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Evolutionary accumulation of *FKS*1 mutations from clinical echinocandin-resistant *Candida auris*

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ABSTRACT

Introduction: Drug resistance to echinocandins, first-line drugs used to treat *Candida auris* infection, is rapidly emerging. However, the accumulation of mutations in genes other than *FKS*1 (before an isolate develops to resistance via *FKS*1 mutations), remains poorly understood. *Methods:* Four clinical cases and 29 isolates associated with the incremental process of echinocandin resistance were collected and analyzed using antifungal drug susceptibility testing and genome sequencing to assess the evolution of echinocandin resistance. Findings: Six echinocandin minimum inhibitory concentration (MIC)-elevated *C. auris* strains and seven resistant strains were isolated from the urinary system of patients receiving echinocandin treatment. Meanwhile, phylogenetic analyses illustrated that the echinocandin-resistant strains were closely related to other strains in the same patient. Genomic data revealed that the echinocandin-resistant strains had *FKS*1 mutations. Furthermore, three categories (ECN-S/E/R) of non-synonymous mutant SNP genes (such as *RBR*3, *IFF*6, *MKC*1, *MPH*1, *RAD*2, and *MYO*1) in *C. auris* appeared to be associated with the three-stage-evolutionary model of echinocandin resistance in *C. glabrata*: cell wall stress, drug adaptation, and genetic escape (*FKS* mutation). Interpretation: Echinocandin-resistant *C. auris* undergoes spatial and temporal phase changes closely related to echinocandin exposure, particularly in the urinary system. These findings suggest that *FKS1* mutations mediate an evolutionary accumulation of echinocandin resistance followed by modulation of chromosome remodelling and DNA repair processes that ultimately lead to *FKS*1 hot spot mutations and the development of drug resistance. This study provides an in-depth exploration of the molecular pathways involved in the evolution of *Candida auris* echinocandin resistance.

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KEYWORDS *Candida auris*; echinocandin; resistance; *FKS*1 mutation; whole genome sequencing

Introduction

Candida auris is classified as a critical priority pathogen by the World Health Organization (WHO). Studies indicate that 90% of *C. auris* isolates are highly resistant to fluconazole, 30% are resistant to polyene amphotericin B, and a small number (<5%) are resistant to echinocandins [1–3]. While echinocandins are the first-line drugs used to treat *C. auris* infections because of their safety, efficacy, and low resistance rates [4], *C. auris*-specific echinocandin resistance continues to emerge [5–7]. From 2020 to 2022, we monitored the prevalence of echinocandin resistance in *C. auris* clinical isolates. Several clinical isolates had elevated minimum inhibitory concentrations (MICs) or even exhibited resistance to micafungin (MCF), caspofungin (CAS), and anidulafungin (ANF).

Research on the mechanism of echinocandin resistance in *C. auris* has primarily focused on mutations in the *FKS*1 gene [8,9] (mainly *FKS*1^{S639Y}, *FKS*1^{S639F}, and *FKS*1^{S639P}), but few studies have assessed the drivers of these *FKS*1 mutations [10]. Importantly, the evolution of echinocandin resistance in multidrug-resistant *Candida glabrata* strains has multiple steps. It remains unclear whether the development of *C. auris*-specific echinocandin resistance involves a similar multistep process.

This study focused on a series of *C. auris* isolates in which resistance emerged following echinocandin treatment. A potential correlation between echinocandin exposure and the emergence of drug resistance was explored. The genome-wide micro evolutionary characteristics of *C. auris* isolates were analyzed to

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Table 1. Antifungal susceptibility data for 29 *Candida auris*.

Drug	MIC (μg/mL)[a]											MIC range (no. of dilutions)[b]	GM μg/mL	MIC50 μg/mL	MIC90 μg/mL	dECV μg/mL	No.(%R or non-WT)
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256					
Micafungin		13	3	4	2			7						0.12–8 (5)	0.46	0.25	8	≥ 4	7(24.1%)
Anidulafungin		3	12	2	3	2	2	5						0.12–8 (7)	0.71	0.25	8	≥ 4	7(24.1%)
Caspofungin		4	9	4	5	4		3						0.12–8 (6)	0.55	0.5	8	≥ 2	7(24.1%)
Fluconazole												2	27	128 to >256 (2)	219.45	>256	>256	64	29 (100%)
Voriconazole				5	9	8	5	2						0.5–4 (5)	1.57	2	4	NA	NA
Itraconazole	1		3	16	7				2					0.06–16 (5)	0.35	0.25	0.5	NA	NA
Posaconazole	12	7	7		2			1						0.06–8 (5)	0.15	0.12	1	NA	NA
Amphotericin B					22	7								1–2 (2)	1.18	1	2	≥2	7 (24.1%)
Flucytosine		11	18											0.12–0.25 (2)	0.19	0.25	0.25	NA	NA

Note: MIC, minimum inhibitory concentration; MIC50/90, MIC that inhibits 50% and 90% of the isolates, respectively; GM, geometric mean; dECV, derivative epidemiological cut-off value; R, resistant; non-WT, non-wild-type; NA, not applicable.

[a]Modal MICs are indicated with underlined numbers and gray shading, and values in parentheses represent the number of isolates with an MIC equal or less than the MIC indicated due to truncation. distribution spanned is given in parentheses. Additional peaks are illustrated by underlining.

