PATHOGEN CHARACTERISTICS

- Budding Yeast
- Growth (from isolate) on Sabouraud Dextrose agar incubated at at 30-37℃ can typically be seen in 24-48hrs
- Heat tolerant; able to grow at temperatures as high as 42℃
- Colonization of skin and various body sites
    - Colonization can persist for years– perhaps indefinitely, and there are currently no protocols for decolonization
    - Colonized individuals are typically asymptomatic and without *C. auris* infection, but can still transmit *C. auris* onto surfaces or objects
- Capable of causing superficial (skin) as well as invasive infection (bloodstream) and is associated with high mortality
    - Causes symptoms similar to that of a bacterial infection with severity depending on body site
- Able to persist on surfaces for several weeks, making it of particular concern in healthcare settings
- Antifungal Resistance
    - There are three main classes of antifungals: azoles, polyenes, and echinocandins
    - >90% of isolates resistant to one class of antifungals
        - Most isolates are resistant to fluconazole, a part of the azole class of antifungals (3)
    - >25% of isolates resistant to two classes of antifungals
    - Occurance of pan-resistant isolates, resistant to all three classes of antifungals, in the US and worldwide
        - Echinocandin resistance is not widespread and is often acquired by patients who have received recent echinocandin treatment. However, there is also evidence of echinocandin-resistance transmission in the absence of previous enchinocandin treatment (3, 4)

DETECTION

- Isolated from blood, urine, wound, respiratory secretions, etc.
- Skin swabs, typically axilla/groin composite, used for surveillance samples
- Real-time PCR for detection from patient skin swabs
- Broth enrichment allows for a higher likelihood of isolation
    - Recommended up to five day incubation period
- Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) for confirmation of identity
- Antifungal susceptibility testing to determine minimum inhibitory concentration (MIC)
- Test for susceptibility and resistance to: Anidulafungin, Caspofungin, Fluconazole, Itraconazole, Micafungin, Posaconazole, Voriconazole, Ibrexafungerp, and Amphotericin B
    - Susceptibility is typically phenotypically assessed using microbroth dilution panels with a predefined gradient of antibiotic concentrations
    - There are currently no *C.auris*-sepcific susceptibility breakpoints, but the CDC has defined MIC values based on other *Candida* species and expert opinion (5)

GENOMIC ATTRIBUTES

- Genome Length: 12.2 - 12.7 Mb
- Chromosomes: 7
- Core genes: 5,494

- Evolutionary Rate: 1.87 x 10^{-5} substitutions per site per year
    - This could be an overestimation due to short time scale of samples used in calculation; more likely represents a spontaneous mutation rate (6)
- Genome Transfer: *C. auris* predominantly propagates via asexual budding but can also undergo sexual reproduction. Each clade expresses only one of the two fungal mating type loci (MTL**a** or MTLα) which suggests recombination could occur in geographical locations where multiple clades coexist, although there are no reported cases of mating between clades or mixed infection with opposite mating types (6)
- Mutations in *ERG11* gene which encodes an azole target and may confer fluconazole resistance; this is a hotspot for azole resistance in *C. albicans*, a related species (3)
    - Y132F
    - K143R
    - F126L
- Copy number variants of *ERG11* are also associated with fluconazole resistance (6)
- *FKS1* gene which encodes for an echinocandin target (6)
    - S639P
    - S639F
    - S639Y
- Isolates without mutations in *ERG11* or *FKS1* are often drug susceptible (3, 6, 7)

WHOLE GENOME SEQUENCING (WGS)

- Culture required for WGS
- Five major clades that cluster by geographic region
    - South Asia (I)
        - Most prevalent globally
        - Known to exhibit increased antifungal resistance (9)
    - East Asia (II)
        - Not the cause of outbreaks
        - Propensity for ear infections
        - Often drug susceptible (6)
    - South Africa (III)
    - South America (IV)
    - Iran (V)
        - Only one known isolate
- Tens of thousands of SNPs between clades with far fewer SNPs between isolates within the same clade, often less than 100 SNPs (8)
- All cases in the US cluster within one of the first four clades suggesting importation from abroad and subsequent local transmission
    - Strict SNP cutoffs for determining transmission are difficult to determine due to dependence on bioinformatic parameters
    - Within an outbreak, 12 or fewer SNPs between patients could suggest recent transmission (8)
- Resistance levels and mutation types cluster based on clade type (6)
    - Y132F and K143R (*ERG11* gene mutations) appear in clade I and IV
    - F126L (*ERG11* gene mutation) found in clade III
    - Increase in copy number predominantly seen in clade III
- WGS allows a broader understanding of transmission events
    - Linking or ruling out cases in an outbreak
    - Distinguishing new introductions of *C. auris* from ongoing transmission in a healthcare facility

- More extensive WGS data collection globally and within the US will give a more accurate picture of the burden of *C. auris* and clade distribution worldwide

