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Candida auris in Singapore: Genomic epidemiology, antifungal drug resistance, and identification using the updated 8.01 VITEK®2 system

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ABSTRACT

Objectives: *Candida auris* (*C. auris*) has globally emerged as a multidrug-resistant pathogen. While it is known that there are four geographic clades, little is known about its genomic epidemiology in the Southeast Asian region. Laboratory identification can be challenging but the VITEK®2 system (version 8.01 software) has recently updated its database to include *C. auris*. This study aimed to investigate the genomic epidemiology of *C. auris* isolated in Singapore and the susceptibility profiles in relation to *ERG11* and *FKS1* mutations.

Methods: Seven *C. auris* isolates from 2012–2018 were analysed using whole-genome sequencing, and antifungal susceptibility testing was performed. The performance of the updated VITEK®2 system in identifying *C. auris* was also evaluated using these *C. auris* strains together with five closely related *Candida* species.

Results: Three clades were identified: South Asian (71.4%), South American (14.3%) and East Asian (14.3%). Local transmission was unlikely as there was no obviously identified cluster and most cases were likely to be imported at different time points following overseas hospitalisation exposure. Three isolates (42.9%) were multidrug-resistant. All South Asian strains were resistant to fluconazole and harboured *ERG11* mutations, which were clade-specific. No *FKS1* mutation was detected. The VITEK®2 system was able to correctly identify most of the South Asian *C. auris* strains but misidentified the East Asian strain and gave a low discrimination result for the South American clade.

Conclusion: This study showed that the introduction of *C. auris* into Singapore was possibly over multiple episodes and from different sources. The VITEK®2 System version 8.01 software has limited abilities in identifying *C .auris*.

1. Introduction

Candida auris (*C. auris*), an emerging multidrug-resistant fungal pathogen, has been implicated in invasive infections, including candidaemia, and is associated with high morbidity and mortality [1]. Since its first description in Japan in 2009, *C. auris* has been detected in multiple countries across six continents and has been responsible for large nosocomial outbreaks in India, the United States and United Kingdom [2,3]. Owing to its multidrug-resistant characteristics, high virulence, difficulty in microbiological identification, and high transmissibility in healthcare settings, *C. auris* is considered to be an emerging serious global public health threat [4]. At present, *C. auris* is separated into four geographic clades: South Asian (clade I), East Asian (clade II), South African (clade III), and South American (clade IV). Genetic analyses from previous studies have indicated the simultaneous emergence of these separate clades of *C. auris* in various geographical regions [1,5].

The genomic epidemiology of *C. auris* has been described on a global level but is lacking in the Southeast Asian region. There was one case report from Malaysia [6]. Previously, a small case series on *C. auris* was reported in Singapore but the clade and genomic relationship of these isolates were unknown [7]. Knowledge of the clades is important, as different clades may have phenotypic

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differences due to large genetic distances [8,9] and all four clades may not be well-represented in the databases of many commercial identification systems. This may compromise the accurate identification of *C. auris* strains. Although the VITEK®2 automated identification system (bioMérieux) has recently included *C. auris* into their database (version 8.01), the performance of the system has not been evaluated. Further, different clades may have different antifungal susceptibility profiles and gene mutations [1,8,9]: *C. auris* isolates from clade II tend to be less invasive and have lower propensity to cause outbreaks [5]. This has infection control implications. Study of the phylogenetic links of locally isolated strains to other geographic clades may provide insights into the introduction of *C. auris* into Singapore, as well as infection control measures to be taken.

This study aimed to investigate the genomic epidemiology and antifungal susceptibility of *C. auris* in Singapore, in relation to specific mutations in the *ERG11* and *FKS1* genes. The secondary objective was to assess the reliability of the VITEK®2 system (version 8.01) in the identification of these *C. auris* isolates.