[b]The number of dilutions each MIC distribution spanned is given in parentheses.

obtain mechanistic insight into echinocandin resistance. The findings provide a theoretical basis for the prevention, control, and treatment of echinocandin-resistant *C. auris* infections.

Methods

C. auris *strains*

The drug sensitivity of *C. auris* clinical isolates to echinocandins was monitored from 1 December 2020, to 31 July 2022. Patients with elevated echinocandin MICs and echinocandin-resistant strains were enrolled in the study. To further investigate the evolutionary characteristics of *C. auris* echinocandin resistance, echinocandin-sensitive *C. auris* strains from these same patients, along with five *C. auris* isolates (15 September 2022) from the skin and hospital environments of these patients were included in the analysis. These isolates were confirmed as *C. auris* using MALDI-TOF mass spectrometry.

Clinical data and ethical statement

The medical histories of the enrolled patients with *C. auris* infection or colonization, including clinical data such as gender, age, department, underlying disease, and antifungal drug use, were obtained from the Hospital Information System (HIS) system of the First Hospital of China Medical University, and assessed by clinicians. This study was reviewed and approved by the Ethics Review Committee of the First Hospital of China Medical University (ERC No. 2024-40) in accordance with the principles of the Declaration of Helsinki.

Drug susceptibility testing

Antifungal susceptibility testing was performed using a commercial chromogenic susceptibility plate (Sensititre YeastOne, Thermo Fisher Scientific). *C. auris* isolates with MICs ≥4 μg/mL for ANF and MCF, or ≥2 μg/mL for CAS were categorized as echinocandin-resistant (ECN-R) based on the provisional MIC breakpoints published by the Centers for Disease Control and Prevention (CDC) or derived epidemiological cut-off values (ECVs) for *C. auris* [11,12]. According to Kordalewska et al., MCF is the most potent echinocandin in MIC testing [8]. Thus, *C. auris* isolates with MCF MICs of 0.5 or 1 μg/mL were categorized as elevated echinocandin MIC (ECN-E) in this study.

Whole genome sequencing

Whole-genome sequencing (WGS) was performed by Shanghai Personal Biotechnology Co., Ltd. (China) using the Illumina NovaSeq platform [3]. Variants that were predicted to alter protein sequences in any

coding sequence (non-synonymous single nucleotide variants (SNVs), stop loss or gain variants, indels) were annotated using ANNOVAR software [13] and the RefSeq *C. auris* B11221 coding sequence.

The cleaned BAM datasets were used to identify copy number variation (CNVs) for each isolate. CNVpytor 1.3.1 was used to identify the genomic regions with CNVs based on the RD analysis approach developed using CNVnator. In brief, this included the following steps: extracting the read depth signal, binning the read depth signal with 100-bp non-overlapping windows across the genome, correcting the signal for GC bias, and segmenting the signal using the mean-shift technique. CNV results with normalized read depth values >1.8 and <0.05 were retained and visualized using R script.

Phylogenetic trees, heat maps, Sankey diagrams, and protein–protein interaction networks

The maximum likelihood method based on the Tamura-Nei model was used to infer evolutionary history [14]. Evolutionary analysis was performed with MEGA7 [15]. A phylogenetic tree was constructed using iTOL software (https://itol.embl.de/). Sanghi diagrams were generated using the SankeyMATIC tool (https://www.chiplot.online/). GenesCloud (https://www.genescloud.cn/) was used to generate heat maps. Protein–protein interaction (PPI) network analysis of non-mutated genes was conducted using the STRING database (https://cn.string-db.org).

Results

Clinical characteristics and drug sensitivity analysis

A total of 29 strains were assessed in this study, including 24 clinical isolates. Detailed strain information is shown in Supplementary 1-1. Seven ECN-R and six ECN-E isolates were identified. Of these, Y0021 was resistant to fluconazole, amphotericin B, and all three echinocandins. Moreover, the echinocandin MICs of in urine specimen isolates from SICU8 increased after treatment. The ANF, CAS, and MCF MICs in *C. auris* A411 were 0.25, 0.5, and 0.12 μg/mL before treatment, respectively. After two rounds of treatment, all three echinocandin MICs in the A458 (27 June 2022) and A466 (9 July 2022) isolates were 0.5 and 8 μg/mL, respectively. However, SICU8 sputum isolates did not show elevated MICs. The ANF, CAS, and MCF MICs in isolate A427 (26 May 2022) were 0.12, 0.25, and 0.25 μg/mL, respectively, while the MICs of each drug in isolate A459 (27 June 2022) were the same. The specific drug sensitivity results are shown in Table 1 and Supplementary 1-1.

Case series data for C. auris infection or colonization

The strains discussed above were isolated from four patients (SICU5, SICU8, RICU38, and RICU40). While SICU5 and SICU8 were admitted to the Surgery Intensive Care Unit ICU (SICU) ward, RICU38 and RICU40 were seen in the Respiratory ICU (RICU) ward. The first echinocandin-sensitive (ECN-S) strain was isolated from case SICU5 at the end of 2020, while the ECN-E strains A357, A359, A360, and A367 were isolated after 1 year of echinocandin treatment. In 2022, ECN-R strains were isolated from cases SICU8, RICU38, and RICU40. The first ECN-E strain from case SICU8, A458, was isolated after the second CAS treatment, and the ECN-R strain, A466, was isolated 12 days later. The ECN-R strain from case RICU38, A382, was isolated after 8 days of MCF treatment, while the ECN-R strain from case RICU40, A430, was isolated after 7 days of MCF treatment. This was followed by the emergence of resistant strains, A441, A447, and A451. All ECN-E and ECN-R strains were isolated from urine samples, while the ECN-S strains were isolated from blood and sputum. Specific case information is shown in Figure 1 and Supplementary 1-2.

