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ORIGINAL ARTICLE

Genotypic and phenotypic characterisation of a nosocomial outbreak of *Candida auris* in Spain during 5 years

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Abstract

Objectives: The investigation of *Candida auris* outbreaks is needed to provide insights into its population structure and transmission dynamics. We genotypically and phenotypically characterised a *C. auris* nosocomial outbreak occurred in Consorcio Hospital General Universitario de Valencia (CHGUV), Spain.

Methods: Data and isolates were collected from CHGUV from September 2017 (first case) until September 2021. Thirty-five isolates, including one from an environmental source, were randomly selected for whole genome sequencing (WGS), and the genomes were analysed along with a database with 335 publicly available genomes, assigning them to one of the five major clades. In order to identify polymorphisms associated with drug resistance, we used the fully susceptible GCA_003014415.1 strain as reference sequence. Known mutations in genes ERG11 and FKS1 conferring resistance to fluconazole and echinocandins, respectively, were investigated. Isolates were classified into aggregating or non-aggregating.

Results: All isolates belonged to clade III and were from an outbreak with a single origin. They clustered close to three publicly available genomes from a hospital from where the first patient was transferred, being the probable origin. The mutation VF125AL in the ERG11 gene, conferring resistance to fluconazole, was present in all the isolates and one isolate also carried the mutation S639Y in the FKS1 gene. All the isolates had a non-aggregating phenotype (potentially more virulent).

Conclusions: Isolates are genotypically related and phenotypically identical but one with resistance to echinocandins, which seems to indicate that they all belong to an outbreak originated from a single isolate, remaining largely invariable over the years. This result stresses the importance of implementing infection control practices as soon as the first case is detected or when a patient is transferred from a setting with known cases.

Juan Vicente Mulet-Bayona and Irving Cancino-Muñoz contributed equally to this work.

KEYWORDS
Candida auris, candidaemia, clinical mycology, fluconazole resistance, molecular epidemiology, phylogenetic analysis

1 | INTRODUCTION

Candida auris was first described in 2009[1] and rapidly emerged as a serious threat for public health due to its propensity to cause large hospital outbreaks, its tendency to produce bloodstream infections (candidaemia) in critically ill patients, its multidrug resistance properties, and its long-term survival in the hospital environment.[2] *C. auris* cases have dramatically increased in Europe since 2016 and Spain is one of the countries with the highest prevalence, being responsible of 76% of all candidiasis cases and the only European country where cases by *C. auris* is currently considered an endemic situation.[3]

Genomic studies, based on whole genome sequencing (WGS) experiments, are increasingly being applied to clinical isolates and are providing insights into how *C. auris* is spreading among facilities, communities, countries and continents.[4] To date, six major genetic clades have been described, separated by many (tens of thousands) single nucleotide polymorphisms (SNPs). Each clade has been associated with a geographical region: clade I (South Asian), clade II (East Asian), clade III (African), clade IV (South American), clade V (based on few isolates from Iran) and, more recently, clade VI (based on two isolates, from Singapore and Bangladesh).[4,5]

C. auris displays phenotypic characteristics that differ from those of the most common *Candida* species, such as its multidrug resistance profile. In addition, an intriguing characteristic is that two different phenotypes have been described, the aggregating and the non-aggregating, the latter having been linked to a greater virulence of the strains.[6]

In September 2017, a *C. auris* outbreak started in Consorcio Hospital General Universitario de Valencia (CHGUV), Spain, which was reported in previous studies.[7–9] Here, we present the results of the WGS analysis and the phenotypic characterisation (aggregation and susceptibility) of 35 *C. auris* strains isolated over 4 years in our setting.

