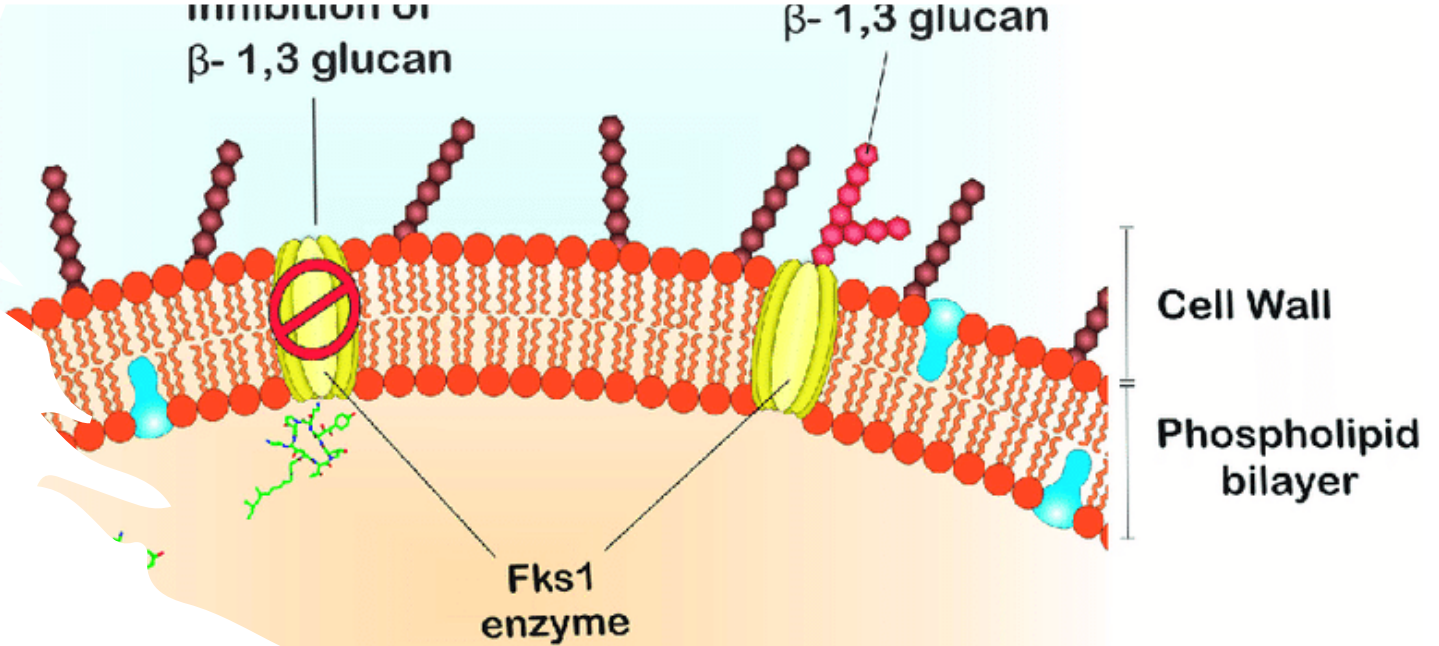
- Three drug classes
  - ~~Azoles~~
    - 80% of *C. auris* isolates are resistant to fluconazole
  - Polyenes
    - 30% of *C. auris* isolates are intrinsically non-susceptible to Amphotericin B
  - Echinocandins



Focus on emerging drug resistance during the outbreak



- Three drug classes
  - ~~Azoles~~
    - 80% of *C. auris* isolates are resistant to fluconazole
      - Mainly clades I, III, & IV
  - Polyenes
    - 30% of *C. auris* isolates are intrinsically non-susceptible to Amphotericin B
  - Echinocandins
    - 2-3% of *C. auris* isolates are non-susceptible to echinocandins
    - Mainly due to an amino acid change in the Fks1 protein subunit of the β-1,3-glucan-synthase
      - Hotspot 1 – Amino acids 639-645

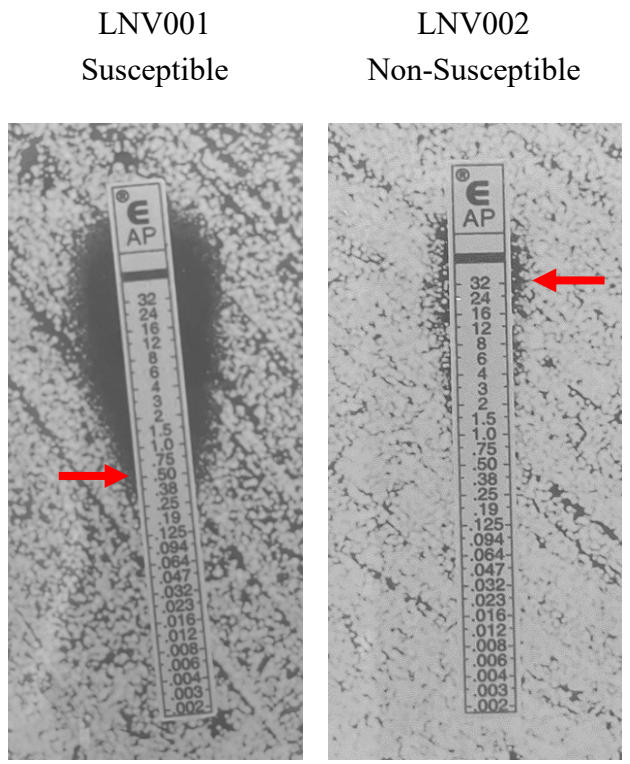


Vignette 1: Amphotericin B resistance

- Isolated in 1955 with medical use starting in 1958
- Grandfathered into medical use with understanding the mechanism of action
- Mechanism is believed to involve interaction with the membrane component ergosterol