2. Materials and Methods

2.1. Candida auris isolates

Seven non-duplicate isolates from seven patients were analysed. These isolates were collected from Singapore General Hospital between 2012 and 2018. Singapore General Hospital is the largest acute care tertiary hospital in the country, accounting for approximately 25% of the total acute hospital beds in the public sector. The microbiology laboratory also receives testing requests from private hospitals in Singapore. The species were identified using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (BrukerDaltonik GmbH, Germany) and supported by other phenotypic tests such as failure to grow in the presence of cycloheximide and the ability to grow at 42°C. Identification was further confirmed by internal transcribed spacer (ITS) sequencing. Isolates were stored in Microbank™ storage vials (Pro-Lab Diagnostics, Round Rock, TX, USA) at −70°C and subcultured twice on sheep blood agar for 24–48 hours at 30 °C prior to use, to ensure adequate growth and purity.

This study was not subjected to a local institutional review board as there was no patient identifiers collected.

2.2. Antifungal susceptibility testing

In vitro susceptibility testing for nine antifungals (fluconazole, voriconazole, itraconazole, posaconazole, caspofungin, anidulafungin, micafungin; amphotericin B, flucytosine) was performed using the Sensititre™ YeastOne® microdilution panel (TREK Diagnostics System Ltd, Thermo Scientific) according to the manufacturer's recommendations. *Candida parapsilosis* American Type Culture Collection (ATCC) 22019 and *Candida krusei* (*Issatchenkia orientalis*) ATCC 6258 were used as quality controls.

There are no species-specific breakpoints for *C. auris* currently established by the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Minimum inhibitory concentrations (MICs) were interpreted in accordance to the United States Centres for Disease Control and Prevention (US CDC) tentative breakpoints [10].

2.3. DNA extraction and whole-genome sequencing

Genomic DNA was extracted and purified from overnight exponential-phase yeast peptone broth cultures with the Qiagen DNeasy PowerSoil Kit (Qiagen Inc., Valencia, CA, USA). Extracted gDNA was quantified using a Qubit™ 2.0 fluorometer and dsDNA BR assay kit (Life Technologies, Carlsbad, CA, USA). Quality control (purity and integrity) of gDNA was conducted using the NanoDrop instrument (Thermo Scientific, Wilmington, MA) and Fragment Analyzer™ (Advanced Analytical Technologies GmbH, Heidelberg, Germany). Purified gDNAs were stored at −20°C until library preparation. Genomic libraries were constructed and barcoded using the TruSeq DNA PCR-Free kit (Illumina, San Diego, CA, USA) in accordance with the manufacturer's instructions. Paired-end sequencing was conducted on the Illumina MiSeq platform (2×250 bp).

2.4. Genome assembly and single-nucleotide polymorphism identification

Quality check was performed on the raw Illumina reads using FastQC (v0.11.7) (Babraham Institute; http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were trimmed using Trimmomatic (v.0.36) [11], removing adapters and poor-quality bases. The filtered reads were subsequently aligned using the Burrows-Wheeler aligner (BWA) maximal exact matches (MEM) algorithm (v0.7.17) [12] to the publicly available genome of the Pakistan isolate B8441 (National Center for Biotechnology Information Sequence Read Archive BioProject PRJNA328792), which was used in the study by Lockhart et al. The output files were then indexed and converted to sorted BAM format using SAMtools (v1.9) [13]. Prior to variant calling, local indel realignment was conducted with Genome Analysis Toolkit (GATK) (v3.8-1) [14]. Mapping statistics were assessed using QualiMap (v2.2.1) [15].