2 | METHODS

2.1 | Sample collection

This study is an update to the previous report conducted during 2017–2019 in the same healthcare setting.[9] *C. auris* isolates were recovered from patients admitted to the CHGUV, from September 2017 until September 2021. Among the 550 cases reported during that period (by considering one isolate per patient), 34 *C. auris* isolates were selected for both WGS and phenotypic characterisation according to the following criteria: the first 2 *C. auris* isolates (S1 and S2) and an echinocandin-resistant isolate from blood (S67) were selected, and the remaining isolates were randomly selected from several days apart, attempting to include at least one sample for each sample type and ward where the pathogen had been identified. Patients' surveillance samples taken in the context of active surveillance studies were also considered for inclusion (in our setting, active surveillance studies consist of collecting axillary-rectal and pharyngeal swabs from patients admitted to the intensive care unit (ICU) once a week). Furthermore, an isolated environmental sample from a bedside table at the ICU was included. All the isolates, previously stored at −70°C, were subcultured onto Sabouraud Dextrose Agar plates (Becton Dickinson, USA), incubated at 37°C for 24 h and identified by matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF, Bruker, USA). The information on the selected isolates is shown in Table 1.

2.2 | Antifungal susceptibility testing and phenotypic characterisation

Antifungal susceptibility testing (AFST) was carried out with Sensititre® YeastOne YO10 (Thermo Fisher Scientific, USA). Tentative breakpoints from CDC were used to interpret the susceptibility of the isolates.[10] Isolates were then classified as aggregating or non-aggregating, as described by Szekely et al.[11] In all cases, type strain ATCC® MYA-5001® was used as a control.

2.3 | DNA extraction and whole genome sequencing

Several colonies of each isolate were introduced into Eppendorf tubes with distilled water and centrifuged at 5000 rpm for 5 min. The supernatant was removed, 180 μL of lysis buffer (ATL buffer; Qiagen) and 40 μL of proteinase K (Qiagen) were added, and the mix was incubated at 56°C overnight. Then, DNA was extracted from the mix using the QIAsymphony® SP DNA kit for the QIAsymphony® SP system (Qiagen). Extracted DNA was quantified using a Tecan Infinite® PRO fluorometer (Tecan, Switzerland) and the concentrations were adjusted to 5 ng/μL.

DNA libraries were prepared with the Illumina DNA Prep kit (Illumina) following the Illumina DNA Prep Reference Guide v10. Input DNA was diluted to 0.2 ng/μL to start the protocol. At the multiplexing step, Nextera DNA CD Indexes (Illumina) were used. Library size selection was performed according to the Illumina library Prep protocol with Sample Purification Beads (SPB) included in the library Prep kit. Library size was validated by using a Fragment Analyser 48-Capillary Array with the HS NGS Fragment Kit (1-6000 bp) (Agilent). Libraries were sequenced with a Nextseq 2×150 pb paired-end reagent kit (NextSeq 500/550 High Output Kit v2.5,

TABLE 1 Isolates selected for genotypic and phenotypic characterisation.

Isolate	Isolation date	Sample type	Hospital ward	Previously stayed in ICU?
S1 (first case)	09/03/17	Urine	Neurosurgery	No
S2 (second case)	10/14/17	Blood	ICU	Yes
S4	10/18/17	Blood	ICU	Yes
S8	12/12/17	Blood	ICU	Yes
S14	02/06/18	Blood	Oncology	Yes
S28	04/30/18	Blood	ICU	Yes
S33	05/27/18	Blood	ICU	Yes
S35	06/28/18	Blood	Cardiac surgery	Yes
S44	08/31/18	Catheter	General surgery	Yes
S56	11/13/18	Cerebrospinal fluid	Neurosurgery	Yes
S62	01/23/19	Catheter	ICU	Yes
S67 (echinocandin resistant)	02/22/19	Blood	Emergencies	No
S72	04/02/19	Urine	Neurosurgery	Yes
S75	05/21/19	Blood	ICU	Yes
S77	06/02/19	Urine	General surgery	Yes
S81	08/16/19	Blood	ICU	Yes
S82	09/05/19	Catheter	ICU	Yes
S86	10/18/19	Blood	ICU	Yes
S94	12/05/19	Blood	ICU	Yes
S99	01/29/20	Blood	Neurosurgery	Yes
S103	02/04/20	Urine	Internal medicine	Yes
S117	03/24/20	Blood	ICU	Yes
S127	04/14/20	Patients' surveillance sample	ICU	Yes
S129	05/04/20	Blood	ICU	Yes
S131	06/09/20	Patients' surveillance sample	Internal medicine	No
S132	07/07/20	Blood	ICU	Yes
S134	08/17/20	Patients' surveillance sample	ICU	Yes
S135	09/09/20	Blood	ICU	Yes
S138	11/30/20	Blood	ICU	Yes
S140	12/18/20	Blood	Neurology	Yes
S141	01/12/21	Patients' surveillance sample	ICU	Yes
S145	02/11/21	Blood	ICU	Yes
S147	04/17/21	Blood	ICU	Yes
S150	05/12/21	Blood	ICU	Yes
S151	06/14/21	Environmental sample (bedside table)	ICU	-

