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Incursions of *Candida auris* into Australia, 2018

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DOI: https://doi.org/10.3201/eid2606.190936

Candida auris is an emerging global healthcare-associated pathogen. During July–December 2018, four patients with *C. auris* were identified in Victoria, Australia, all with previous overseas hospitalization. Phylogenetic analysis revealed putative transmission between 2 patients and suspected overseas acquisition in the others. Vigilant screening of at-risk patients is required.

The fungal pathogen *Candida auris* is an emerging global health threat associated with a range of invasive infections, most commonly candidemia; it is often resistant to multiple antifungal drugs (*1*). First identified in Japan in 2009, *C. auris* has been reported across all 6 populated continents with outbreaks in healthcare settings, particularly intensive care and high-dependence units (*1*,*2*). Four genetic lineages of *C. auris* with phylogeographic variation have been identified (*3*).

Before July 2018, only 1 case of *C. auris* had been reported in Australia, none in the state of Victoria (population 6.5 million) (*4*); no centralized surveillance or mandatory reporting has been implemented on a state or national level, and local screening policies are limited. However, Victoria has experienced large interfacility and intrafacility healthcare-associated outbreaks of other multidrug-resistant organisms (*5*) and has increasingly implemented genomics in both the investigation of outbreaks and routine surveillance (C.R. Lane et al., unpub. data, https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3498431).

In July 2018, *C. auris* was cultured from a patient hospitalized in a Victoria healthcare facility. In response, the Victoria Department of Health and Human Services (DHHS) convened an incident management team and issued a Chief Health Officer alert to all health services and laboratories recommending admission screening for patients with recent overseas hospitalization. Also recommended was consideration of *C. auris* in patients with cultured non–*C. albicans* species and risk factors for fungal infection, including diabetes mellitus and recent antimicrobial drug use. The alert specified that all *C. auris* and nonspeciated non–*C. albicans* isolates from high-risk patients be referred to Victorian Public Health laboratories for speciation and characterization and reported to the DHHS (*6*). We report on the use of genomics to investigate putative transmission of *C. auris* in Victoria during July 1–December 31, 2018.

Isolates of *C. auris* were referred to the Victorian Infectious Diseases Reference Laboratory, where they underwent species identification and antimicrobial susceptibility testing. All isolates were then referred to the Microbiological Diagnostic Unit Public Health Laboratory for DNA extraction, whole-genome sequencing, and bioinformatic analysis.

During July–December 2018, we identified *C. auris* in 4 patients. Three patients (patients 1, 2, and 4 chronologically) were identified through clinically indicated samples, whereas patient 3 was screened on transfer from an overseas healthcare facility. Patients 1 and 2 were admitted to the same hospital at specimen collection, and patients 3 and 4 were admitted to different facilities. All patients reported previous overseas hospitalization (Figure).

We obtained 7 *C. auris* isolates from these 4 patients and performed core genome phylogenetic analysis on all isolates (Appendix, https://wwwnc.cdc.gov/EID/article/26/6/19-0936-App1.pdf). We downloaded publicly available *C. auris* sequences and included those meeting quality control metrics (Appendix Table 1). Phylogenetic analysis revealed all 7 isolates fell within the previously described South Asian clade (Figure, panel A).

We identified putative transmission during a concurrent hospital stay between patients 1 and 2 (Figure, panel B); transmission was epidemiologically validated and reported elsewhere (*7*). Because both patients reported overseas hospitalization, it is unclear which constituted the index case. Isolates from patients 3 and 4 were not closely related to each other, or any other included isolates, consistent with independent overseas acquisition.