Phylogenetic analysis was conducted by comparing the sequences in this study with the representative sequences from the four major clades (I–IV) to assess for sequence similarity. These were obtained from the study by Lockhart et al. [1]. Consensus genotypes at each loci/position for the *C. auris* genomes were determined based on the output of the mpileup commands as implemented in BCFtools (v1.9). Single nucleotide polymorphisms (SNPs) for each *C. auris* genome were then identified as genotype calls that differed from the DNA base in the B8441 reference genome. The same filtering criteria as those in the study by Lockhart et al. were used (i.e. SNP calls were filtered if they were identified in repetitive regions, supported by $< 90\%$ of the base calls at that position by the reads mapped, and did not have a minimum read depth coverage of 10). Based on the combined set of SNP positions identified across the *C. auris* isolates, the DNA bases at these positions were retrieved for each sample to construct a concatenated FASTA sequence, which was used as input to the program FastTree (http://www.microbesonline.org/fasttree/) for inference analysis of maximum-likelihood phylogenetic tree generated based on the generalised time reversible model. Read data were deposited into the National Center for Biotechnology Information's Sequence read Archive under BioProject PRJNA540907.

2.5. Resistance gene analyses

Azole and echinocandin resistance have been found to be closely related to specific mutations in the *ERG11* and *FKS1* genes, respectively, and are recommended as diagnostic markers for drug-resistant *C. auris* [16]. Orthologous sequences to *Candida albicans ERG11* and *FKS1* (strain SC5314) (Candida Genome Database; http://www.candidagenome.org/) were extracted from each sample and aligned using the https://www.megasoftware.net/web_help_10/Part_II_Assembling_Data_For_Analysis/Building_Sequence_Alignments/ClustalW/About_ClustalW.htm/ ClustalW alignment program in MEGA software (v7.0.26). Isolates were screened for amino acid substitutions, which were known to confer resistance in *C. auris* (azole resistance – F126, Y132, K143; echinocandin resistance S639) [17–19].

2.6. VITEK®2 automated identification system

All seven molecularly identified *C. auris* isolates were grown on sheep blood agar for 48 hours and tested using the VITEK®2 yeast identification card (YST) according to the manufacturer's instructions. The updated software version 8.01 was used in the analysis. Purity check was performed for each isolate. Of note, quality control for YST identification card was performed using *C. albicans* ATCC 14053 and it passed satisfactorily. Two *Candida haemulonii* (*C. haemulonii*) and three *Candida duobushaemulonii* (*C. duobushaemulonii*) isolates were also included in the evaluation of the updated VITEK®2 database (bioMérieux, version 8.01). The identification of these closely related *Candida* species was confirmed by ITS sequencing.

3. Results

3.1. Phylogenetic analysis by whole-genome sequencing

A total of seven *C. auris* isolates were analysed by whole-genome sequencing and the assembled genome size was 12.5 Mb, which was similar to previous studies [1]. Reference-based phylogenetic analysis was performed and three distinct geographic clades were demonstrated, which were separated by tens of thousands of SNPs. Of the seven isolates from the collection, five (71.4%) were identified to be South Asian clade, one (14.3%) was identified to be South American clade, and one (14.3%) belonged to the East Asian clade (Table 1, Figure 1). There was no South African clade. The SNP differences amongst the tested isolates ranged from 22–119 792. The lowest SNP difference was observed between ca03 and ca04 from the South Asian clade, which were isolated from 2016 and 2017, respectively.

3.2. ERG11 *and* FKS1 *mutations analysis*

All *C. auris* isolates were screened for F126, Y132, K143 and S639, which were known to confer resistance to azole and echinocandin, respectively. *ERG11* mutations were detected in all tested South Asian *C. auris* strains, with Y132F detected in three isolates and K143R detected in two isolates. There was no mutation detected at F126. The *FKS1* mutation was absent in all the tested isolates at S639 (Table 1).