Illumina) on a Illumina Nextseq sequencer, according to manufacturer's instructions.

2.4 | Bioinformatic analysis

In order to place our samples into a global phylogenetic context, we downloaded 334 publicly available *C. auris* genomes from all the continents, including the reference assemblies for all the major clades ('GCA_002759435.2' for Clade I, 'GCA_003013715.2' for Clade II, 'GCF_002775015.1' for Clade III, 'GCA_003014415.1' for Clade IV and strain 'IFRC2087' for clade V.[5]) Based on these assemblies, sequencing reads were simulated and created using the ART programme.[12] In summary, we built a database with 334 publicly available genomes along with our 35 isolates. In total, we analysed 369 genomes. All the information regarding the analysed genomes is available in Table S1.

Raw sequencing reads were trimmed and filtered using fastp[13] with the following parameters '--cut_tail, --cut_window_size=10, --cut_mean_quality=20, --length_required=50, --correction, and –dedup'. Variant calling was carried out using snippy software (https://github.com/tseemann/snippy) with the parameters '--mincov 10

--minfrac 0.9 --minqual 60'. Raw sequences were deposited in the European Nucleotide Archive public server under Bioproject PRJEB70513.

2.5 | Sequence alignment and phylogenetic analysis

Different SNP-based multiple sequence alignments (MSAs) were constructed depending on the analytical purpose. First, an MSA including all previously described global genomes ($n=369$) was constructed to put our samples into a global phylogenetic context. The second MSA was built using only genomes belonging to Clade III ($n=268$), including samples from two previous nosocomial outbreaks.[14,15] Lastly, a MSA which included those isolates that were genetically close to our outbreak ($n=40$) was constructed. These MSAs consisted of 190,611, 803 and 105 variable positions, respectively.

MSAs were constructed from the SNPs detected by mapping against the reference genome of *C. auris* Clade III (GCF_002775015.1). Those polymorphisms were called using the Snippy program (https://github.com/tseemann/snippy) with a minimum coverage of 10× and a minimum frequency of 90%. The detected SNPs were then concatenated using the 'snippy-core' option in Snippy and masked to those polymorphisms detected in duplicated and repeated regions of the reference genome (approx. 3.2% of the entire genome). Pairwise SNP distances between isolates belonging to clade III were calculated with the R package ape.

All maximum-likelihood phylogenetic trees were constructed using IQ-TREE2[16] under the GTR substitution model with 1000 bootstrap replicates, and considering the invariant sites of the whole genome alignment.

2.6 | Identification of antifungal resistance mutations

In order to identify polymorphisms associated with drug resistance, we used the fully susceptible GCA_003014415.1 strain as reference sequence. SNPs were annotated using snpEff.[17] Known mutations in genes ERG11 and FKS1 were detected for fluconazole and echinocandins, respectively.[18]