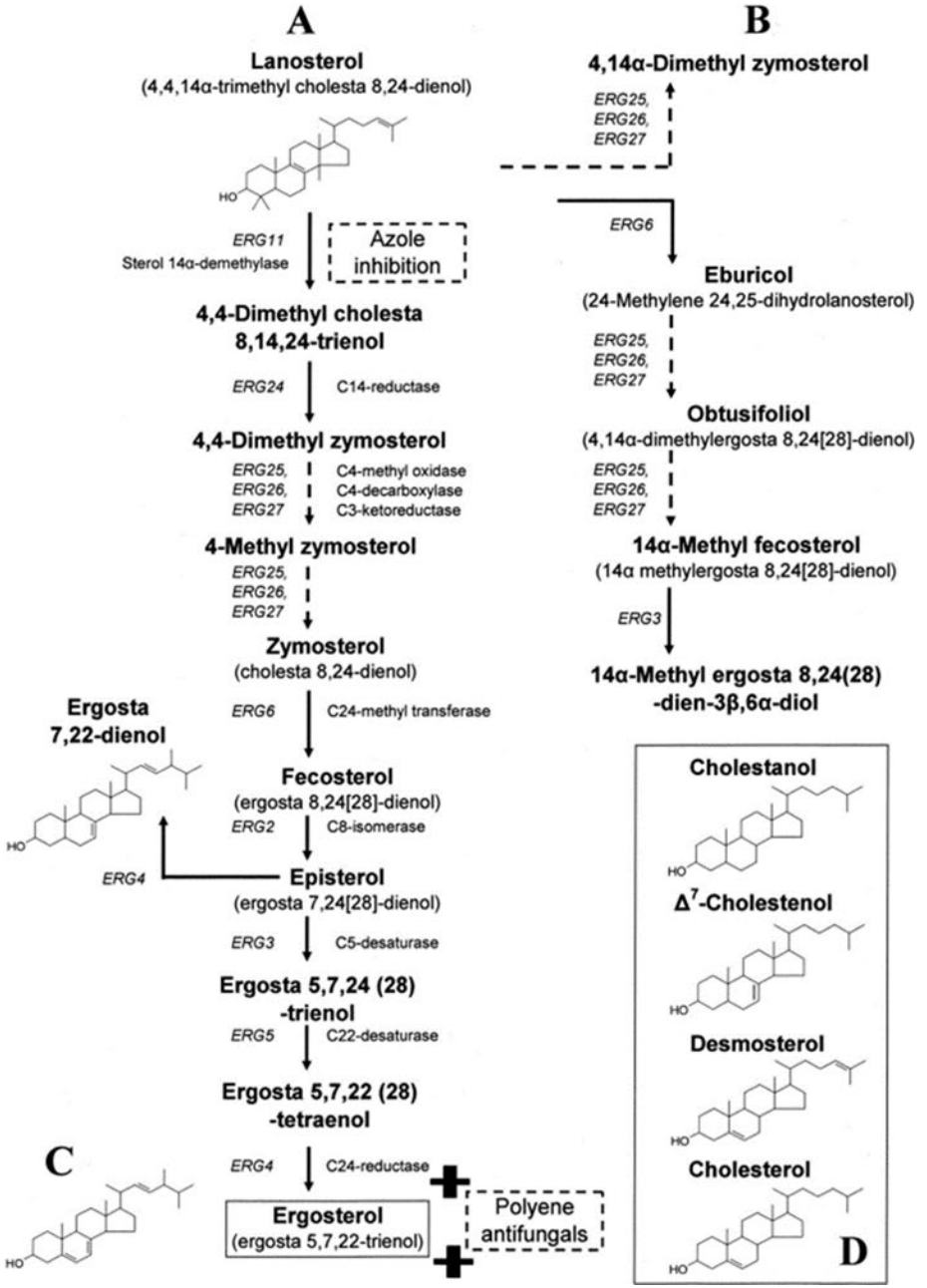
AmpB resistance isolate contains *ERG3* and *ERG4* mutations

Isolate	Collection Date	Isolation Site	AmpB MIC (μg/ml)	SNPs vs LNV001
LNV001	July 2022	Bronchial Lavage	0.5	0
LNV002	Sept 2022	Urine	> 32	9



