Abstract

Background: *Candida auris* is a multidrug-resistant, healthcare-associated fungal pathogen comprising of five known geographical clades. It was first reported in South Africa in 2014 and then detected in a previously misidentified case in 2009 through a retrospective study. C. auris has been reported in over 40 countries since its first detection and has caused nosocomial outbreaks in healthcare facilities including those in neonates. We describe the molecular epidemiology of C. auris, using whole-genome sequencing (WGS) over four years at a large tertiary academic hospital in South Africa with a focus on the hospital's neonatal unit which had a large outbreak during this period.

Methods: Cases of culture-confirmed C. auris infection or colonisation were identified through laboratory surveillance across the entire hospital from March 2016 through July 2020 and viable isolates were submitted to a reference laboratory. Phenotypic characterisation included the assessment of pseudohyphae production and aggregate formation using microscopy techniques. Antifungal susceptibility testing was performed using commercial broth microdilution and gradient diffusion methods. Molecular analysis was based on the WGS data of isolates; the quality of read data was assessed and bioinformatics analysis was performed. Phylogenetic analysis was used to confirm the clade assignments while phylodynamic analysis was used to determine the most common recent ancestor and transmission routes of the outbreak strains.

Results: Of 287 cases, 207 (72%) had viable isolates and 200 non-contaminated isolates were available for further phenotypic experimentation. Cases across the hospital had a median age of 1.4 years (interquartile range: 22 days – 21 years), with a large proportion diagnosed in the neonatal unit (91/287, 31.7%). Most isolates demonstrated moderate aggregation capabilities

(124/200, 62%) while 33% (65/200) had extensive aggregation and only 6% showed no aggregation. Only three isolates produced pseudohyphae. All strains grew at 37°C, 40°C and 42°C. Most isolates belonged to clade III (63%, 118/188) or clade IV (37%, 70/188). All 181 fluconazole-resistant isolates (minimum inhibitory concentration ≥32 μg/mL) had an ERG11 gene mutation. Additionally, five fluconazole-susceptible isolates also carried a mutation in the ERG11 gene. All clade III isolates had the VF125AL ERG11 mutation (118/118, 100%) while isolates in clade IV (68/70, 97%) had K177R/N335S/E343D mutations. We use Bayesian molecular clock phylogeny and dated the emergent time for the most recent common ancestor (TMRCA) for clade III in this hospital to early 2014 and in the neonatal unit to 2018. Phylodynamic analysis showed multiple introductions of C. auris into the neonatal unit.

Conclusion: Clades III and IV co-circulated in the hospital, with clade III causing all but one case in the neonatal unit. Most isolates contained previously-described clade-specific mutations related to azole resistance. The estimated emergence of the TMRCA for the hospital and neonatal unit clade III isolates was roughly consistent with the first cases reported. While the large neonatal unit outbreak may have originated from cross-unit transmission by infected/colonised patients, colonised healthcare workers or contaminated equipment, we could not exclude the possibility of transmission events from other healthcare facilities or from the community.