Genome sequencing and phylogenetic analysis

Consistent with previous phylogenetic and population structure analyses, all isolates in this study belonged to the South African *C. auris* clade [3]. A single nucleotide polymorphism (SNP) phylogenetic tree was constructed by combining the 29 *C. auris* isolates from this study with another 29 isolates from our previous study, including 28 strains from five echinocandin-treated patients and one isolated from the hospital environment (Supplementary 1-3). Of those isolates obtained from our prior study, 25 of 29 were clustered in clade A. Meanwhile, the 29 isolates isolated in this study formed clade B (including B1, B2, and B3 subclades; Figure 2(A)). Clade B also included four isolates (RICU4 A7, RICU4 A8, RICU9 A52, and C12 A109) from our previous study that may be genetically related to the 29 strains in this study. In addition, all isolates from case RICU40 including his skin strains (Y0021, Y0024), and the Y0006 and Y0010 isolates from a neighbouring patient, case RICU37, were in the B1 subclade and showed a close genetic association. Isolates from SICU5 and his skin strain (Y0013) were in the B2 subclade, while isolates from SICU8 clustered in the B3 subclade. Phylogenetic analysis based on the B1, B2, and B3 subclades suggested that *C. auris* isolates from the same ward and the same patient were most closely related.

The ECN-R strains were most closely related to other strains in the same patient. Seven ECN-R strains

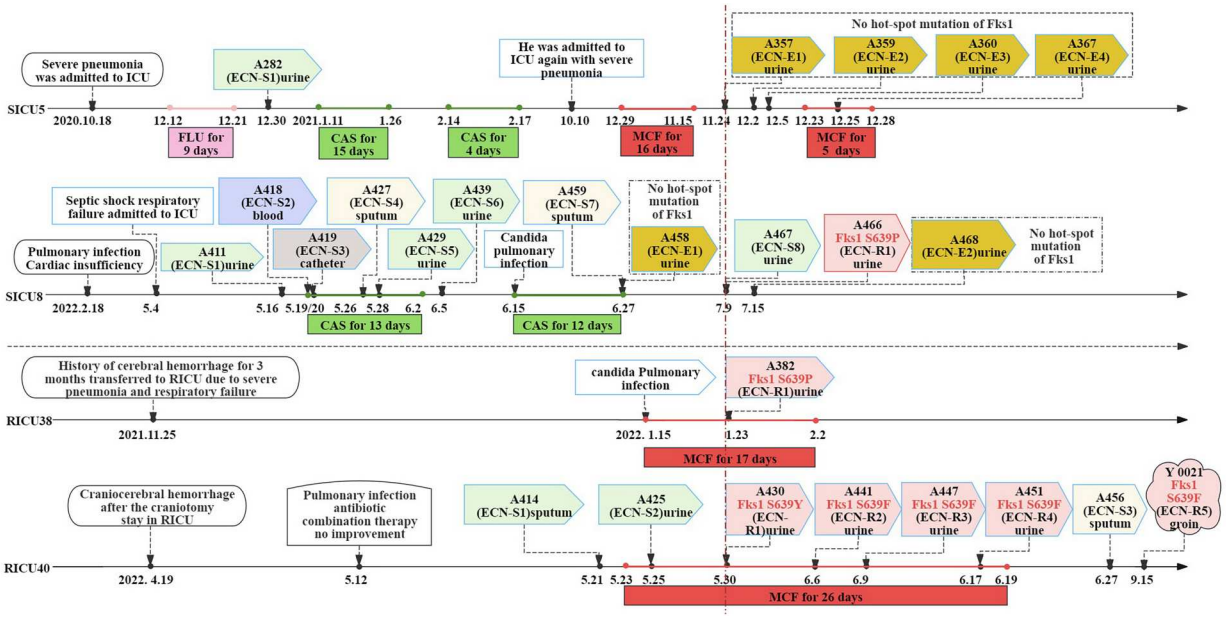

Figure 1. Microbiological data and antifungal treatment schedule of the four **study** patients, showing different intervals of **the** *C. auris* strains and echinocandin treatment. Abbreviations ECN-S: echinocandin-sensitive strain; **ECN-E**: **elevated echinocandin MIC** strain; ECN-R: echinocandin-resistant strain; CFG: Caspofungin, 50 mg, qd, iv; MFG: Micafugin, 150 mg, qd, iv; FLU: Fluconazole, 200 mg, qd, iv.

in this study harboured *FKS*1 mutations (i.e. S639P in A382 and A466, S639Y in A430, and S639F in A441, A447, A451, and Y0021). In addition, eight of the nine ECN-R strains were obtained from urine specimens (two of which were derived from a previous study), and one (Y0021) was derived from the environment (the patient's groin). Six other urine isolates (A357, A359, A360, A367, A458, and A468) were ECN-E (Figure 2(B)).