3.3. Antifungal susceptibility testing

Antifungal susceptibility testing was performed on all *C. auris* isolates and the results are shown in Table 1. Based on the US CDC tentative breakpoints, the majority (71.4%) of the tested isolates were resistant to fluconazole (MIC ≥ 256 μg/mL) with a corresponding voriconazole MIC range of 1–2 μg/mL. These five isolates belonged to the South Asian clade. The other two (one from South American clade and one from East Asian clade) isolates were susceptible to fluconazole (MIC 8 μg/mL) with a corresponding lower MIC for voriconazole at 0.06 μg/mL as compared with the South Asian strains. All the isolates were susceptible to echinocandins, except for one South Asian strain that displayed phenotypical resistance to caspofungin (MIC ≥ 8 μg/mL). Resistance to amphotericin B was detected in four isolates (57.1%; three from South Asian clade and one from South American clade) with the South American strain displaying the highest MIC (4 μg/mL) amongst the tested isolates. Three isolates (42.9%; all from the South Asian clade) were resistant to at least two classes of antifungals (multidrug-resistant) with one of them demonstrating resistance to fluconazole, caspofungin and amphotericin B.

Table 1
Characteristics and antifungal susceptibility profiles of *Candida auris* isolates in Singapore.

Isolate No.	Patient	Nationality/history of overseas hospitalisation	Year of isolation	Specimen site	Clade	Antifungal MIC in μg/mL (Interpretation)[a]								*ERG11* mut-ation[b]	*FKS1* mut-ation[c]
						FLU	VRC	ITC	POS	CAS	AFG	MFG	AMB	5FU	
ca01	1	Local/yes	2012	Femur tissue	South Asian	256 (R)	1 (NA)	0.25 (NA)	0.06 (NA)	≥ 8 (R)	0.5 (S)	0.25 (S)	2 (R)	0.25 (NA)	Y132F	ND
ca02	2	Foreigner/unknown	2015	Blood	South Asian	> 256 (R)	1 (NA)	0.25 (NA)	0.12 (NA)	0.25 (S)	0.25 (S)	0.12 (S)	2 (R)	0.12 (NA)	K143R	ND
ca03	3	Foreigner/yes	2016	Blood	South Asian	> 256 (R)	1 (NA)	0.12 (NA)	0.06 (NA)	0.06 (S)	0.12 (S)	0.12 (S)	2 (R)	≤ 0.06 (NA)	Y132F	ND
ca04	4	Foreigner/yes	2017	Blood	South Asian	256 (R)	2 (NA)	0.12 (NA)	0.06 (NA)	0.12 (S)	0.12 (S)	0.06 (S)	1 (S)	≤ 0.06 (NA)	Y132F	ND
ca05	5	Foreigner/yes	2018	Blood	South American	8 (S)	0.06 (NA)	0.25 (NA)	0.06 (NA)	0.25 (S)	0.25 (S)	0.12 (S)	4 (R)	≤ 0.06 (NA)	ND	ND
ca06	6	Foreigner/unknown	2018	Scrotal wound	South Asian	256 (R)	2 (NA)	0.5 (NA)	0.25 (NA)	0.12 (S)	0.12 (S)	0.12 (S)	1 (S)	≤ 0.06 (NA)	K143R	ND
ca07	7	Local/no	2018	Ear swab	East Asian	8 (S)	0.06 (NA)	0.06 (NA)	0.015 (NA)	0.25 (S)	0.12 (S)	0.06 (S)	1 (S)	0.5 (NA)	ND	ND

[a] There are currently no established susceptibility breakpoints for *C. auris*. Interpretation is based on the US CDC tentative MIC breakpoints for *C. auris*
[b] Only Y132, K143, F126 were screened for azole resistance
[c] Only S639 was screened for echinocandin resistanceFluconazole ≥ 32 μg/mL (R), amphotericin B ≥ 2 μg/mL (R), anidulafungin ≥ 4 μg/mL (R), caspofungin ≥ 2 μg/mL (R), micafungin ≥ 4 μg/mL (R)Abbreviations: S, susceptible; R, resistant; NA, not applicable; FLU, fluconazole; VRC, voriconazole; ITC, itraconazole; POS, posaconazole; CAS, caspofungin; AFG, anidulafungin; MFG, micafungin; AMB, amphotericin B; 5FU, 5-flucytosine; ND, not detected