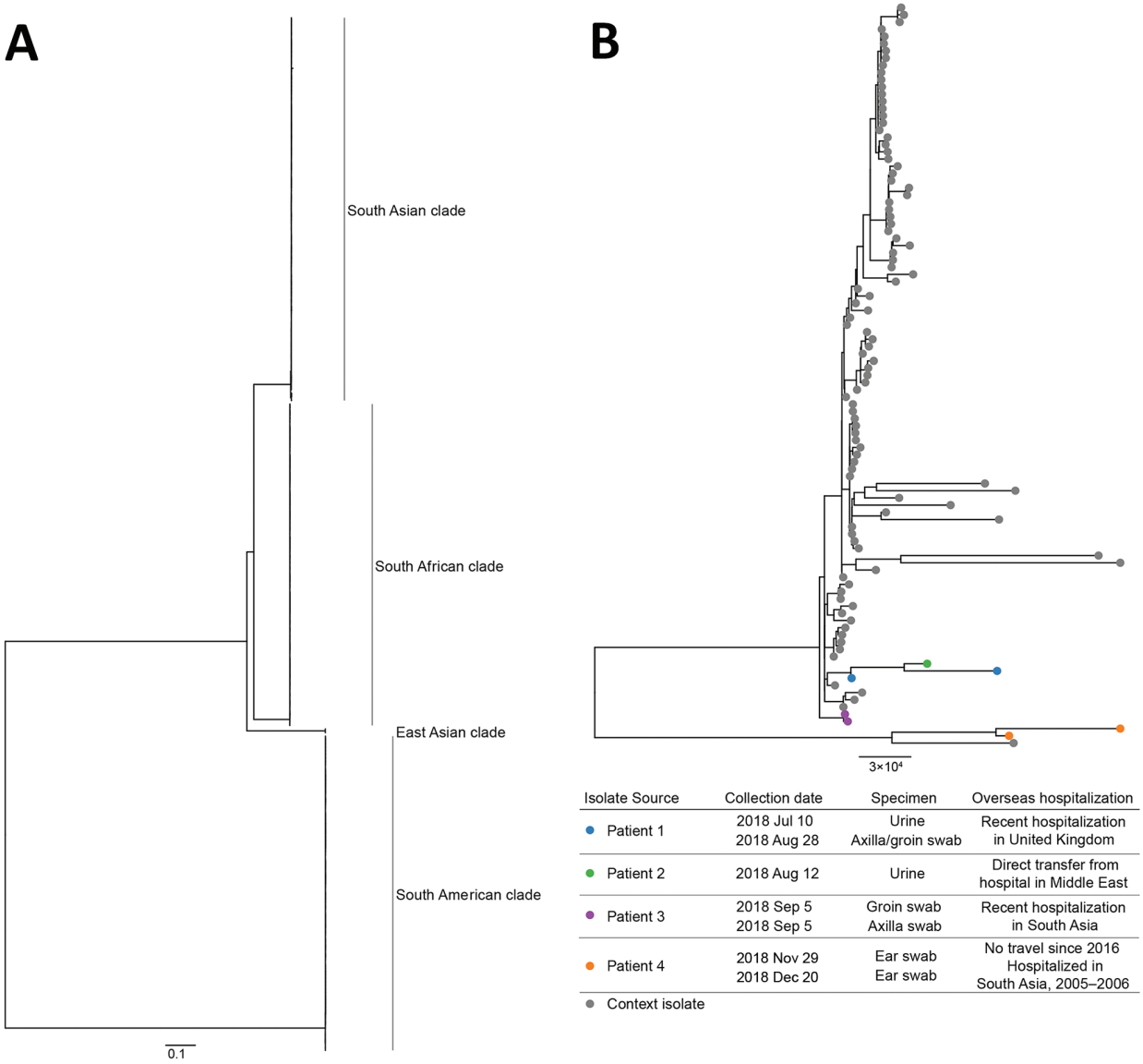

Figure. Maximum-likelihood phylogenetic trees of *Candida auris* isolates from Victoria, Australia, in the context of international publicly available genomes. A) Complete tree; B) South Asian clade. Isolates from 4 patients in Victoria are indicated by colored dots on the inset tree; isolate details and patient travel history are provided in the key. Scale bars indicate substitutions per site.

