Laboratory Investigations of Multidrug-Resistant *Candida auris* – Impact & Lesson Learned

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# What do we know about *Candida*

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Candida auris</th>
</tr>
</thead>
<tbody>
<tr>
<td>• They are gut bugs</td>
<td>• Skin bug</td>
</tr>
<tr>
<td>• Mostly antifungal susceptible</td>
<td>• Mostly resistant to antifungals</td>
</tr>
<tr>
<td>• Rarely cause outbreak</td>
<td>• Frequently causing outbreaks</td>
</tr>
</tbody>
</table>
Why are we concerned about *Candida auris*?

- Highly drug-resistant
- Patients can become colonized and develop invasive infections
- Spreads in healthcare settings
C. auris cases reported in >35 countries
Spreads after introductions from abroad

- Cases are a result of introductions from abroad followed by local transmission
- Majority of cases don’t have direct links to healthcare abroad
Four Genotypes/Clades

Fifth Genotype/Clade

>200,000 SNPs apart

Chow NA et al Emerg Infect Dis 2019, 2019;25(9):1780-1781

Chow NA et al Lancet ID 2018, 18:1377
A Mysterious Infection, Spanning the Globe in a Climate of Secrecy

The rise of Candida auris embodies a serious and growing public health threat: drug-resistant germs.

By Matt Richtel and Andrew Jacobs

April 6, 2019

Last May, an elderly man was admitted to the Brooklyn branch of Mount Sinai Hospital for abdominal surgery. A blood test revealed that he was infected with a newly discovered germ as deadly as it was mysterious. Doctors swiftly isolated him in the intensive care unit.
Wadsworth Center Laboratories
Division of Infectious Diseases

- Arbovirology
- Bacteriology
- Biodefense
- Bloodbore Viruses
- Cellular Immunology
- Diagnostic Immunology
- Mycobacteriology
- **Mycology**
- Parasitology
- Rabies
- Virology

Hazen & Brown (1955)

**NYSTATIN**

Morris Gordon

Cryptococcal antigen test

1963
Mycology Laboratory @ Wadsworth Center: Scope

• **Reference Services (Fungal ID)**
  - Culture
  - MALDI-TOF MS (Bruker) - 2013
  - ITS-PCR/Sequencing - 2010
    - E-test (Yeasts)
    - Microbroth Dilution (Yeasts & Molds)
    - YO9 (Yeasts)
  - Real time PCR assays:
    - *Histoplasma capsulatum* (2011)
    - *Blastomyces dermatitidis* (2011)
    - *Exserohilum rostratum* (2013)
    - *Candida spp.* (2016)

• **Applied Research**
  - Fungal virulence mechanisms (NIH)
  - Antifungal test innovation – (Industry Contracts)
  - Molecular Test Development (WC CLRS)
  - *Pseudogymnoascus* (‘Bat White-Nose’) (NSF & FWLS)

1,100 to 1,300 Samples/Year (99.9% isolates)
CDC–Antibiotic Resistance Laboratory Network (2016...)

Mycology Lab
Candida (glabrata)
Resistance Testing
What did we find?
Unprecedented outbreak of *Candida auris* in NY

What did we do?
180 degree turn around and re-focus

- Writeup of protocols for shipping instructions, sample processing, report release, etc.
- Writeup of advisories with Epi to educate healthcare professionals on *C. auris*
- Enrichment of in-house MALDI library for *C. auris* ID
- Molecular assay development for rapid *C. auris detection* from surveillance samples
- Provid SOPs - *C. auris* culture, MALDI & real-time PCR to Clinical/ Public/Private Laboratories in NY and other states in the US
- Weekly/bi-weekly/monthly conference calls/meetings with NYSDOH Epi/ CDC/WC
- Staff recruitment
Culture/Identification - *C. auris* Surveillance Samples

**Laboratory Workflow Pre-PCR Era**

![Image of laboratory workflow](image)

- **Surveillance Samples (Swabs & Sponges)**
  - **Non Selective Agar (SAB+)**
    - Growth → **MALDI**
      - *Candida auris & Candida spp.*
    - Sanger Sequencing for Phylogenetic analysis
    - Antifungal susceptibility testing for resistance profile
  - **Selective Agar (Salt + Dextrose → Dulcitol)**
    - Growth → **MALDI**
      - *Candida auris*
    - No Growth → **Negative culture**
    - **Cloudy**
  - **Selective Broth (Salt + Dulcitol)**
    - No Growth → **SAB+**
    - Growth → **MALDI**
      - *Candida auris*

**Welsh et al, 2017. JCM, 55:2996**

**Culture + ID = 4 to 14 days**
## Total Number of Samples Processed
(August 2016 to April 2019)

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Isolates suspected of <em>C. auris</em></td>
<td>746</td>
</tr>
<tr>
<td>Surveillance (Patient)</td>
<td>9,676</td>
</tr>
<tr>
<td>Surveillance (Environmental)</td>
<td>4,123</td>
</tr>
<tr>
<td>Admission Screening</td>
<td>4,871</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15,453</strong></td>
</tr>
</tbody>
</table>
Surveillance Sample Testing Strategies

Axilla, Groin, Nares (August 2016)
Axilla/Groin & Nares (November 2016)
Nares/Axilla/Groin (January 2018)

Point Prevalence Screening

Surveillance Samples (11,035)
931+ve for *C. auris* (8.4%)
Candida auris in NY from August 2016... Endemic or Transient?