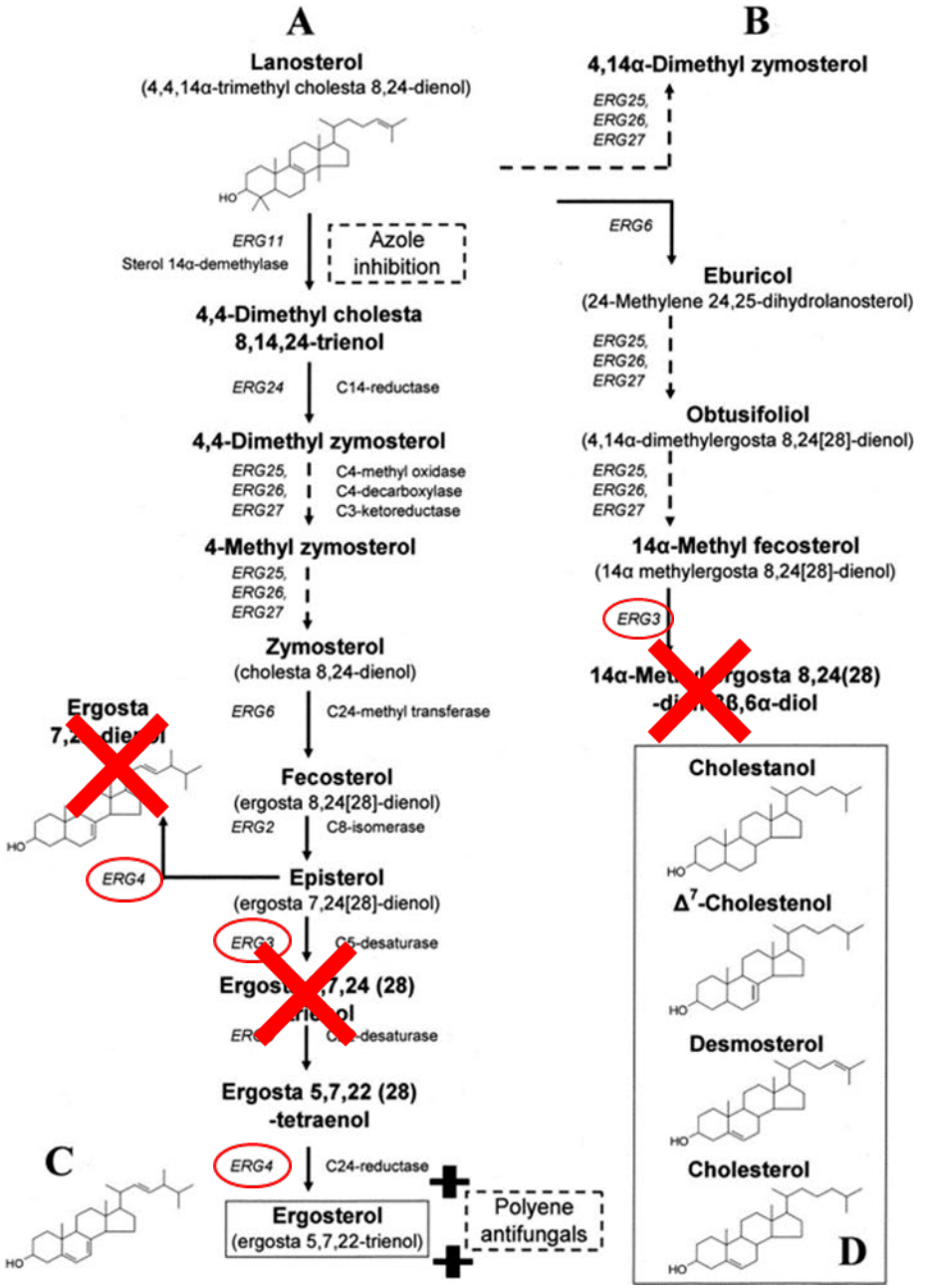
- 76-year old female
- 2 isolates taken over a two-month span
- The Sept 2022 isolate was highly resistant to Amphotericin B
- WGS revealed two candidate mutations in *ERG3* and *ERG4*