We reported results of phylogenetic analyses prospectively and concurrently to the incident management team and affected facilities. Because all patients reported overseas hospitalization, the combined analysis of genomic and epidemiologic data enabled assessment of alternative hypotheses, identifying putative local transmission between patients 1 and 2 and excluding patients 3 and 4 from the outbreak. These results justified targeted infection control measures.

In late 2015, Victoria introduced combined phylogenetic and epidemiologic surveillance for carbapenemase-producing *Enterobacterales*, a similar low-prevalence multiresistant organism (*8*). Using a search-and-destroy approach, this method enabled early identification of local transmission and complemented standard laboratory, screening, and outbreak control measures across the state. Leveraging from this work, a similar system was introduced for the control of *C. auris* in September 2019, with statewide mandatory notification of *C. auris* introduced in December 2019 (*9*,*10*). These measures address limitations in the current study, such as the inability to identify patients with *C. auris* because of noncompliance with screening recommendations, nonreporting, or decreased sensitivity of laboratory methods for the detection of *C. auris*.

A representative sample of international isolates enables inference of local transmission through local cluster identification and can indicate the source of local strains. The emergence of *C. auris* highlights the need for greater surveillance of nonbacterial multiresistant organisms and international data sharing. Sequences from our study were submitted to GenBank.

Our findings demonstrate the importance of proactive screening programs and of strict isolation and containment actions. Despite Australia's geographic isolation, vigilance is necessary to ensure that patients hospitalized overseas are identified and screened for the presence of multiresistant organisms such as *C. auris* upon hospital admission.

About the Author

Ms. Lane is an epidemiologist and PhD candidate at the University of Melbourne. Her primary research interest is the use of genomics in the surveillance of antimicrobial resistance and pathogens of public health concern.

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Article DOI: https://doi.org/10.3201/eid2606.190936

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Appendix

Methods

Identification and Antifungal Susceptibility Testing

We confirmed isolates as *C. auris* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF; Bruker Biotyper, https://www.bruker.com), and performed antifungal susceptibility testing by broth microdilution (Sensititre YeastOne, https://www.thermofisher.com).

DNA Extraction and Whole-Genome Sequencing

We performed DNA extraction and whole-genome sequencing (WGS) of study isolates at the Microbiological Diagnostic Unit Public Health Laboratory at the University of Melbourne. Genomic DNA was extracted from a single colony using a QIAsymphony DSP DNA Mini Kit (QIAGEN, https://www.qiagen.com) according to manufacturer's instructions, and WGS was performed on an Illumina NextSeq 500 platform (Illumina, https://www.illumina.com) with 150 bp paired-end reads or Illumina MiSeq platform with 75 bp paired-end reads. We resequenced isolates that had sequencing depth ≥50× and a minimum Phred quality score of 30. Reads are available from the NCBI Sequence Read Archive (BioProject, https://www.ncbi.nlm.nih.gov/bioproject).

All *C. auris* sequences available on public databases were downloaded and assessed against the quality metrics above; we excluded sequences not meeting these targets.

SNP and Phylogenetic Analysis

We initially compared study genomes and publicly available genomes with *Candida auris* strain B8441 using Snippy version 4.3 (https://github.com/tseemann/snippy). We performed alignment using BWA MEM version 0.7.17-r1188 (1) and called single nucleotide polymorphisms (SNPs) using Freebayes version 1.2.0 (E. Garrison et al., unpub.data,

https://arxiv.org/abs/1207.3907), requiring a minimum read coverage of 10, minimum base quality of 13 and 90% read concordance at a site to report SNPs. The resulting core SNP alignment consisted of 151,484 sites. We inferred maximum likelihood (ML) phylogenetic trees using RAxML version 8.2.12 with 100 pseudo-bootstrap replicates and using a generalized time-reversible model, a Gamma distribution to model site-specific rate variation (the GTR + Γ substitution mode: GTRGAMMA in RAxML).