3 | RESULTS

3.1 | Description of the outbreak

The first isolation of *C. auris* in our setting was in September 2017 from a urine sample of a patient transferred from a hospital located in the same city (Hospital Universitari i Politècnic La Fe; HUiP La Fe, Valencia, Spain). HUiP La Fe had recently reported four *C. auris* candidaemia cases, which later also became an outbreak.[19,20] One month later, *C. auris* was isolated in a blood culture from another patient hospitalised in the ICU, therefore being the first candidaemia case. Since then, candidaemia cases have kept being reported, in spite of having implemented the recommended infection control practices, such as isolation/cohorting of patients, enhanced cleaning with chlorine-based disinfectants or screening studies for detecting colonised patients. The screening studies were implemented in ICU patients, at admission and once a week, and consisted of culturing axillary-rectal and pharyngeal swabs. Until September 2021 (4 years of follow-up), the outbreak affected 550 patients, considering both colonised and infected patients. The most frequent infection was candidaemia, with 86 cases. The first-occurring episodes of *C. auris* colonisation or infection ($n=550$) are shown in Figure 1.

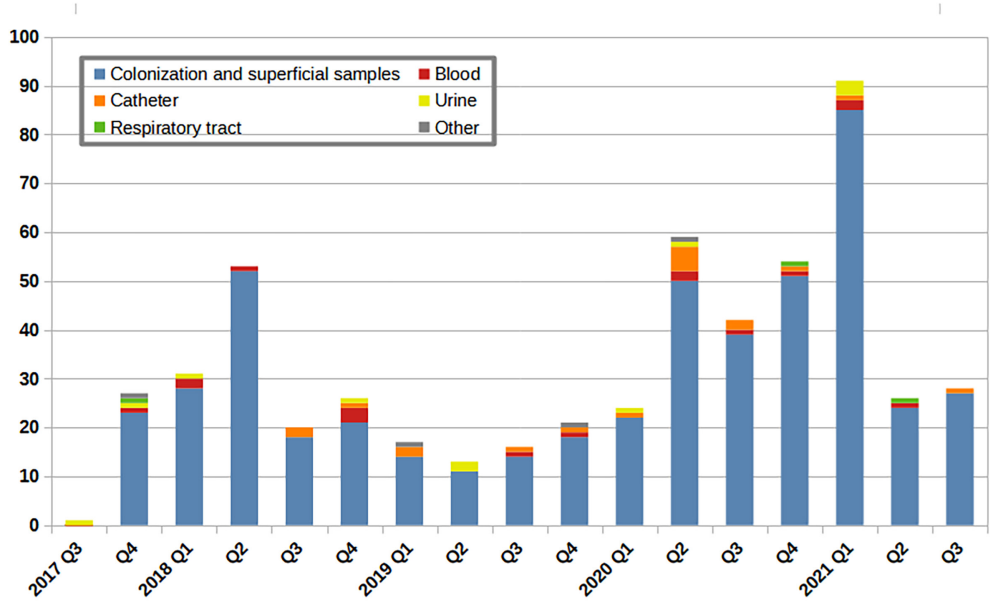

FIGURE 1 First-occurring episodes of *Candida auris* colonisation or infection ($n=550$ patients) from September 2017 (first case) until September 2021. Q: calendar quarter. Isolates from every calendar quarter have been selected for sequencing.

The pathogen has been identified among individuals admitted in nearly all wards within the facility, being the ICU/Reanimation Unit the most affected ward with 481 cases (87.5%), including active surveillance samples. The remaining cases were scattered across several wards, with 45 cases (8.2%) in medical wards and 24 cases (4.3%) in surgical wards. It is noteworthy that the outbreak worsened from June 2020 to February 2021, due to the COVID-19 pandemic, probably due to the over-occupancy of the ICU together with the higher workload of healthcare workers.[8] The 30-day crude mortality rate for candidaemia cases was 32.6%.[9]

3.2 | Genotypic and phenotypic characterisation of the outbreak

Among the 35 isolates that we sequenced, all carried the VF125AL mutation (also referred to as F126L) in the ERG11 gene associated with fluconazole resistance, being consistent with their AFST profile. Moreover, isolate S67 also harboured the S639Y mutation related to echinocandin resistance in the FKS1 gene, also being consistent with its AFST profile (Table S1). All isolates were phenotypically characterised as non-aggregating (Figure 2).