Ergosterol Biosynthesis Pathway



Hull et al., 2012

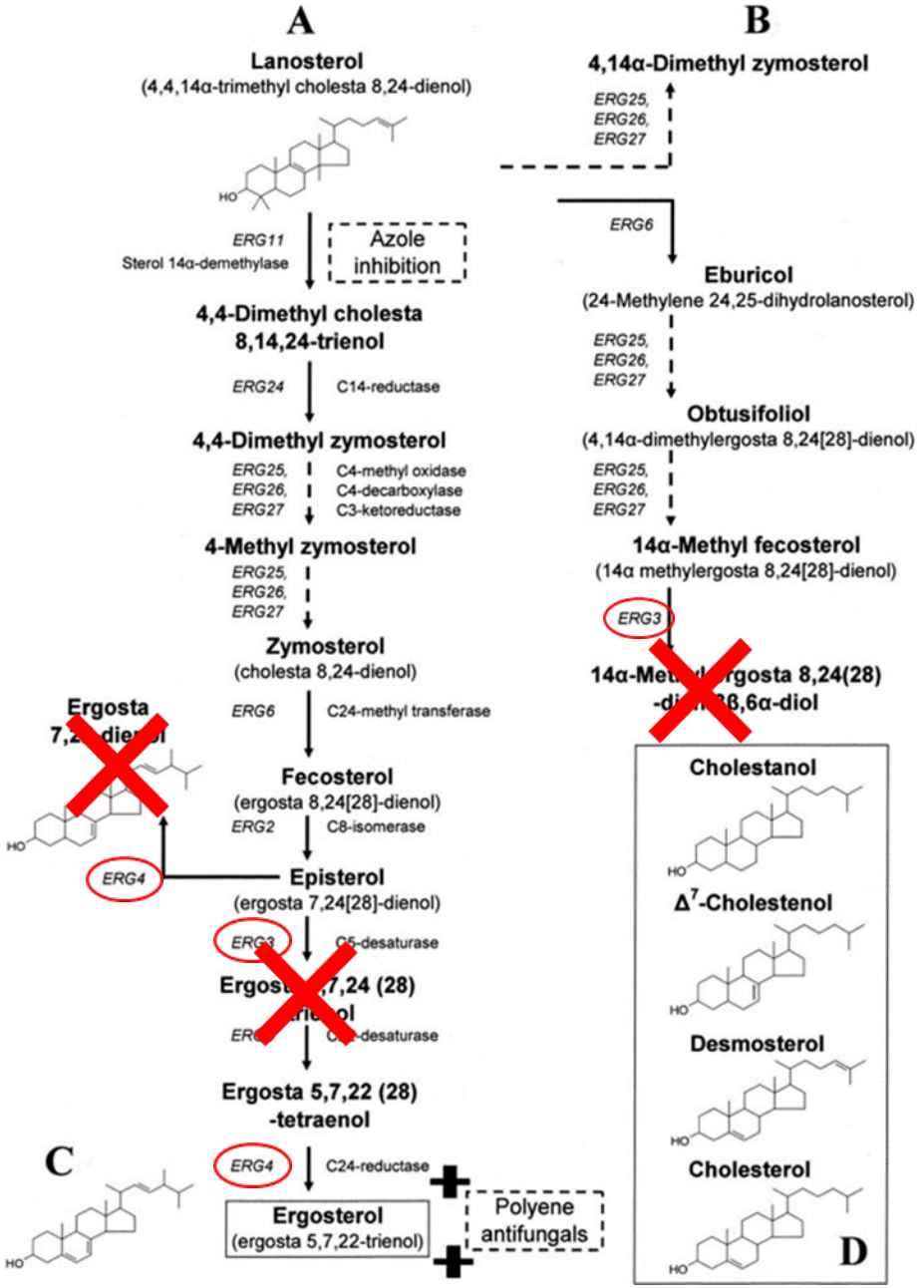
Ergosterol Biosynthesis Pathway



Hull et al., 2012

Ergosterol Biosynthesis Pathway

Does LNV002 produce ergosterol?



Hull et al., 2012

LNV002 does not produce ergosterol

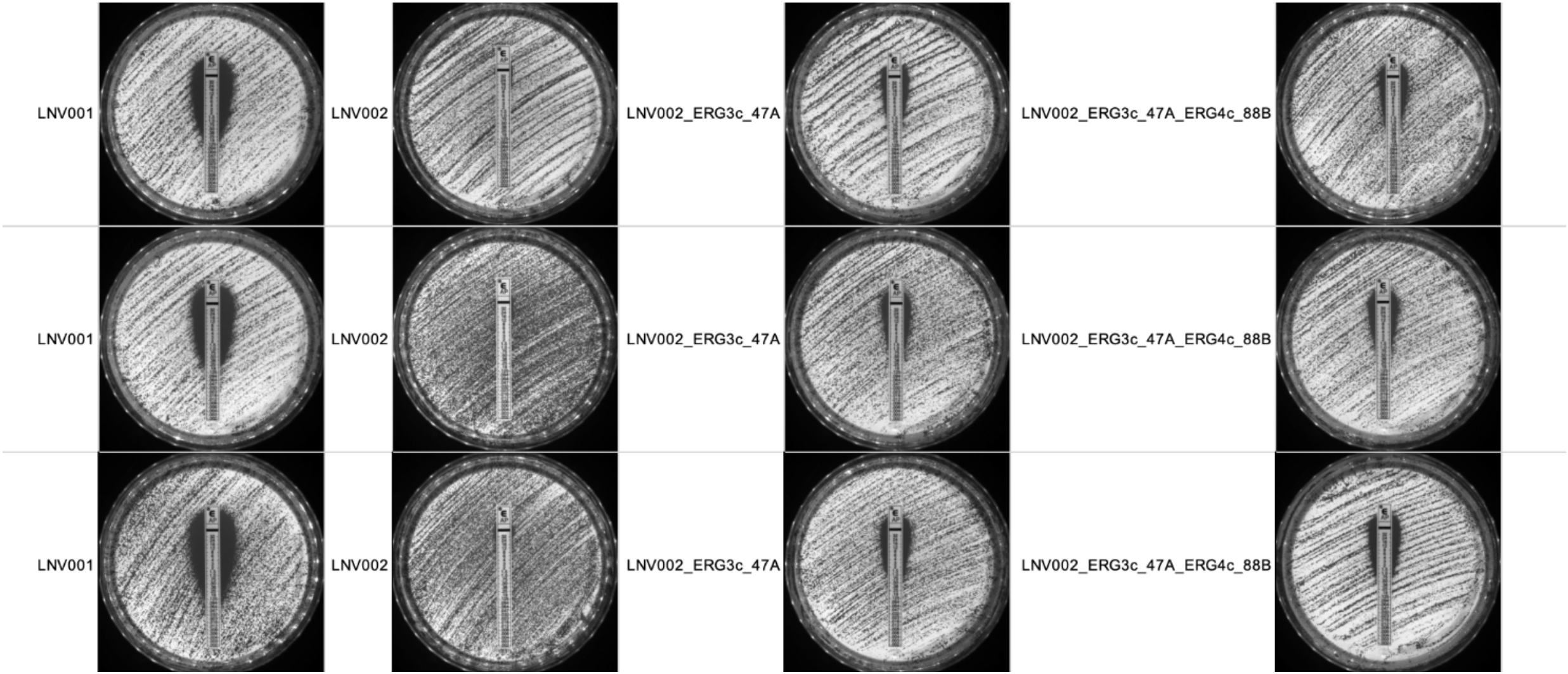
	Percentage of total sterol			
	LNV001 (S)		LNV002 (NS)	
Sterol	**mean**	**±st.dev.**	**mean**	**±st.dev.**
Ergosta-5,8,22,24(28)-tetraenol	0.3	0.1		
Ergosta-5,8,22-trienol	0.1	0.0		
Ergosta-8,22,24(28)-trienol			1.5	0.0
Zymosterol	0.3	0.0		
Ergosterol	**45.2**	**6.4**		
Ergosta 7,22 dienol	0.6	0.1		
unidentified sterol			1.1	0.1
4,14-dimethyl zymosterol	0.3	0.1	2.8	1.0
Fecosterol (Ergosta-8,24(28)-dienol)			0.4	0.3
Ergosta-7,22,24(28)-trienol			**53.7**	**8.6**
14-methyl fecosterol	2.0	0.5	3.2	1.0
Ergosta-5,7-dienol	5.7	7.6		
unidentified sterol			0.6	0.1
Episterol (Ergosta-7,24(28)-dienol)			**17.3**	**4.8**
Ergosta-7-enol	0.3	0.2		
4,4 dimethyl-ergosta 8,14,24(28)-trienol	0.2	0.0	0.1	0.0
14-methyl ergosta-8,24(28)-dien-3-6-diol	0.2	0.0		
Lanosterol	**39.7**	**1.3**	**17.3**	**2.1**
4-methyl ergosta-8,24(28)-dienol	0.7	0.5		
4,4-dimethyl cholesta-8,24-dienol	1.4	0.7	0.8	0.2
4,4-dimethyl cholesta-8,14,24-dienol			0.3	0.1
Eburicol	3.0	0.5	0.9	0.3
Total	**100.0**		**100.0**	