We performed subanalysis of the identified South Asian clade, including all locally sequenced isolates, using the methods above. The resulting core SNP alignment consisted of 103 sites.

We filtered these alignments for phage regions identified using Phaster (2) and recombination regions identified using Gubbins version 2.3.4 (*3*), and extracted core SNPs using SNP-sites (*4*). The resulting trees were midpoint-rooted with *phangorn* (v2.3.1) (*5*) and rendered using *ggtree* (1.8.1) (*6*). We calculated pairwise SNP distances using *harrietR* (https://github.com/andersgs/harrietr).

Appendix Table 1. Publicly available *Candida auris* isolates included in phylogenetic analysis, Victoria, Australia

Accession number	Year	Country	Accession no.	Year	Country
ERR2299885	Unknown	United Kingdom	ERR2300789	Unknown	Unknown
ERR2299874	Unknown	United Kingdom	ERR2300809	Unknown	Unknown
ERR2299868	Unknown	United Kingdom	ERR2300771	Unknown	Unknown
ERR2299870	Unknown	United Kingdom	ERR2300797	Unknown	Unknown
ERR2299877	Unknown	United Kingdom	ERR2300772	Unknown	Unknown
ERR2299887	Unknown	United Kingdom	ERR2300796	Unknown	Unknown
ERR2299892	Unknown	United Kingdom	SRR7976616	2015	United Kingdom
ERR2299893	Unknown	United Kingdom	SRR7976613	2017	United Kingdom
ERR2299891	Unknown	United Kingdom	SRR7976550	2016	United Kingdom
ERR2299882	Unknown	United Kingdom	SRR7976606	2017	United Kingdom
ERR2299884	Unknown	United Kingdom	SRR7976580	2017	United Kingdom
ERR2299881	Unknown	United Kingdom	SRR7976597	2016	United Kingdom
ERR2299872	Unknown	United Kingdom	SRR7976581	2017	United Kingdom
ERR2299883	Unknown	United Kingdom	SRR7976611	2017	United Kingdom
ERR2299871	Unknown	United Kingdom	SRR7976549	2015	United Kingdom
ERR2299869	Unknown	United Kingdom	SRR7976604	2017	United Kingdom
ERR2299873	Unknown	United Kingdom	SRR7976593	2017	United Kingdom
ERR2299875	Unknown	United Kingdom	SRR7976569	2017	United Kingdom
ERR2299886	Unknown	United Kingdom	SRR7976603	2017	United Kingdom
ERR2299880	Unknown	United Kingdom	SRR7976614	2016	United Kingdom
ERR2299878	Unknown	United Kingdom	SRR7976579	2015	United Kingdom
ERR2299889	Unknown	United Kingdom	SRR7976570	2017	United Kingdom
ERR2299890	Unknown	United Kingdom	SRR7976558	2017	United Kingdom
ERR2299876	Unknown	United Kingdom	SRR7976599	2017	United Kingdom
ERR2299888	Unknown	United Kingdom	SRR7976556	2017	United Kingdom
SRR3883466	2012	Venezuela	SRR7976594	2017	United Kingdom
SRR3883468	2012	Venezuela	SRR7976562	2017	United Kingdom
SRR3883464	2013	Venezuela	SRR7976565	2017	United Kingdom
SRR3883465	2012	Venezuela	SRR7976575	2017	United Kingdom
SRR3883467	2012	Venezuela	SRR7976560	2017	United Kingdom
SRR7140043	2016	Colombia	SRR7976589	2017	United Kingdom
SRR7140042	2016	Colombia	SRR7976577	2016	United Kingdom
SRR7140052	2016	Colombia	SRR7976559	2017	United Kingdom