3.3 | Phylogenetic analysis of the outbreak

The global phylogenetic analysis of the 35 *C. auris* genomes obtained (Figure S1) showed that all the genomes from our setting belong to the Clade III (South African). Given that the median genetic distance (SNPs) between the samples of our outbreak and the other clades was very large (Clade I, 21,469 SNPs; Clade II, 30,916 SNPs; Clade IV, 79,368 SNPs; and Clade V, 126,769 SNPs), we decided to zoom in on the samples genetically most closely related to it. The phylogenetic analysis of this dataset revealed four isolates genetically and temporally related to our outbreak isolates, with a median distance of 5 SNPs (range 1–9 SNPs). Three of these were from 2016 and other hospital (HUiP La Fe) located in the same city as our setting (strains 'AA-194', 'AA-200' and 'AA-214'), and were phylogenetically ancestors of our isolates. Interestingly, a case from Austria reported in 2021[21] was also genetically related to our outbreak with a median distance of 6 SNPs (Strain 'Cau4') (Figure 3). Based on the epidemiological information of this case, the patient was admitted to a hospital in Valencia, so it is very likely that the infection occurred during that period of time.

The population structure of the outbreak suggests that the pathogen has a single common source (monophyletic cluster), probably originating in 2016 in another regional hospital (HUiP La Fe). From there, the pathogen was introduced to our hospital in September 2017 by a patient referred from HUiP La Fe ('S1' strain) and from there it has dispersed throughout the hospital during the study period. The maximum genetic distance within the outbreak is 12 SNPs, suggesting recent transmissions within an ongoing outbreak[22] (Figure 4). Interestingly, we found that the most closely genome related to our outbreak was an isolate from Indiana, USA (strain 'B12631'). However, because of the median genetic distance observed (26 SNPs, range 22–36), and because we found no epidemiological link between this strain and the rest of the outbreak, the idea of transmission was discarded.

3.4 | Determining transmission

By analysing the genetic distance as a marker of recent transmission within the same facility (≤12 SNPs), we observed that our outbreak fell within this threshold; however, this approximation did not fit with other nosocomial outbreaks. Examining the median genetic distance within other previously described outbreaks (one from the UK[14] and one from China[15]), we observed that the maximum distance was 22 SNPs for the case of the UK outbreak, and 19 SNPs for the China outbreak (Figure 5). In contrast, when analysing the range of distances between the rest of the genomes that have not been described in nosocomial outbreaks, we can observe