Genome-wide SNP locus analysis

WGS analysis was performed on 29 strains from the *C. auris* series, of which 47 mutated genes were identified. A total of 35 genes had non-synonymous mutations (Figure 3(A)), of which 30 had SNPs, five had multiple nucleotide polymorphisms (MNPs), *IFF6* (M175I, Y49F), RBR3 (G1385D, S80F), *NMA111* (L314F, L811H), *AMN1* (C282Y, S123X), *FKS1* (S639P, S639F, S639Y), and 12 had synonymous mutations. Almost all the genes had mutations at 1–2 loci of RBR3 and *IFF6*, but the mutation rate was generally low (i.e. 0.14–0.43), and the non-synonymous mutation rate of the other genes was close or equal to 100%. In addition, the SNP non-synonymous mutant genes in this study did fall into the CNV region, except for one gene, RBR3 (Supplementary Table 1). Of the non-synonymously mutated genes, *IFF6* was mutated in all strains, and RBR3 was mutated in most strains. Isolates from RICU40 and RICU37 had seven mutated genes in common, and four urinary isolates from RICU40 (A425, A441, A447, and A451) along with his groin strain (Y0021) had a mutation in *AMN1*. There were 23 non-synonymously mutated genes in SICU5 and SICU8, of which eight were co-mutated. In addition, SICU5 had 10 unique mutated genes, of which *MPH*1 and *UPC2* were only mutated in ECN-E strains, while SICU8 had five unique mutated genes, of which *FKS*1 and *CaO19.1946* were only mutated in the ECN-R strain, A466. Protein interactions were assessed in 35 non-synonymously mutated genes, of which 31 had interactions. The association analysis of *LRG*1, *MKC*1 and *GSC*1/*FKS*1 was experimentally determined, text mining and co-expression, The association analysis of *MYO*1, *IFF*6, *LRG*1, *MKC*1 and *GSC*1/*FKS*1 was experimentally determined and text mining, while the association analysis of *FAA*4 and *FKS*1 was only co-expression (Figure 3(B)).

Traceability analysis of the characterized genes

Traceability of strain information and plotting of non-synonymously mutated genes of known function in the series of strains from four cases showed that *FKS*1, *ALR*2, *SLD*1, *UTP*22, and *MRPL*10 mutations only occurred in the ECN-R strains (Figure 4). Mutations in *MET*15, *MPH*1, *CDR*1, *UPC*2, and *IRA*2 appeared in ECN-E strains while mutations in RBR3, *IFF6, YOR1, THR4, PRO41, RAD2, ECM22, PWP2, MYO1, LRG1, PIN3, MKC1, FAA4, SCH9, NSP1,* and *AMN1* were present in both ECN-S and ECN-R strains.

*IFF*6 and *RBR*3 were mutated in almost all the early isolates (MIC = 0.12 μg/mL) from the four patients as well as their subsequent isolates. In SICU patients,