Accession number	Year	Country	Accession no.	Year	Country
SRR7140069	2016	Colombia	SRR7976608	2017	United Kingdom
SRR7140058	2016	Colombia	SRR7976553	2017	United Kingdom
SRR7140059	2016	Colombia	SRR7976587	2017	United Kingdom
SRR7140010	2016	Colombia	SRR7976584	2016	United Kingdom
SRR7140009	2016	Colombia	SRR7976543	2017	United Kingdom
SRR7140022	2016	Colombia	SRR7976601	2017	United Kingdom
SRR7140020	2016	Colombia	SRR7976612	2017	United Kingdom
SRR7140076	2016	Colombia	SRR7976576	2017	United Kingdom
SRR7140006	2016	Colombia	SRR7976605	2017	United Kingdom
SRR7140044	2016	Colombia	SRR7976590	2017	United Kingdom
SRR7140025	2016	Colombia	SRR7976547	2017	United Kingdom
SRR7140063	2016	Colombia	SRR7976586	2017	United Kingdom
SRR7140032	2016	Colombia	SRR7976602	2017	United Kingdom
SRR7140007	2016	Colombia	ERR2300804	Unknown	Unknown
SRR7140001	2016	Colombia	ERR2300783	Unknown	Unknown
SRR7140035	2016	Colombia	SRR7976566	2017	United Kingdom
SRR7140039	2016	Colombia	SRR7976583	2017	United Kingdom
SRR7140045	2016	Colombia	SRR7976564	2017	United Kingdom
SRR7140061	2016	Colombia	SRR7976600	2017	United Kingdom
SRR7140004	2015	Colombia	SRR7976552	2017	United Kingdom
SRR7140054	2016	Colombia	SRR7976609	2017	United Kingdom
SRR7140033	2015	Colombia	SRR7976610	2017	United Kingdom
SRR7140038	2016	Colombia	SRR7976541	2017	United Kingdom
SRR7140013	2016	Colombia	SRR7976572	2017	United Kingdom
SRR7140040	2016	Colombia	SRR7976544	2017	United Kingdom
SRR7140030	2016	Colombia	SRR7976617	2017	United Kingdom
SRR7140082	2015	Colombia	SRR7976598	2017	United Kingdom
SRR7140017	2016	Colombia	SRR7976545	2017	United Kingdom
SRR7140024	2016	Colombia	SRR7976555	2017	United Kingdom
SRR7140047	2016	Colombia	SRR7976573	2017	United Kingdom
SRR7140003	2016	Colombia	SRR7976585	2017	United Kingdom
SRR7140068	2016	Colombia	SRR7976582	2015	United Kingdom
SRR7140074	2016	Colombia	SRR7976540	2017	United Kingdom
SRR7140041	2015	Colombia	SRR7976567	2017	United Kingdom
SRR7140029	2015	Colombia	SRR7976546	2017	United Kingdom
SRR7140080	2016	Colombia	SRR7976548	2017	United Kingdom
SRR7140056	2015	Colombia	SRR7976615	2017	United Kingdom
SRR7140070	2015	Colombia	SRR7976591	2016	United Kingdom
SRR7140015	2015	Colombia	SRR7976561	2017	United Kingdom
SRR7140012	2015	Colombia	SRR7976557	2016	United Kingdom
SRR7140071	2015	Colombia	SRR7976568	2017	United Kingdom
SRR7140077	2016	Colombia	SRR7976574	2017	United Kingdom
SRR7140062	2015	Colombia	SRR7976592	2017	United Kingdom
SRR7140002	2016	Colombia	SRR3883473	2015	Pakistan
SRR7140055	2015	Colombia	SRR3883439	Unknown	India
SRR7140075	2016	Colombia	SRR3883437	Unknown	India
SRR7140008	2015	Colombia	SRR3883451	Unknown	India
SRR7140078	2016	Colombia	SRR3883436	Unknown	India
SRR7140066	2016	Colombia	ERR899743	Unknown	Unknown
SRR7140065	2016	Colombia	SRR3883434	Unknown	India
SRR7140026	2016	Colombia	ERR1519358	Unknown	Unknown
SRR7140023	2016	Colombia	ERR1519357	Unknown	Unknown
SRR7140049	2016	Colombia	SRR3883435	Unknown	India
SRR7140073	2016	Colombia	SRR3883440	Unknown	India
SRR7140027	2016	Colombia	SRR3883449	2014	Pakistan
SRR7140072	2016	Colombia	SRR3883429	2015	Pakistan
SRR7140014	2016	Colombia	SRR3883471	2015	Pakistan
SRR7140037	2016	Colombia	SRR3883430	2015	Pakistan
SRR7140021	2015	Colombia	SRR3883438	2014	Pakistan
SRR7140064	2016	Colombia	ERR1519359	Unknown	Unknown
SRR7140036	2015	Colombia	SRR1664628	2013	India
SRR7140067	2015	Colombia	SRR1664626	2013	India
SRR7140016	2016	Colombia	SRR1664627	2013	India
SRR7140031	2015	Colombia	SRR3883460	2014	Pakistan
SRR7140011	2016	Colombia	SRR3883470	2014	Pakistan
SRR7140028	2016	Colombia	SRR3883426	2014	Pakistan
SRR7140034	2016	Colombia	SRR3883432	2015	Pakistan
SRR7140051	2016	Colombia	SRR3883474	2015	Pakistan
SRR7140019	2016	Colombia	SRR3883427	2014	Pakistan