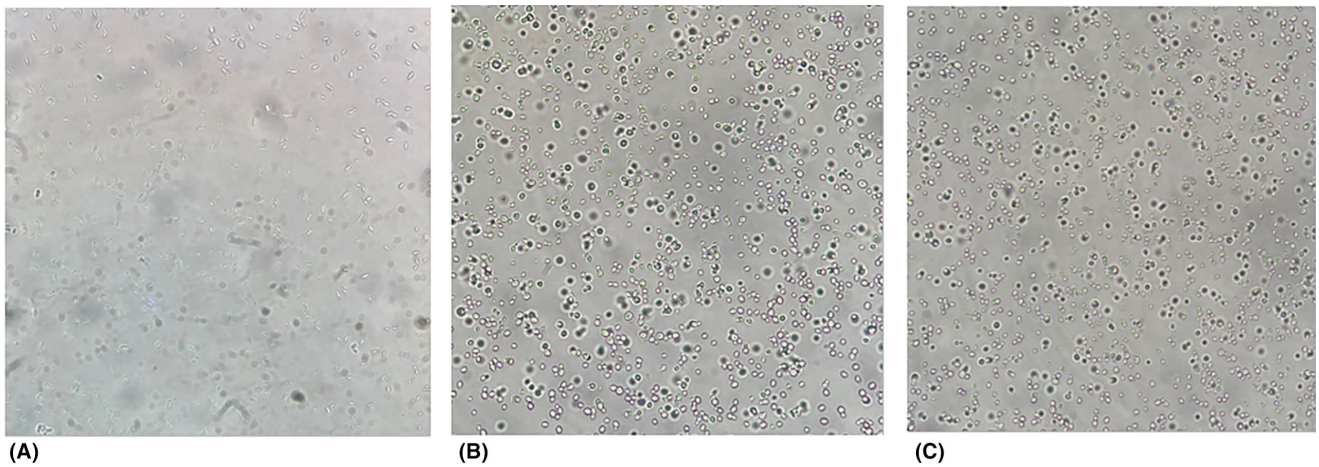

FIGURE 2 Microscopic appearance of isolates MYA-5001 (type strain, A), S1 (B) and S151 (C), they all showing disperse cells and, therefore, being characterised as non-aggregating (×400 magnification). All 35 isolates showed the same non-aggregating phenotype.