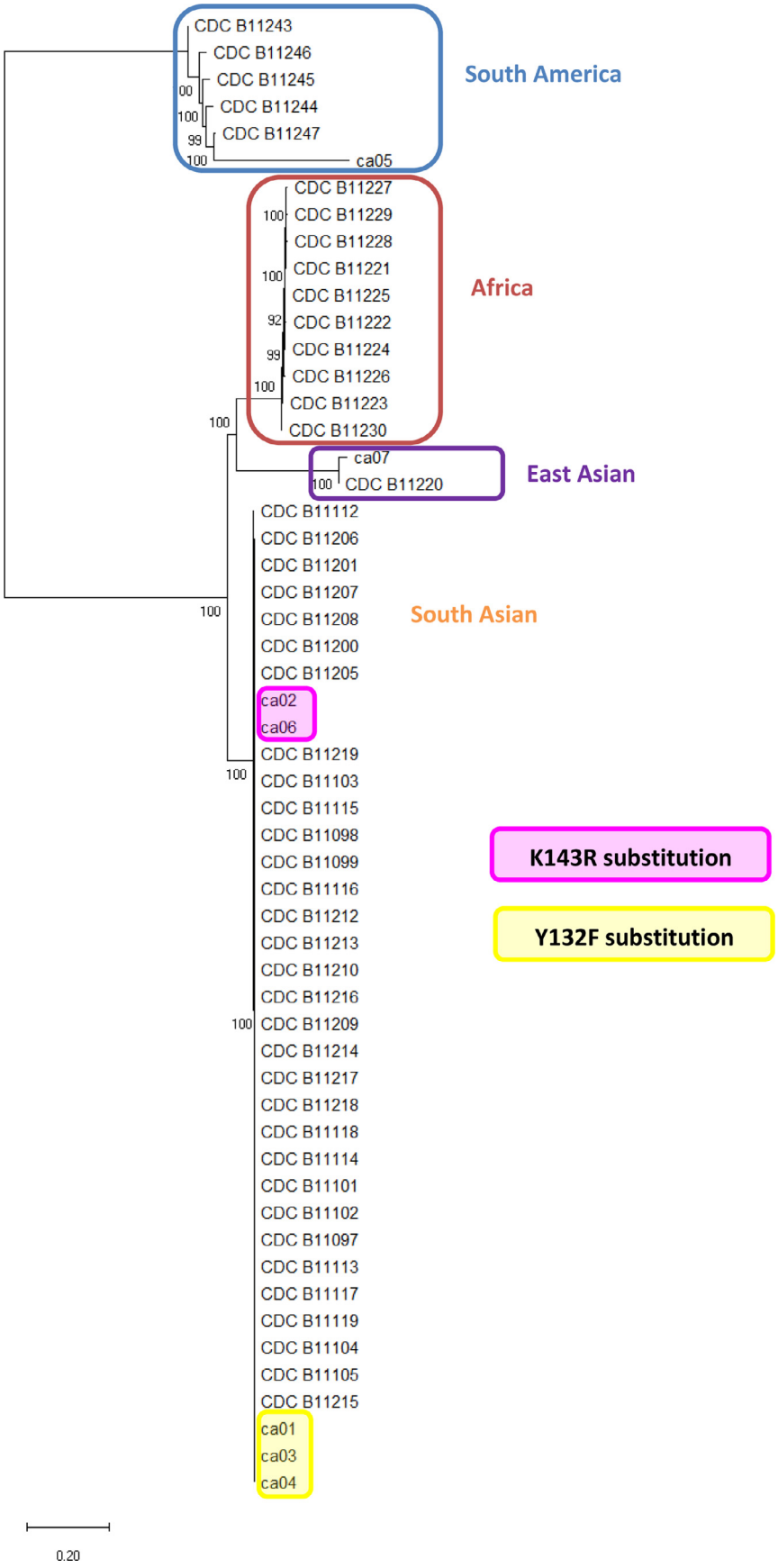

Figure 1. Phylogenetic relationships among isolates.