LNV002 does not produce ergosterol

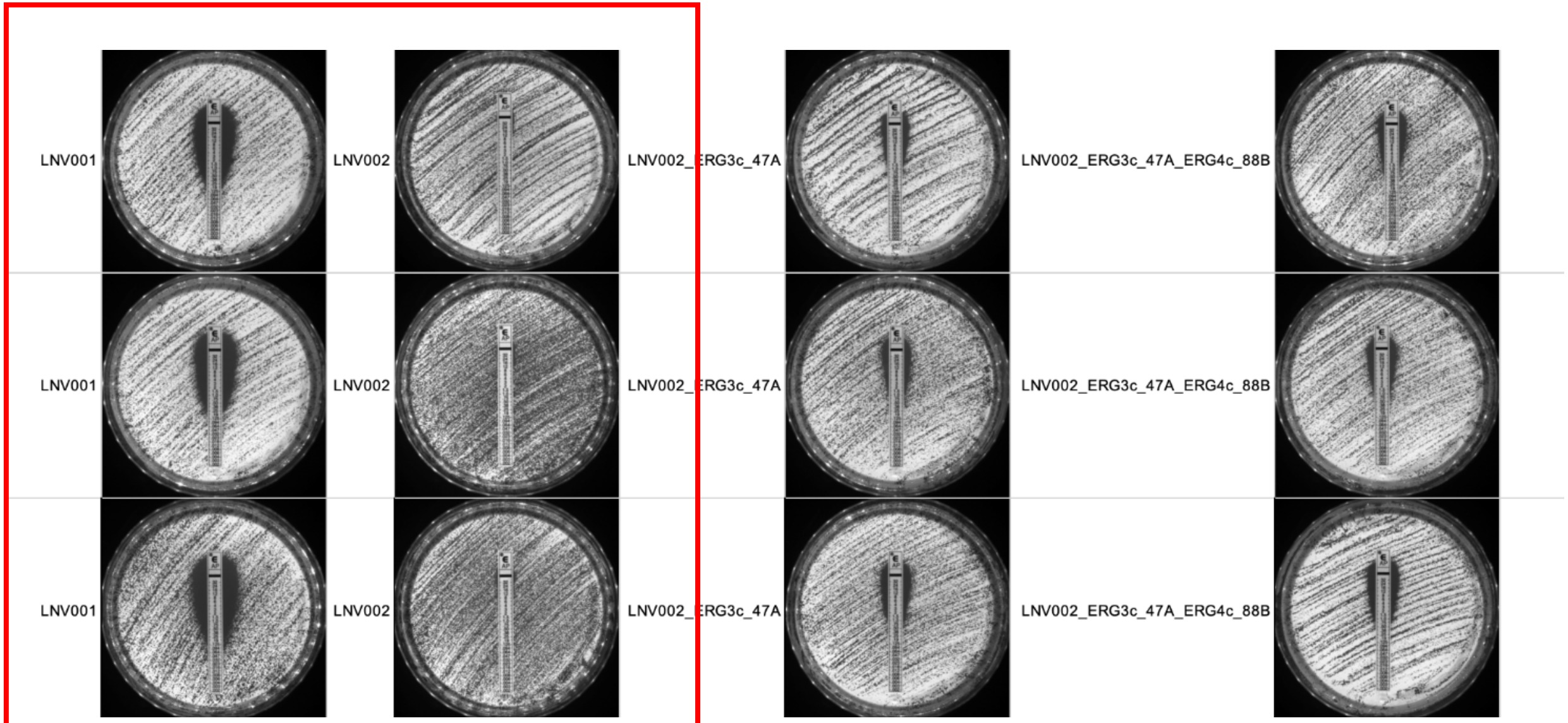
	Percentage of total sterol			
	LNV001 (S)		LNV002 (NS)	
Sterol	**mean**	**±st.dev.**	**mean**	**±st.dev.**
Ergosta-5,8,22,24(28)-tetraenol	0.3	0.1		
Ergosta-5,8,22-trienol	0.1	0.0		
Ergosta-8,22,24(28)-trienol			1.5	0.0
Zymosterol	0.3	0.0		
Ergosterol	**45.2**	**6.4**		
Ergosta 7,22 dienol	0.6	0.1		
unidentified sterol			1.1	0.1
4,14-dimethyl zymosterol	0.3	0.1	2.8	1.0
Fecosterol (Ergosta-8,24(28)-dienol)			0.4	0.3
Ergosta-7,22,24(28)-trienol			**53.7**	**8.6**
14-methyl fecosterol	2.0	0.5	3.2	1.0
Ergosta-5,7-dienol	5.7	7.6		
unidentified sterol			0.6	0.1
Episterol (Ergosta-7,24(28)-dienol)			**17.3**	**4.8**
Ergosta-7-enol	0.3	0.2		
4,4 dimethyl-ergosta 8,14,24(28)-trienol	0.2	0.0	0.1	0.0
14-methyl ergosta-8,24(28)-dien-3-6-diol	0.2	0.0		
Lanosterol	**39.7**	**1.3**	**17.3**	**2.1**
4-methyl ergosta-8,24(28)-dienol	0.7	0.5		
4,4-dimethyl cholesta-8,24-dienol	1.4	0.7	0.8	0.2
4,4-dimethyl cholesta-8,14,24-dienol			0.3	0.1
Eburicol	3.0	0.5	0.9	0.3
Total	**100.0**		**100.0**	