Accession number	Year	Country	Accession no.	Year	Country
SRR7140048	2016	Colombia	SRR3883431	2015	Pakistan
SRR7140057	2015	Colombia	SRR3883428	2015	Pakistan
SRR7140079	2016	Colombia	SRR3883433	2015	Pakistan
SRR7140018	2016	Colombia	SRR3883444	Unknown	India
SRR7140046	2016	Colombia	ERR2300793	Unknown	Unknown
SRR7140050	2016	Colombia	ERR2300806	Unknown	Unknown
SRR7140060	2016	Colombia	ERR2300790	Unknown	Unknown
SRR7140053	2016	Colombia	ERR2300792	Unknown	Unknown
SRR7140005	2016	Colombia	ERR2300805	Unknown	Unknown
SRR7140081	2016	Colombia	ERR2300784	Unknown	Unknown
ERR2300774	Unknown	Unknown	ERR2300803	Unknown	Unknown
SRR3883452	2009	Japan	ERR2300794	Unknown	Unknown
SRR3883463	2014	South Africa	ERR2300791	Unknown	Unknown
SRR3883457	2014	South Africa	ERR2300799	Unknown	Unknown
SRR3883455	2012	South Africa	SRR3883441	Unknown	India
SRR3883453	2012	South Africa	SRR3883450	Unknown	India
SRR3883454	2012	South Africa	SRR3883446	Unknown	India
SRR3883461	2014	South Africa	SRR7507279	2017	USA
SRR3883456	2012	South Africa	SRR3883472	2015	Pakistan
SRR3883458	2014	South Africa	SRR6220384	2015	Pakistan
SRR3883459	2014	South Africa	SRR3883447	Unknown	India
SRR3883462	2014	South Africa	SRR3883442	Unknown	India
ERR2300770	Unknown	Unknown	SRR3883445	Unknown	India
ERR2300798	Unknown	Unknown	SRR3883443	Unknown	India
ERR2300810	Unknown	Unknown	SRR3883448	Unknown	India
ERR2300769	Unknown	Unknown			

Appendix Table 2. Antimicrobial susceptibility profile of isolates of *Candida auris* identified in Victoria, Australia, July– December 2018

		Specimen	Minimum inhibitory concentration (μg/mL)				
Patient	Collection date	source	Amphotericin B	Fluconazole	Voriconazole	Micofungin	Anidulafungin
1	10-Jul-18	Urine	0	256	1	0.12	0.12
2	12-Aug-18	Urine	4	256	2	0.25	0.25
3	5-Sep-18	Groin swab	2	256	1	0.12	0.12
	5-Sep-18	Axilla swab	2	256	1	0.12	0.12
4	29-Nov-18	Ear swab	0.5	16	0.5	2	0.5

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