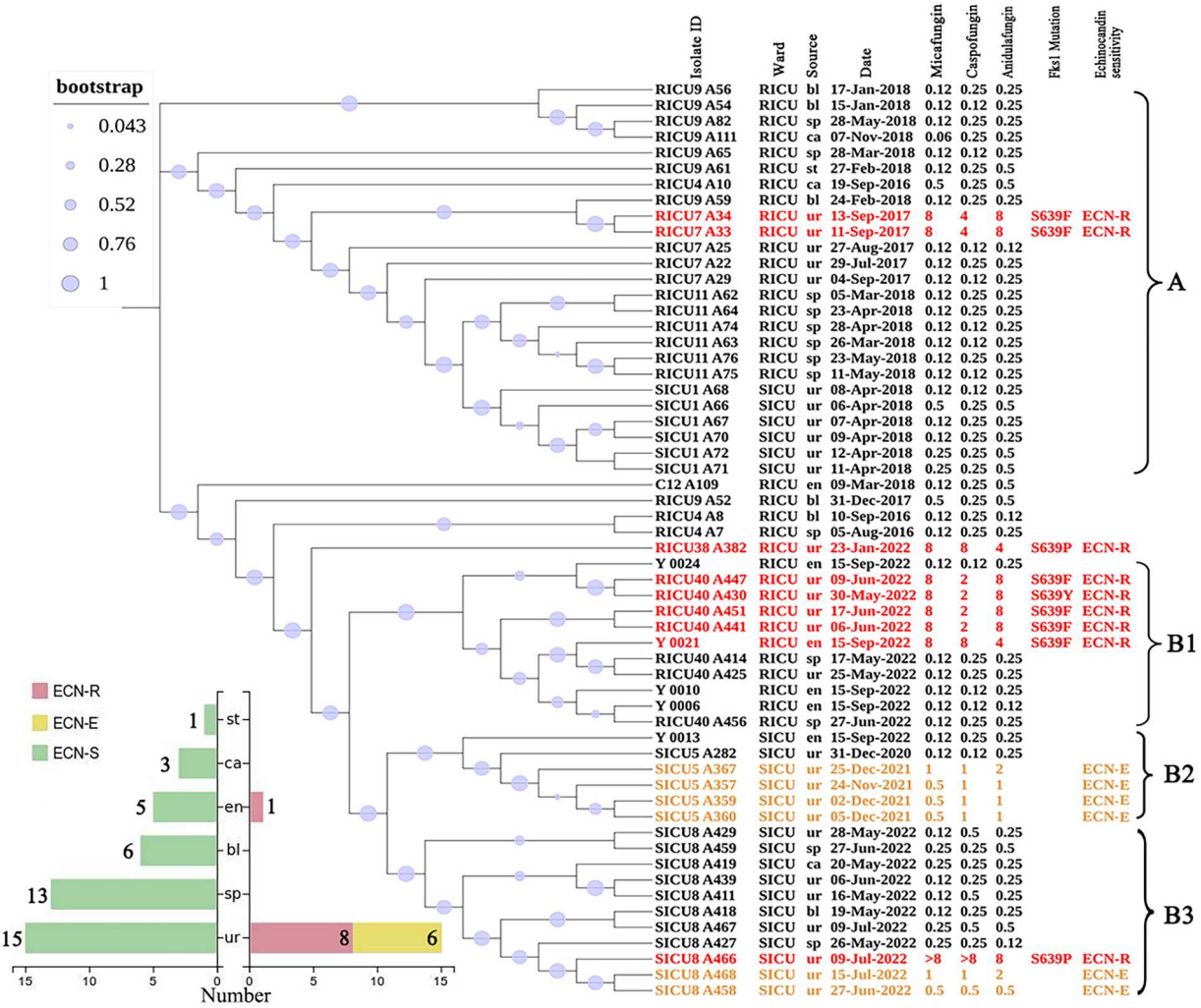

Figure 2. Phylogenetic analysis of the *C. auris* clinical isolates. (A) Molecular phylogenetic analysis and other relevant information. Phylogenetic trees were generated using the maximum likelihood method of MEGA7 and phylogenetic trees were constructed and composed **using** iTOL software. All **58** *C. auris* isolates were obtained from our hospital, half **during** the present study and half **during** the previous study. Each sample **corresponds** to labelled wards, source, date, MICs of MCF, CAS, and ANF, as well as *FKS*1 mutation site and echinocandin susceptibility status. Abbreviations ECN-S: echinocandin-sensitive strain; **ECN-E**: **elevated echinocandin MIC** strain; ECN-R: echinocandin-resistant strain. ECN-R strains and their sample information are marked in red. **(B)** Statistical plot of the 58 isolates included in the phylogenetic analysis **by** type, with nine ECN-R strains (red; two from a previous study), six **ECN-E** strains (yellow), and the remaining ECN-S strains (green). Abbreviations st: stool, ca: catheter, en: environment, bl: blood, sp: sputum, ur: urine.

genes that were co-mutated in cases SICU8 and SICU5, *YOR1* and *THR4*, appeared in case SICU5 strain, A282 (MIC = 0.12 μg/mL), which was collected on 31 December 2020, and were also present in the four ECN-E strains collected from the same patient in 2021 and all isolates collected from case SICU8 in 2022. Early isolate, A282 (MIC = 0.12 μg/mL), from case SICU5 had mutations in *MYO*1, *LRG*1, and *PIN*3, early isolate, A441 (MIC = 0.12 μg/mL), from case SICU8 had mutations in *MKC1, FAA4* and *SCH9*, and early isolate, A425 (MIC = 0.12 μg/mL), from case RICU40 had an *NSP1* mutation. *MET*15, *MPH*1, *CDR*1, and *UPC*2 mutations occurred earliest in caseSICU5 strains with MIC = 0.5 μg/mL (A357, A359, A360), and the *IRA*2 mutation occurred earliest in case SICU5 strains with MIC = 1 μg/mL (A367). The seven isolates with *FKS*1 mutations, SICU8 A466, RICU40 A441, RICU40 A447, RICU40 A451, RICU40 A430, RICU38 A382, and Y0021, were resistant to MCF (MIC = 8 μg/mL). In addition to *FKS1*, RICU38 isolate A382 (MIC = 8 μg/mL) had mutations in the *UTP*22, *SLD1*, and *MRPL10* genes. The *AMN1* mutation, which occurred only in urinary isolates from case RICU40 and its inguinal strain, appeared in the susceptible strain A425 (MIC = 0.12 μg/mL) and its four subsequent ECN-R strains, A430, A441, A447, A451 and Y0021 (MIC = 8 μg/mL).

Three categories (ECN-S/E/R) of the non-synonymous mutant SNP gene in *C. auris* appears to be associated with the three-stage evolutionary model of echinocandin resistance in *C. glabrata*: cell wall stress, drug adaptation, and genetic escape (*FKS* mutation) [10]. Prior research indicates that the non-synonymous mutant genes, *RBR*3, *IFF*6, *MKC*1, and