Are *ERG3* and/or *ERG4* the causal mutations of AmpB resistance?

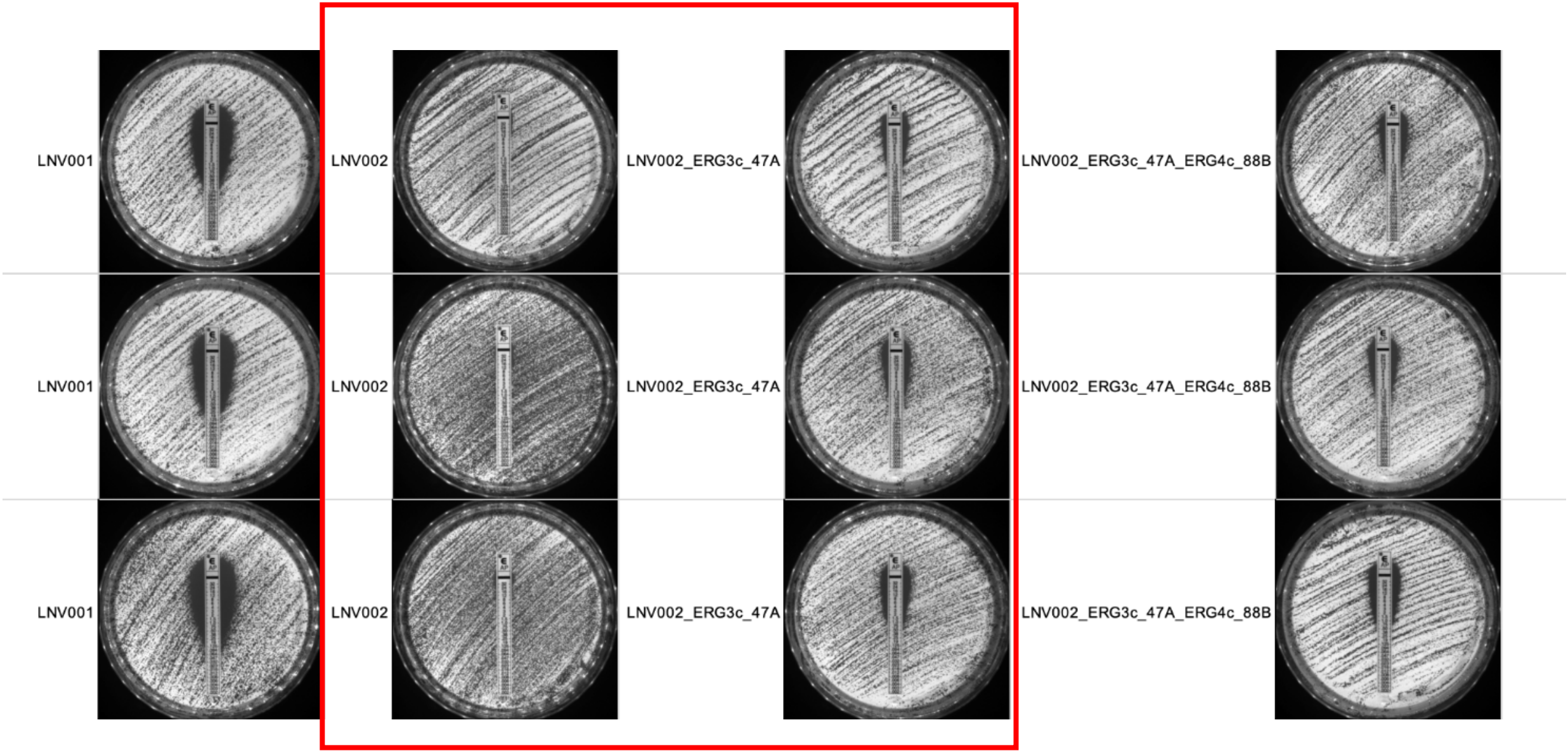
CRISPR correction of the *ERG3* mutation decreases AmpB resistance phenotype