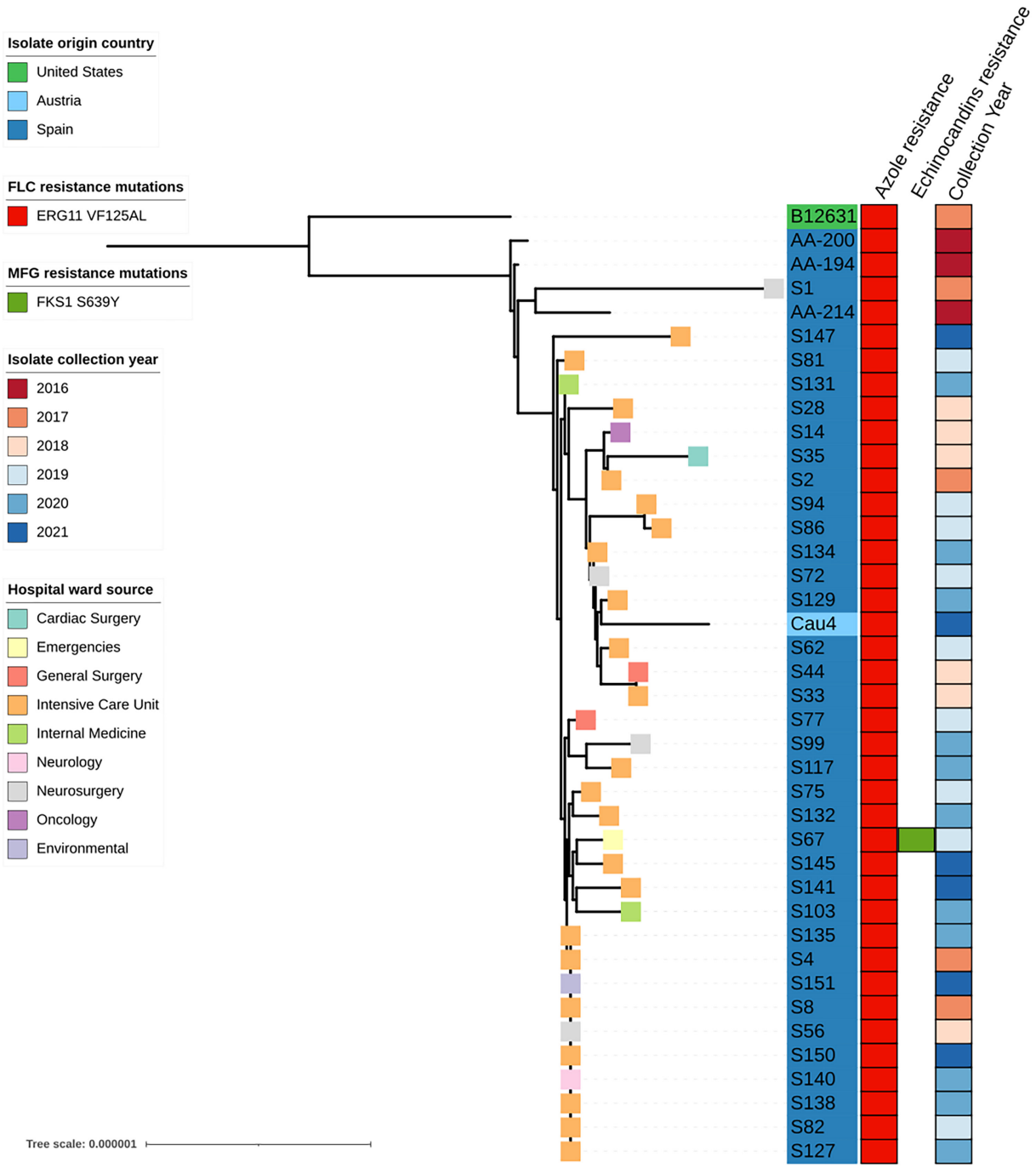

FIGURE 3 Phylogeny of the Valencia outbreak. The tree was contructed using the 35 *Candida auris* genomes from our setting (strains from 'S1' to 'S151') and five publicly available genomes. The strain 'B12631' was used as an outgroup. Country of origin, azole- and echinocandin-resistant associated mutations and collection year are shown. The rectangle shape at the branch tip indicates the hospital ward origin.

that there are pairs of isolates that have compatible distances with this category of recent transmission (range 0–53 SNPs).

4 | DISCUSSION

C. auris outbreaks have been difficult to control worldwide, with some of them lasting for over a year, as the one occurred in our setting. The characterisation of the isolates show that they are genotypically related and phenotypically non-aggregating, which seems to indicate that they all belong to a single outbreak originated from a single isolate, remaining largely invariable over the years. It is noteworthy that the first patient was not admitted to the ICU, while the outbreak started 1 month later in the ICU ward; no epidemiological relationship was found. Since then, the pathogen has mostly affected patients at the ICU or recently discharged from it, being the