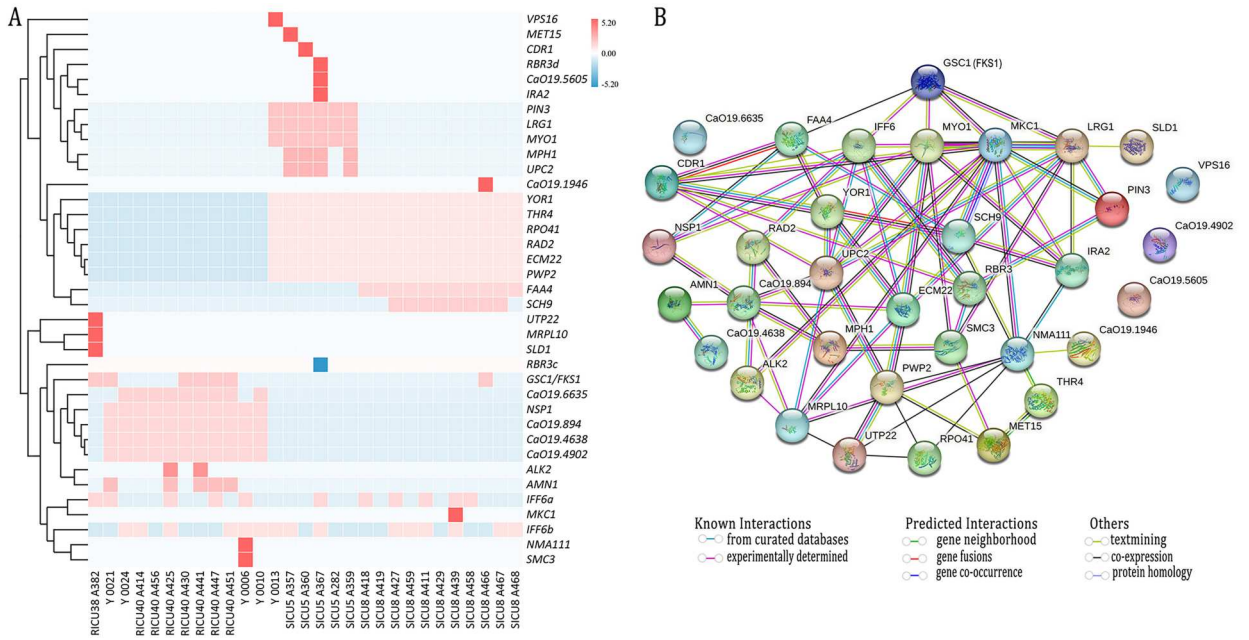

Figure 3. (A): Heatmap of the 29 *C. auris* strains isolated in this study plotted against their 35 non-synonymously mutated genes using heatmap tools in the genescloud platform (https://www.genescloud.cn). The data are normalized by z-scores. Except for two SNPs in the *NMA111* gene of strain Y0006, one SNP appeared in all other genes (shown in red). IFF6 has two mutation sites and is named *IFF6a* and *IFF6b*. RBR3 has two mutation sites and is named *RBRc* and *RBRd*. (B) Amino acid sequences encoded by 31 non-synonymously mutated genes were used in protein-protein interaction network analysis. *Candida albicans* SC5314 homologous protein was used as a reference. The identity ranges from 84.5% (*GSC*1) to 24% (*CDR*1). Each node in the figure represents a protein, and the edges between the circle nodes represents the interaction relationship between the two proteins linked by the line. Different colours correspond to different interaction types.

LRG1, which show morphological changes such as cell aggregation in response to drug exposure and other stressors, may be involved at the cell wall stress stage (cell wall integrity or PKC pathways) [16–22]. Meanwhile, RBR3 and *IFF6* encode GPI-anchored proteins, which are important cell wall proteins [23]. The *MKC1* gene encodes mitogen-activated protein (MAP) kinase, a key component of the PKC pathway [24]. The *LRG1* gene encodes Lrg1p, which inhibits the GTPase, Rho1, an activator of the PKC1 pathway [25]. In vivo drug resistance may also involve host environmental stressors [16–22]. The non-synonymous mutant genes, *MYO*1, *MPH1*, and *RAD*2 may be involved in the drug-adaptation stage of echinocandins. The *MYO*1 gene encodes the heavy chain of myosin [26]. *MYO*1 is associated with chitin deposition, and *MYO*1-deficient mutants are sensitive to CAS [27]. *MPH1* encodes a DNA fusion enzyme that unwinds the structure of DNA molecules and participates in the DNA repair and recombination process [28]. *RAD*2 encodes DNA endonuclease, which is involved in nucleotide cleavage and repair [29]. Finally, the genetic escape stage is associated with the *FKS*1 mutation.

Discussion

The first echinocandin-resistant *C. auris* was isolated in China (Shenyang) in 2017 [30] and *C. auris*-specific echinocandin resistance has been closely monitored ever since. This led to the discovery that isolates from four patients, seen from December 2020 to July 2022, had elevated MIC values or echinocandin resistance. Fungal strains from these patients were also monitored at various time points, with particular focus on identifying *C. auris* clinical isolates before, during, and after echinocandin treatment. Surprisingly, there was a shift from echinocandins-sensitive to drug-resistant strains, indicating that clinical isolates of *C. auris* in China (Shenyang) were undergoing resistance evolution to echinocandins. Moreover, *C. auris* (Y0021) isolated from the body surface of RICU40 was resistant to fluconazole, echinocandins, and amphotericin B, indicating a trend toward pan-resistance. These data represent a red flag that requires urgent clinical attention.