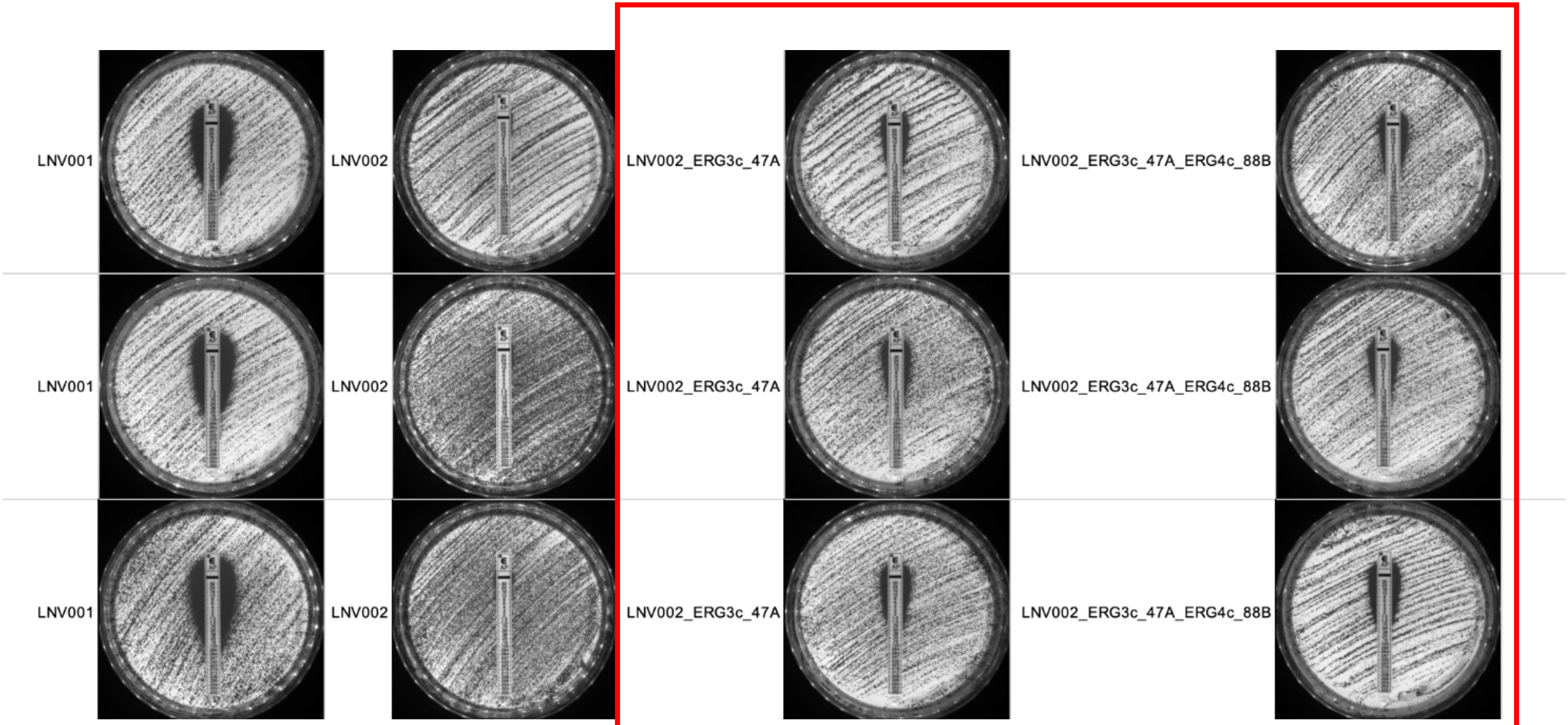
CRISPR correction of the *ERG3* mutation decreases AmpB resistance phenotype



CRISPR correction of the *ERG3* mutation decreases AmpB resistance phenotype



CRISPR correction of the *ERG3* mutation decreases AmpB resistance phenotype



Summary of AmpB resistance isolate

- Patient isolate displayed high-level of Amphotericin B resistance by e-test
- *ERG3* and *ERG4* mutations suggested a mechanism of resistance
- Sterol profiling demonstrates the resistance isolate does not produce ergosterol
- CRISPER analysis supports *ERG3* as the major resistance locus

Vignette 2: Pan resistance isolate investigation

- On 05/28/2024 the NVSPHL received a *C. auris* isolate that had alert values for all three antifungal classes
- Fluconazole: MIC >256 μg/ml
- Anidulafungin: MIC 4 μg/ml; Micafungin: MIC 4 μg/ml
- Amphotericin B: MIC 2 μg/ml

Vignette 2: Pan resistance isolate investigation

- Patient was 75 years of age and hospitalized in Feb 2024
- Between Feb and June of 2024, 12 clinical *C. auris* isolates were taken from this patient
- 9 of these have been sequenced
- 11 were tested by our ARLN for antifungal susceptibility

Pan resistance isolate investigation

patient sample	Seq	date collected	site	Fluc.	Anid.	Mica.	AmpB	ERG11, FUR1 and FKS1 mutations
01	yes	2024-02-22	Skin Swab					ERG11 (Lys143Arg),FUR1 (Arg101Leu)
02	yes	2024-03-17	Urine	>256	0.25	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
03	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
04	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.25	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
05	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
06	yes	2024-03-27	Wound- Lt. Heel	>256	0.12	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
07	yes	2024-04-19	Urine	>256	4	4	0.5	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
08	yes	2024-05-06	Wound- Lt. Elbow	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
09	no	2024-05-06	Blood	256	0.5	0.12	0.5	
10	yes	2024-05-28	Urine	>256	4	4	2	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
11	no	2024-06-08	Blood	>256	1	0.5	0.5	
12	no	2024-06-10	Wound	>256	8	8	2	

