THE LANCET Infectious Diseases

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Chow NA, Gade L, Tsay SV, et al, on behalf of the US Candida auris Investigation Team. Multiple introductions and subsequent transmission of multidrug-resistant *Candida auris* in the USA: a molecular epidemiological survey. *Lancet Infect Dis* 2018; published onlined Oct 4. http://dx.doi.org/10.1016/S1473-3099(18)30597-8.

APPENDIX

SUPPLEMENTARY METHODS

Storage and processing of C. auris *isolates*

A 100 ul aliquot of the Amies storage medium from the BD-eswab were inoculated into enrichment broth and incubated while shaking at 250 RPM at 40C for up to 7 days or until the broth became turbid. Then the broth cultures were plated on CHROMagar Candida Chromogenic agar and incubated at 37C for up to 7 days. Pink or pale colonies were selected and confirmed.

Antifungal susceptibility testing

Antifungal susceptibility testing was performed as outlined by CLSI guidelines.[(1)] Custom prepared microdilution plates (Trek Diagnostics) were used for the echinocandins (anidulafungin, caspofungin, and micafungin) and the azole fluconazole. Interpretive breakpoints for *C. auris* were defined based on a combination of those breakpoints which have been established for other closely related *Candida* species, epidemiologic cutoff values, and the biphasic distribution of MICs between the isolates with and without known mutations for antifungal resistance. Resistance to anidulafungin and micafungin was set at ≥4 μg/mL, caspofungin at ≥2 μg/mL, and fluconazole at ≥32 μg/mL. Etests (BioMerieux) were used for the polyene Amphotericin B, and resistance was set at ≥2 μg/mL. Isolates were considered to be resistant to a class if resistance was documented for at least one drug within the specified class.

Paired-end whole-genome sequencing

In preparation for WGS, DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. Beta-mercaptoethanol was added to the Fungal/Bacterial Binding buffer to a dilution of 0.5% (v/v) prior to extraction as

suggested by the manufacturer. Genomic libraries were constructed and barcoded using the NEBNext Ultra DNA Library Prep kit for Illumina (New England Biolabs, Ipswich, MA, USA) and following manufacturer's instructions. Genomic libraries were sequenced on either the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA) using the HiSeq Rapid SBS Kit v2 500-cycles (Illumina) or the MiSeq platform (Illumina) using the MiSeq Reagent Kit v2 500-cycles (Illumina).

Single nucleotide polymorphism (SNP) analysis

Paired-end sequences that had at least 50X coverage were used for downstream analyses. FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and PRINSEQ[(2)] were used to assess quality of read data and perform read filtering. Read data were aligned against a previously described reference isolate from a patient in Pakistan[(3)] using BWA[(4)]. SNP variants were identified using SAMtools[(5)] and filtered using the publically available SNP analysis pipeline NASP (http://tgennorth.github.io/NASP/) to remove positions that had less than 10x coverage, less than 90% variant allele calls, or that were identified by Nucmer[(6)] as being within duplicated regions in the reference.

Phylogenetic analysis and principal component analysis (PCA)

Phylogenetic analysis was performed on SNP matrices using MEGA and the *ape* R package. Bootstrapping was performed under default settings for 500 iterations. A SNP matrix was used as a Hamming distance input for the R package *adegenet*.

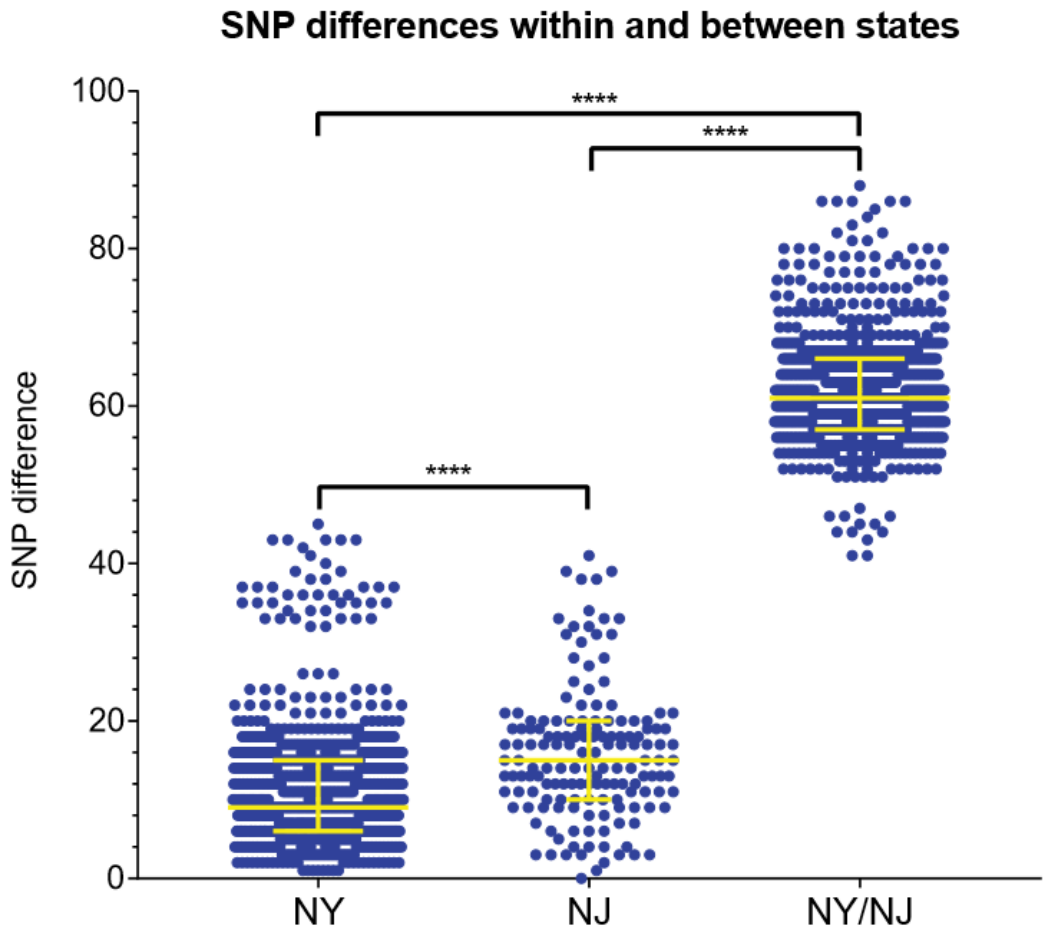
SUPPLEMENTARY TABLE

Collection dates for *C. auris* isolates by state.

State	*Collection date(s)*
California	August 13, 2017
Connecticut	June 22, 2017
Florida	April 26, 2017
Illinois	May 13, 2016–Dec 28, 2016
Indiana	March 17, 2017
Massachusetts	July 16, 2017–July 27, 2017
Maryland	April 11, 2016
New Jersey	July 12, 2015–June 14, 2017
New York	May 11, 2013–May 10, 2017
Oklahoma	April 27, 2017

SUPPLEMENTARY FIGURE AND FIGURE LEGEND

Supplemental Figure. Number of SNP differences for each pairwise comparison among NY clinical cases (N=42), each pairwise comparison among all NJ clinical cases (N=17), and each pairwise comparison between NY and NJ clinical cases.



SUPPLEMENTARY REFERENCES

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