Our case series focusing on the evolution of echinocandin resistance in *C. auris* strains revealed three distinguishing clinical features. First, echinocandin resistance in *C. auris* is on the rise [3]. Of eight patients monitored during 2016–2020, only one case of echinocandin resistance (RICU7) was noted. However, from the end of 2020 to the end of 2021, ECN-E *C. auris* strains were isolated from patient SICU5, and from the end of 2021 to June 2022, three patients (RICU38, RICU40, and SICU8) were monitored for the presence of ECN-R strains. Second, the evolution of echinocandin resistance in *C. auris* strains is closely

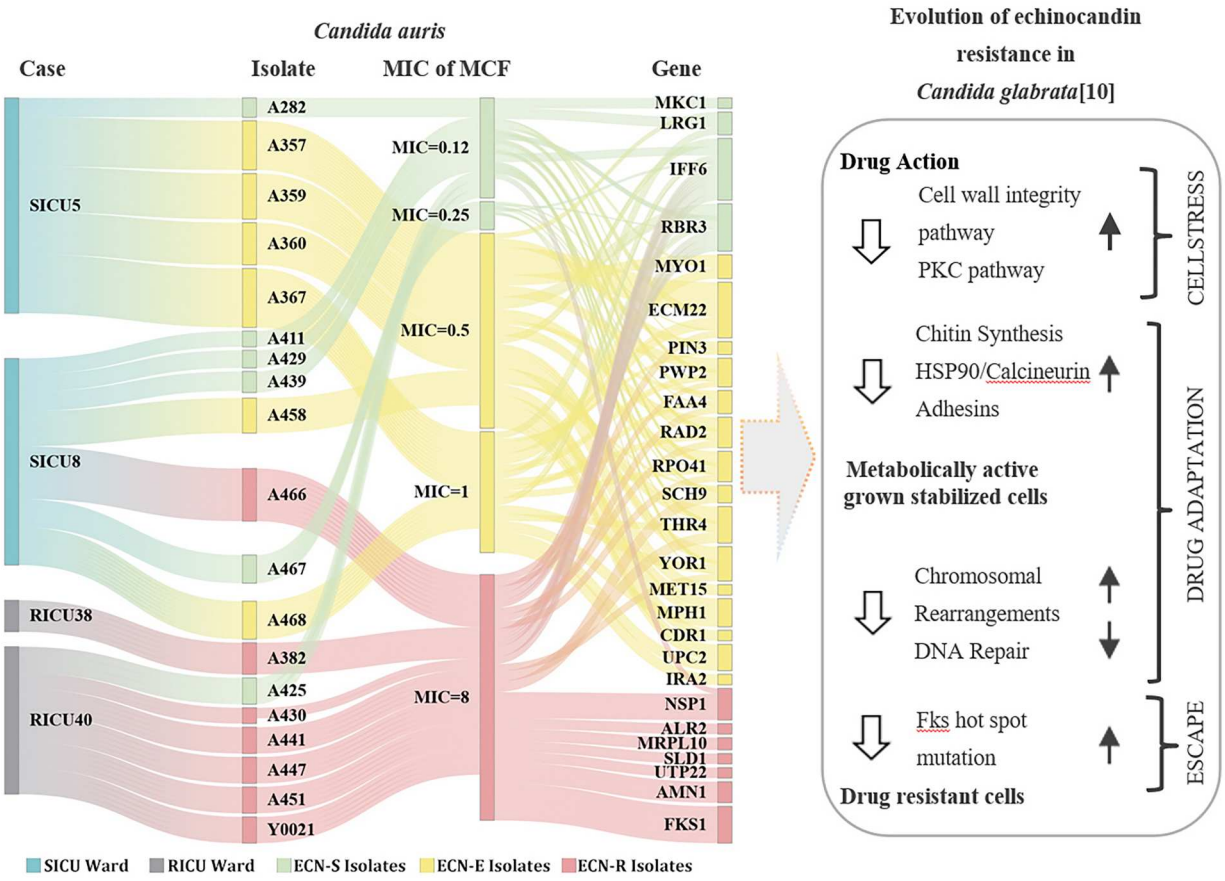

Figure 4. Sankey diagram showing the summary analysis of genes from case to isolate to MCF MIC and then to non-synonymous mutant SNPS. Two cases (SICU5, SICU8) were SICU ward patients (grey) and two cases (RICU38, RICU40) were RICU ward patients (dark red). Eighteen clinical strains (urine) and one groin specimen, Y0021, were isolated from these four cases. According to the MCF MIC values, a MIC = 0.12/0.25 is regarded as ECN-S (green), MIC = 0.5/1 is regarded as ECN-E (yellow), and MIC = 8 is regarded as drug resistant (red). The non-synonymous mutant SNP gene is present in the strains from three categories (ECN-S/E/R). In addition, the multistep evolution model of *C. glabrata* echinocandin resistance is associated.