Pan resistance isolate investigation

patient sample	Seq	date collected	site	Fluc.	Anid.	Mica.	AmpB	ERG11, FUR1 and FKS1 mutations
01	yes	2024-02-22	Skin Swab					ERG11 (Lys143Arg),FUR1 (Arg101Leu)
02	yes	2024-03-17	Urine	>256	0.25	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
03	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
04	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.25	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
05	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
06	yes	2024-03-27	Wound- Lt. Heel	>256	0.12	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
07	yes	2024-04-19	Urine	>256	4	4	0.5	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
08	yes	2024-05-06	Wound- Lt. Elbow	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
09	no	2024-05-06	Blood	256	0.5	0.12	0.5	
10	yes	2024-05-28	Urine	>256	4	4	2	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
11	no	2024-06-08	Blood	>256	1	0.5	0.5	
12	no	2024-06-10	Wound	>256	8	8	2	

- The only mutation unique to samples 7 and 10 is *FKS1* (Ser639Phe) known for echino. resistance

Pan resistance isolate investigation

patient sample	Seq	date collected	site	Fluc.	Anid.	Mica.	AmpB	ERG11, FUR1 and FKS1 mutations
01	yes	2024-02-22	Skin Swab					ERG11 (Lys143Arg),FUR1 (Arg101Leu)
02	yes	2024-03-17	Urine	>256	0.25	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
03	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
04	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.25	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
05	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
06	yes	2024-03-27	Wound- Lt. Heel	>256	0.12	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
07	yes	2024-04-19	Urine	>256	4	4	0.5	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
08	yes	2024-05-06	Wound- Lt. Elbow	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
09	no	2024-05-06	Blood	256	0.5	0.12	0.5	
10	yes	2024-05-28	Urine	>256	4	4	2	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
11	no	2024-06-08	Blood	>256	1	0.5	0.5	
12	no	2024-06-10	Wound	>256	8	8	2	

- Only two coding mutations unique to sample 10, both are involved in cell membrane functioning
  - AmpB targets ergosterol, a component of the cell membrane

Pan resistance isolate investigation

- So what happened?
- We are working for clearance on the medical records in this case

Pan resistance isolate investigation

- So what happened?
- We are working for clearance on the medical records in this case

- But, a compelling narrative is presented by the existing data
- This narrative is consistent with the standard of care
- This narrative does not imply any mistakes in patient care

Progression of resistance is aligned with standard of care

patient sample	Seq	date collected	site	Fluc.	Anid.	Mica.	AmpB	ERG11, FUR1 and FKS1 mutations
01	yes	2024-02-22	Skin Swab					ERG11 (Lys143Arg),FUR1 (Arg101Leu)
02	yes	2024-03-17	Urine	>256	0.25	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
03	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
04	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.25	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
05	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
06	yes	2024-03-27	Wound- Lt. Heel	>256	0.12	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
07	yes	2024-04-19	Urine	>256	4	4	0.5	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
08	yes	2024-05-06	Wound- Lt. Elbow	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
09	no	2024-05-06	Blood	256	0.5	0.12	0.5	
10	yes	2024-05-28	Urine	>256	4	4	2	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
11	no	2024-06-08	Blood	>256	1	0.5	0.5	
12	no	2024-06-10	Wound	>256	8	8	2	

- Colonized skin infection would not prompt anti-fungal treatment
- Either the late March bladder infection or wound colonization would prompt Echinocandin treatment
- Approximately one month later the first alert value for Echinocandins is observed

Progression of resistance is aligned with standard of care

patient sample	Seq	date collected	site	Fluc.	Anid.	Mica.	AmpB	ERG11, FUR1 and FKS1 mutations
01	yes	2024-02-22	Skin Swab					ERG11 (Lys143Arg),FUR1 (Arg101Leu)
02	yes	2024-03-17	Urine	>256	0.25	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
03	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
04	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.25	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
05	yes	2024-03-27	Skin Swab	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
06	yes	2024-03-27	Wound- Lt. Heel	>256	0.12	0.12	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
07	yes	2024-04-19	Urine	>256	4	4	0.5	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
08	yes	2024-05-06	Wound- Lt. Elbow	>256	0.5	0.25	0.5	ERG11 (Lys143Arg),FUR1 (Arg101Leu)
09	no	2024-05-06	Blood	256	0.5	0.12	0.5	
10	yes	2024-05-28	Urine	>256	4	4	2	FKS1 (Ser639Phe),ERG11 (Lys143Arg),FUR1 (Arg101Leu)
11	no	2024-06-08	Blood	>256	1	0.5	0.5	
12	no	2024-06-10	Wound	>256	8	8	2	

- Three weeks later the patient presents with Candidemia
- The physician would have access to the Echinocandin alert value, Amphotericin B would be SOC
- Approximately three weeks later the pan resistant isolate is observed

Pan resistance isolate investigation

- This case demonstrates that as few as 2-3 mutations could transform a susceptible *C. auris* infection into a pan resistant infection
- This case suggests that a pan resistant isolate can emerge from complications of long-term colonization even when following the standard of care
- WGS is critical for tracking and understanding the emergence of these resistant isolates

Acknowledgements

- Nevada State Public Health Laboratory
  - Lauryn Massic
  - Mark Pandori
  - Lauren Siao
  - Clarissa Martins
  - Adam Vazquez
  - Danielle Siao

- WA ARLN facility
- CDC (especially OAMD)
- APHL
- Ryback Lab (CRISPR)
- Kelly Lab (Sterol profiles)