Table 2
Identification by VITEK®2 system (Version 8.01) for *Candida auris* and closely related species.

Isolate No.	Identification (ITS)[a]	Clade[b]	Identification (Vitek®2 v8.01)	Probability (%)
ca01	*Candida auris*	South Asian	*Candida duobushaemulonii*	94
ca02	*Candida auris*	South Asian	*Candida auris*	99
ca03	*Candida auris*	South Asian	*Candida auris*	99
ca04	*Candida auris*	South Asian	*Candida auris*	99
ca05	*Candida auris*	South American	*Candida auris/duobushaemulonii*	Low discrimination
ca06	*Candida auris*	South Asian	*Candida auris*	95
ca07	*Candida auris*	East Asian	*Candida duobushaemulonii*	99
ca08	*Candida duobushaemulonii*	NA	*Candida duobushaemulonii*	95
ca09	*Candida duobushaemulonii*	NA	*Candida duobushaemulonii*	97
ca10	*Candida duobushaemulonii*	NA	*Candida duobushaemulonii*	93
ca11	*Candida haemulonii*	NA	*Candida haemulonii/ Candida haemulonii* var *vulnera*	Low discrimination
ca12	*Candida haemulonii*	NA	*Candida haemulonii/ Candida haemulonii* var *vulnera*	Low discrimination

[a] ITS, internal transcribed spacer sequencing.
[b] NA, Not applicable.

3.4. VITEK®2 yeast identification system (version 8.01) evaluation

The identification results of the updated system for *C. auris* and its closely related species are shown in Table 2. The overall correct identification to the species level by VITEK®2 system is 58.3% (seven of 12 isolates) with a probability range of 93–99%. Low discrimination results were obtained for three isolates (25%) that involved all the tested *C. haemulonii* and one *C. auris* South American strain. The VITEK®2 system was unable to differentiate between *C. auris* and *C. duobushaemulonii* for the South American strain. Although low discrimination results were obtained for both *C. haemulonii* isolates, the updated system was able to identify the correct species level.

Two of the *C. auris* isolates, consisting of one South Asian and the East Asian strain, were misidentified as *C. duobushaemulonii* with high probabilities of 94% and 99%, respectively. The updated software was able to identify most *C. auris* South Asian strains with 80% (four of five isolates) of the tested isolates belonging to this clade being correctly identified. All three (100%) *C. duobushaemulonii* isolates were also correctly identified with a probability range of 93–97%.

4. Discussion

Candida auris was first detected in Singapore in 2012 [7]. Phylogenetic analysis based on whole-genome SNPs revealed that the tested isolates from Singapore did not possess their 'own lineage' but were found to be in three of the four major clades: South Asian, South American, and East Asian. Most of the strains were isolated from the bloodstream. Patients 1 and 7 were locals, while the rest were foreigners largely from the South Asian region. Patient 1 was a local-born Chinese who was transferred from an Indian hospital after encountering an accident there [7]. Patient 7 was a local elderly man who presented with chronic otitis media. He had no known history of a recent overseas stay. Patients 3–5 were transferred directly from overseas hospitals for further medical management. The history of overseas hospitalisation was unknown for Patients 2 and 6 (Table 1). Most of the cases were likely to have been imported at different time points. All five strains belonging to the South Asian clade were isolated in different years. There was one South American clade strain from the collection belonging to Patient 5 and one East Asian clade strain isolated from an ear swab from Patient 7 (Table 1). Interestingly, the isolation of the East Asian clade was similar to the first case of *C. auris* reported from Japan in 2009 [2]. In a recent Korean study by Kwon et al., it was found that > 93% of the clade II isolates was cultivated from ear infections. It appears that the East Asian clade has a propensity for the ear and is less invasive when compared with other clades [5,9]. These epidemiological findings suggested that the local introduction of *C. auris* was via multiple novel introductions of the pathogen into healthcare settings. Local transmission was highly unlikely. This was further supported by the number of SNP differences (range 22–119 792) between isolates. According to Chow et al., an SNP difference of ≤ 12 is more likely to indicate recent transmission [20]. An SNP difference of 22 between ca03 and ca04 may have been due to low genetic diversity within the same clade [1] or linked to the same overseas hospital, which both patients were transferred from at different years. There was no locally isolated *C. auris* in between the admissions of these two cases and the next identified *C. auris* belonged to the South American clade isolated in 2018 (Table 1, Figure 1). It is thus important to interpret genomic data with epidemiological information that would otherwise confound the findings.