Clinical Cases (422)
Colonized Cases (621)
Colonized → Clinical (63)
Environmental = (152)

190 facilities including 69 hospitals, 117 NH, 1 LTACH, 2 Hospices, & 1 VA Health System
Candida auris Cases in New York State by Month, May 2013 - September 2019

Month of First Positive Culture/PCR for Candida

C. haemulonii  →  C. auris
Heavy Colonization of Skin & Mucosa of 350 Colonized Cases

Unpaired Samples

Paired Samples
Heavy Colonization of Hospital Surfaces

C. auris CFU/Sponge

Porous
- Linen
- Carpet
- Gowns

Non-porous
- Bed rail
- Window sill
- Bathroom surface
- Call bell
- Counter top
Antifungal Resistance Pattern of NY C. auris isolates

- FLU = Fluconazole
- AMB = Amphotericin B
- ECHI = Echinocandins

Pan Resistant Isolates = 3

- FLU: 99%
- FLU+AMB: 61%
- FLU+ECHI: 1%

Mutation:
- ERG11
- FKS1/FKS2
Candida auris Prevalence

Patients

- Candida parapsilosis: 12.15%
- Candida albicans: 8.90%
- Candida glabrata: 8.90%
- Candida auris: 10.07%
- Mold: 1.00%
- UNSAT Not Tested: 1.33%
- Bacterial: 7.17%
- No Growth: 51.50%

Environment

- Negative
- Other yeasts (3.5%)
- C. auris (3.0%)
- C. parapsilosis (4.5%)
NY Outbreak is dominated by South Asia Clade I

Sanger Sequencing of Ribosomal genes
Highly Sensitive (one *C. auris* CFU/PCR reaction)

Highly Specific (No cross-reaction to yeasts/molds/bacteria/parasites)

Rapid (4 h)

Drawback- Manual nature of the assay

![Flowchart Diagram](image)
Bruker Expands Portfolio for Testing of Candida Auris, an Emerging, Multidrug-Resistant Pathogen in Human Healthcare

Fungiplex Candida Auris RUO Real-Time PCR Kit

PCR reagents for test + controls

Leach et al 2018, JCM, 56:E01223-17
A high-throughput and rapid method for accurate identification of emerging multidrug-resistant Candida auris.

Ahmad A¹, Spencer JE¹, Lockhart SR², Singleton S², Petway DJ¹, Bagarozzi DA Jr¹, Herzegh OT¹.
A Rapid and Automated Sample-to-Result *Candida auris* Real-Time PCR Assay for High-Throughput Testing of Surveillance Samples with the BD Max Open System

L. Leach, A. Russell, Y. Zhu, S. Chaturvedi, V. Chaturvedi

**Sensitivity**

= One *C. auris* CFU/PCR Reaction

**TAT**

= 2 h

**Total samples**

= 100-150 samples/Day
Modified Workflow Post PCR Era

**PCR**

**Negative**
- No Further Test

**Positive**
- Culture (Dulcitol Broth)
- MALDI
- **C. auris**

**Significant cut down on**
- Efforts
- Supplies
- $ amount
Testing doesn’t end with real-time PCR assay!

• Need *C. auris* isolate with confirmed ID to do antifungal susceptibility testing, genotyping, etc.

Bruker MALDI-TOF MS – FDA approved database April 2018

bioMérieux VITEK MS - FDA approved database December 2018
Continuing Challenges

- Mycology training/re-training needed in Clinical, Public Health & Commercial Laboratories
- Availability of selective medium (Dulcitol) is restricted to one vendor [https://s2cm.com/product/salt-sabouraud-dulcitol-broth-ssdb/](https://s2cm.com/product/salt-sabouraud-dulcitol-broth-ssdb/)
- LDT for C. auris is not FDA approved
- MALDI-TOF MS expensive technology-not easily available
- AST – 50% inhibition by naked eye –need extensive practice
Antimicrobial Resistance Laboratory Network
Northeast Regional *Candida auris* Training Workshop
Wadsworth Center – Mycology Laboratory
Albany, NY
November 4 - 6, 2019

Swab and Sponge Testing by PCR and Culture
Antifungal Susceptibility Testing by Microbroth and E-test
MALDI-TOF MS Identification of Yeasts
Packaging and Shipping of Surveillance Samples

Offered by: New York State Department of Health-Wadsworth Center Mycology Laboratory
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SUMMARY

- Total Surveillance samples tested 20,661 including 15,026 point prevalence (10,521 swabs & 4,505 sponges), & 5,635 admission screening.

- Clinical cases 422 & colonized cases 623 as of November 5, 2019. Approximately 10% of colonized cases converted into clinical, a major concerning factor.

- Successful use of one swab of Nares/Axilla/Groin for all PPS (January 2018).

- Development of PCR assays (manual & automated) and their impact on infection control practices.

- Relatively heavier colonization of nares than axilla/groin.

- Predominance of South Asia Clade I.

- Isolation of three Pan-resistant isolates.
Candida auris in the U.S.

May 2017

July 2018

July 2019
Think Fungus

Fungal diseases cost an estimated $7.2 BILLION annually in the US

- $4.5 billion for hospitalizations
- $2.7 billion for outpatient visits.

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