PEOPLE AT INCREASED RISK

- Healthy individuals are **not** at risk of *C. auris* infection but may be colonized on their skin, allowing for transmission (10)
- Individuals with severe underlying medical conditions that require complex care are more likely to be affected by *C. auris* (16)
- Outbreaks often occur in healthcare settings since *C. auris* can persist in the environment
  - Long-term acute care hospitals (LTACHs)
  - Ventilator-capable skilled-nursing facilities (vSNFs)
- Those colonized with other multidrug-resistant organisms
- Those who have recently received antibiotic or antifungal treatment
- Ventilator-dependent individuals
- Those who have undergone tracheostomies or have indwelling devices
- Those with wounds or breaks in the skin (portal of entry)
- Those who have prolonged health care encounters
- Those with a history of healthcare outside of the US or in a region of the US that has a high burden of C. auris

EPIDEMIOLOGICAL OBJECTIVES

- Tracking spread of antifungal resistance *C. auris* in healthcare settings
  - Currently, community-wide spread is not considered a threat. Healthcare transmission is responsible for most, if not all, transmission seen in the US (12)
- Determining related cases in outbreaks is imperative to establish effective infection control and prevention measures
  - Patients that have been determined to be positive for *C. auris* should be isolated from patients who are not colonized, and more aggressive control measures should be put in place to prevent further spread
  - Identification of multiple cases testing positive for *C. auris* within the same setting but with divergent whole genome sequences would support that infection control measures are working, but that multiple introductions are occurring
  - Similarly, whole genome sequences from multiple cases with high sequence identity would likely indicate intra-facility transmission
- Clade identification of isolates to determine the origin of an outbreak
  - When assessed with other relevant information, such as recent travel activity or healthcare received abroad, clade identification can help put together the pieces of potential origin of the outbreak. However, most recent US cases reflect local transmission.

INVESTIGATION QUESTIONS

- What mutations are factors in antifungal resistance?
    - WGS aids in the detection, identification, and monitoring of recurring and novel mutations that could confer resistance mechanisms
    - These investigative efforts are currently being done by the Mycotic Diseases Branch at the CDC and health research instututions worldwide
- What are the transmission dynamics of *C. auris* and how can we effectively detect new introductions?
- What is the *C. auris* clade distribution in the US worldwide?
    - Characterization of subclusters within clades found through WGS analysis
- What is the source/cause of recent *C. auris* emergence (greater burden of antifungal use, environmental factors, etc)?

TOOLS

Tools able to identify *C. auris* and generate relatedness metrics from whole genome sequencing data (13):

- **TheiaEuk**: performs assembly, quality assessment, and genomic characterization of fungal genomes (14)
    - Accepts Illumina paired-end sequencing data primarily, but allows other optional inputs for user customization of workflow
    - Performs de novo assembly, species taxon identifcation, and taxa-specific workflows
    - Performs clade typing, giving a clade assignment and clade-specific annotated genome which is then passed through an antifungal resistance detection module
    - Alignment to annotated reference genome, variant calling is followed by a query for any that occur within genes that are known to contain resistance-conferring mutations (allows for detection of novel mutations)
    - For C. auris, TheiaEuk queries for strings matching know resistance genes (*FKS1*, *ERG11*)
    - Produces assemblies that can be used as inputs for kSNP3 to produce phylogenetic tree and SNP matrix
- **MycoSNP** (GitHub - CDCgov/mycosnp-nf: CDCgov/mycosnp-nf)
    - Open source bioinformatic pipeline used to call variants and construct a phylogeny from sequencing data
    - Input includes raw read files (FASTQ) from an Illumina paired-end sequencing run and reference genome (FASTA)
    - Performs alignment to reference genome which includes masking repeat regions
    - Outputs a consensus sequence for each sample in the form of a multi-FASTA to be used as an input to phylogenetic tree tools
    - VCF files can be fed into MycoSNP_Tree workflow to produce phylogenetic tree and SNP matrix
- **Nullabor** (GitHub - tseemann/nullarbor: :floppy_disk: "Reads to report" for public health and clinical microbiology)
    - Reads to report bioinformatic pipeline
    - Requires sequence reads as an input; additional inputs include an array of sample names and a reference genome
    - No clade typing module; reference genome should therefore be clade-specific
    - Performs species identification, de novo assembly, alignment to reference genome
    - Produces a core genome, SNP matrix, and phylogenetic tree

BEAST (Bayesian estimation analysis sampling trees): analytical tool for evolutionary inference

MicroReact (Introduction - Microreact): web-based, interactive data visualization tool

Microbetrace (Home · CDCgov/MicrobeTrace Wiki · GitHub): web-based data visualization tool

NCBI (National Center for Biotechnology): NIH database for sharing sequencing data

- **Genbank**: publicly accessible collection of DNA sequences
- **Pathogen Detection**: centralized system that integrates bacterial and fungal pathogen genomic sequences from ongoing surveillance and research efforts

FungiNet: global network to advance pathogen genomic surveillance and epidemiology of fungal diseases.

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