Gaining insights into the introduction of *C. auris* in Singapore will help to devise appropriate infection control and preventive measures. The majority of the cases were from patients with a recent history of overseas hospitalisation in areas with high incidence of *C. auris* and carbapenemase-producing Gram-negative bacteria. Co-colonisation with these two pathogens is not uncommon, as demonstrated by the first three reported *C. auris* cases [7]. Hence, it may be appropriate to screen patients who have been admitted to overseas healthcare facilities in the previous year, especially from a country reported to have *C.* auris, as recommended by the US CDC [21]. It may also be worthwhile to explore if the knowledge of the clade will determine strategies in *C. auris* surveillance. This is because the East Asian clade (clade II) has been linked with a lower propensity to cause an outbreak as compared with other clades and is generally less resistant [5]; however, this requires further validation in subsequent studies.

Candida auris can be multidrug-resistant, with some isolates displaying resistance to all three major antifungal classes [22]. Isolates are typically resistant to fluconazole but this appears to be clade-specific and can vary widely [23,24]. According to the US CDC guidelines, *C. auris* isolates with fluconazole MIC ≥ 32 μg/mL are more likely to harbour *ERG11* mutations and, hence, less likely to respond to fluconazole [10]. Lockhart et al. demonstrated that mutations were detected in the *ERG11* gene in most of the isolates with raised MICs to fluconazole and voriconazole. The amino acid substitutions were strongly clade-associated: *F126T* in the South African clade, *Y132F* or *K143R* in the South Asian clade, and *Y132F* in the South American clade [1]. Interestingly, Kwon et al. found 7% of the tested fluconazole-resistant isolates from Korea to have mutations in the *ERG11* gene [9]. It appears that clade II is less resistant compared with other geographic clades [5]. Similar observations were seen in the current study: all five isolates belonging to the South Asian clade had higher MICs to fluconazole and voriconazole than the South American and East Asian strains; with *Y132F* or *K143R* mutations detected in the *ERG11* gene (Table 1).

These mutations were not found in other clades. No mutation was detected at F126 in all the tested strains, which was not surprising as this mutation is strongly associated with the South African clade, which was lacking in this collection. However, it is important to note that there are other possible mechanisms for azole resistance such as the upregulation of drug efflux pumps (e.g. *CDR1* and *MDR1*) and oligopeptide transporters [24,25].

There were also studies that reported high MICs to caspofungin and amphotericin B [22,26]. The current study only screened for *FKS1* mutations, particularly *S639F* amino acid substitution as this was the main mechanism of resistance to echinocandin [24]. There was no *FKS1* gene mutation detected in all the tested *C. auris* isolates, although there was one isolate (ca01) with a raised MIC value to caspofungin but susceptible to micafungin and anidulafungin (Table 1). This may have been due to the Eagle effect [24]. The broth microdilution method may not be reliable for testing caspofungin. Instead, micafungin is a better indicator for echinocandin resistance or the detection of *FKS1* mutations [24]. The mechanism of resistance for amphotericin B in *C. auris* is less well established. Escandon et al. described that there was a higher rate of amphotericin B resistance in the northern part of Columbia compared with the central region [26]. Coincidentally, the only South American strain in the current collection had higher amphotericin B MIC than the rest of the clades (Table 1).