linked to drug exposure, which is increasing over time. Similar to *C. glabrata* [10], which is prone to develop multidrug resistance, the four case isolates identified in the present study had elevated MICs or even developed resistance to echinocandins after treatment (Figure 1). One case, SICU5, was first isolated as a sensitive strain at the end of 2020. After repeated echinocandin exposure over 1 year, SICU5 isolates had a significantly elevated MIC. Subsequently, three cases were identified in 2022 that developed resistance over a significantly shorter period than was seen previously. For example, RICU40 developed echinocandin resistance after 7 days of MCF treatment. In contrast, only one of the eight patient strains in our previous study developed resistance after echinocandin treatment. Based on these results, we hypothesized that after 2022, *C. auris* acquired a unique potential to develop rapid echinocandin resistance, or even multiresistance, following treatment. Third, the evolution of resistance to echinocandins in *C. auris* appeared to originate in the urinary system. Almost all monitored ECN-E and ECN-R strains originated from urine, except for the multi-resistant strain (Y0021), which originated from the inguinal area of RICU40. This may be due to differences in the distribution of echinocandins in each region of the body. To clear infection, high concentrations of echinocandins are concentrated in the blood and sputum [31]. Meanwhile, these drugs are distributed at lower concentrations in the urinary system, which is not sufficient to clear *C. auris*. In addition, *C. auris* can colonize the urinary system continuously, and repeated exposure to low drug concentrations may induce resistance. Poor echinocandin penetration in *C. glabrata* contributes to resistance [32] and suggests that low levels of drug exposure may accumulate additional mechanisms of echinocandin resistance [10]. In agreement with other studies [33,34], the use of echinocandins to treat urinary tract infections or colonization caused by *C. auris* is not recommended due to the low concentration distribution of echinocandins in the urinary tract. Even when echinocandins are used to treat infections in other regions of the body, close attention should be paid to the development of *C. auris* echinocandin resistance in the urinary system.

Consistent with prior studies, *C. auris* echinocandin resistance is directly related to mutations in

*FKS*1 [35]. In this study, *FKS1*^{S639P} (two strains), *FKS1*^{S639Y} (one strain), and *FKS1*^{S639F} (four strains) mutations occurred in seven resistant strains, suggesting that Chinese (Shenyang) strains of *C. auris* have *FKS*1 resistance polymorphisms. Of these, the S639F or S639Y mutation was produced only after MCF treatment, while the S639P mutant strains resulted after CAS or MCF treatment. The *FKS*1 polymorphic variations may be related to exposure to different echinocandin drug types, but further investigation using a larger sample size is required to confirm this.

Most importantly, this study identified the potential drivers of *FKS*1 mutations in clinical *C. auris* isolates. Mutated genes from four series of strains that were evolving echinocandin resistance were collected, allowing the identification of 35 non-synonymously mutated genes. Notably, while *IFF6* and RBR3 encode cell wall proteins and were commonly mutated in the four series of strains, they were not mutated in any pre-studied strains [3]. This suggests that variation in the *IFF6* and RBR3 genes may impact the evolution of *C. auris* echinocandin resistance. Further study is required to assess this. Six strains of ECN-E *C. auris* were identified from the SICU ward, and two SICU ward patients (SICU5 and SICU8) had active non-synonymously mutated gene variants, along with the appearance of 23 non-synonymous mutated genes (e.g. *MKC1* and *MYO1*). Given the evolution of *C. glabrata* resistance to echinocandins [10], *MYO*1, and other genes may be involved in adaptive changes. Of these, *RBR*3, *IFF*6, *MKC*1, and *LRG*1, among others, may be involved in cell wall integrity /PKC pathways, *MYO*1 may be involved in chitin synthesis [26], and *MPH*1 and *RAD*2 may be involved in DNA repair and chromatin remodelling [28,29]. These genes may help *C. auris* to develop echinocandin resistance, allowing the pathogen to tolerate these drugs and provide time for the formation of *FKS* hotspot mutations needed for drug evasion. Subsequently, six echinocandin-resistant strains with *FKS*1 mutations were detected in two patients in the RICU wards (RICU38 and RICU40). *UTP*22, *SLD*1, *MRPL*10 and *ALK*2 were only mutated in the resistant strains, and their involvement in the evolution of echinocandin resistance in *C. auris* requires further investigation. *AMN*1 was mutated in five ECN-R strains in RICU40, suggesting that *AMN*1 may be closely related to the *FKS*1 mutation in this patient.

The *C. auris* strains isolated during 2020–2022 were divided into two major evolutionary branches that differed from the isolates obtained before 2020, suggesting that *C. auris* strains in China (Shenyang) are changing. The *C. auris* strains isolated in this study were more closely related to blood isolates from the two patients in our previous study, indicating that those *C. auris* strains that cause bloodstream infections are more likely to disseminate. These findings highlight the importance of strictly controlling the prevalence of *C. auris* from blood sources to effectively prevent and control widespread dissemination among patients and in the environment. The data also showed that ECN-R strains were more closely related genetically to other isolates from the same patient, suggesting that resistance may evolve independently within individual patients rather than spreading from person to person through a single clone.

This study had some limitations. First, the case number was small, limiting our ability to assess the evolutionary genes and their ability to predict echinocandin resistance. The study findings require validation in follow-up *in vitro* and *in vivo* studies. Second, the evolution of *C. auris* resistance to amphotericin B, which was described in another study, was not explored. Third, the current method (fungus 3) for detecting fungal drug sensitivity does not include echinocandins, which inevitably leads to errors in the detection of echinocandin-resistant strains and underestimates the rate of echinocandin resistance.

In conclusion, there is an urgent need to address the continued emergence of *C. auris* resistance to echinocandin. Routine and active surveillance should be strengthened, with particular attention paid to *C. auris* urinary colonization or infection. Echinocandins should be prescribed judiciously to prevent the development of *C. auris* resistance.

Author contributions

Sufei Tian, Yusheng Wu, Hailong Li, Chen Rong, Na Wu, and Yunzhuo Chu have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; Ning Jiang and Jingping Zhang have been involved in drafting the manuscript; and Hong Shang **has** given final approval of the version to be published.

Data availability statement

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA015722 and CRA015753) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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