Up to 42.9% of the tested isolates in this study were multidrug-resistant and these isolates belonged to the South Asian clade. To date, all *C. auris* isolates were generally susceptible to the echinocandin class, such as anidulafungin and micafungin, which remained the first-line therapy for *C. auris* infection [27]. In the near future, a novel long-acting echinocandin CD101 may provide an alternative in *C. auris* treatment [28]. However, it is important to note that it is ineffective against biofilms [29]. Although most of the tested strains displayed high MICs to fluconazole and voriconazole, the MICs to posaconazole and itraconazole remained relatively low. This may have been due to the difference in the chemical structures of the various azoles [23]. The decision to treat with another azole apart from fluconazole is on a case-by-case basis [10].

Laboratory identification of *C. auris* in diagnostic laboratories can be challenging. Most commercial systems do not have *C. auris* in their databases, resulting in misidentification of this pathogenic yeast. Previously, the VITEK®2 identification system used to misidentify *C. auris* as *C. haemulonii*, as the older software version did not include *C. auris* and *C. duobushaemulonii*. With the updated version 8.01, both yeasts are now included in the database, thus enabling routine microbiology laboratories to identify these yeasts without any errors. However, there have been limited studies on the performance of this updated identification system. The identification results of the updated system for *C. auris* and its closely related species shown in Table 2 were not entirely surprising. Sharp et al. conducted a small survey on VITEK®2 systems in the identification of *C. auris* in the United Kingdom and found that the updated version was unable to correctly identify *C. auris* belonging to the East Asian clade [30]. This was similar to the current finding. East Asian clade isolates are phenotypically unique, as they do not assimilate N-acetylglucosamine (NAG), unlike the South Asian clade isolates [9]. It appears that not all the clades are well-represented in the database and it is highly possible that routine microbiology laboratories may still misidentify *C. auris* with this updated software, which is clade-dependent. It has limited abilities in correctly identifying the South American and East Asian *C. auris* strains. Despite this, the identification of *C. auris* can be confirmed if the VITEK®2 identification system (version 8.01) reports a good score. Confirmation by other methods such as MALDI-TOF and/or ITS sequencing is only required if the score is poor. The US CDC also recommends all *C. duobushaemulonii* identified by the system be further worked up to exclude *C. auris* [31]. Although low discrimination results were obtained for both *C. haemulonii* isolates, the updated system was able to correctly identify to the species level (Table 2).

This study had some limitations. First, it only screened for mutations that were known to confer azole and echinocandin resistance at the *ERG11* and *FKS1* genes, respectively. Second, the number of studied *C. auris* isolates was small and there was no South African clade isolate with which to evaluate the updated VITEK®2 version 8.01 software.

In conclusion, *C. auris* remains uncommon in Singapore. Only isolated cases of *C. auris* have been detected and most of them are likely to be imported from patients with a history of overseas hospitalisation in regions that have a high incidence of *C. auris*. There was no evidence of a locally established outbreak. Echinocandin remains a good option for first-line therapy. Microbiology laboratories that use the VITEK®2 identification system as part of their routine testing have to be alerted that the updated version 8.01 software is unable to identify some of the clades of *C. auris*. This is important to avoid misdiagnosis, which has implications for infection control practices. The knowledge of the clades is useful in understanding the degree of pathogenicity, outbreak propensity and antifungal resistance profile of *C. auris*. The current phylogenomic analyses suggests that the local introduction of *C. auris* was via multiple novel introductions of the pathogen into healthcare settings with South Asian, East Asian and South American clades identified in local isolates. Continued surveillance of this pathogen is essential to monitor the emergence of *C. auris* in Singapore. Vigilance is needed to prevent this fungus from gaining a foothold in healthcare settings and cause potential outbreaks.

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Declarations

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Ethical Approval: Not required.

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