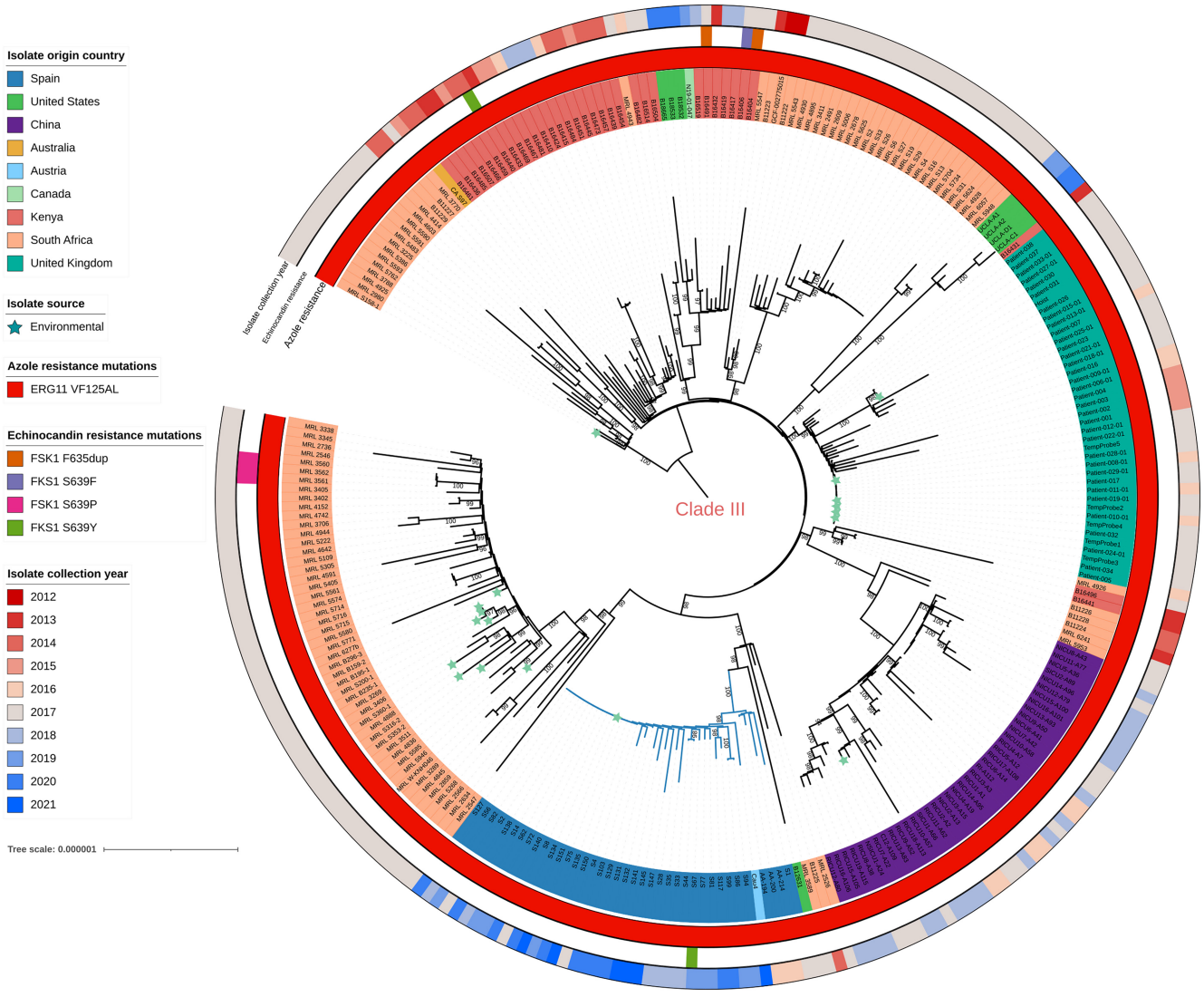

FIGURE 4 Maximum likelihood phylogenetic tree of 35 *Candida auris* genomes from our setting (S1 to S151) and 268 publicly available *C. auris* genomes from Clade III ($n = 803$ variant positions). Country of origin, azole- and echinocandin-resistant associated mutations, and collection year are shown from inner to outer circles. The star shape indicates isolates of environmental origin.

probable main epidemiological source, although other wards cannot be excluded as epidemiological sources, as screening colonisation studies are only performed at the ICU and there may be undetected colonised patients in other wards, who do not develop as many invasive infections due to less risk factors. Environmental screening was also performed in some cases at the ICU, finding the pathogen in beds, bed rails, bedside tables, computer keyboards and mouse, and several medical devices. In fact, it is widely recognised that invasive candidiasis, such a *C. auris* candidaemia, has a propensity to affect critically ill patients in ICUs.[20]

Some *C. auris* outbreaks have been attributed to recent spread to other continents, as the first European outbreak occurred in the United Kingdom in 2015,[23] whose isolates analysed by WGS were found to belong to clade I (with an Asian origin). *C. auris* was isolated for the first time in our setting in September 2017, in a patient transferred from HUiP La Fe, which had an ongoing outbreak.[19,20] Amplified fragment length polymorphism (AFLP) analysis in samples from HUiP La Fe revealed that the isolates were genetically similar to South African isolates (clade III), except one isolate which was grouped in the Venezuelan cluster (clade IV).[20] Three isolates from this outbreak were also sequenced (isolates AA-194, AA-200 and AA-214) and assigned to clade III.[4] Although the most likely hypothesis was that our outbreak originated from the outbreak of HUiP La Fe, the possibility that *C. auris* would have been introduced to our setting from other source did exist. In fact, Chow et al. found that multiple clades of *C. auris* were introduced into the United States, some of which even several times.[22] However, phylogenetic analysis of the 35 isolates form CHGUV showed that they all belonged to clade III, and closely clustered with the 3 isolates from HUiP La Fe, which were also ancestors of our isolates, making the first hypothesis more probable. These results make Spain the only country in the European Union with persistent transmission of a single clade *C. auris*, which is a remarkable finding since it would be expected that such prolonged outbreaks

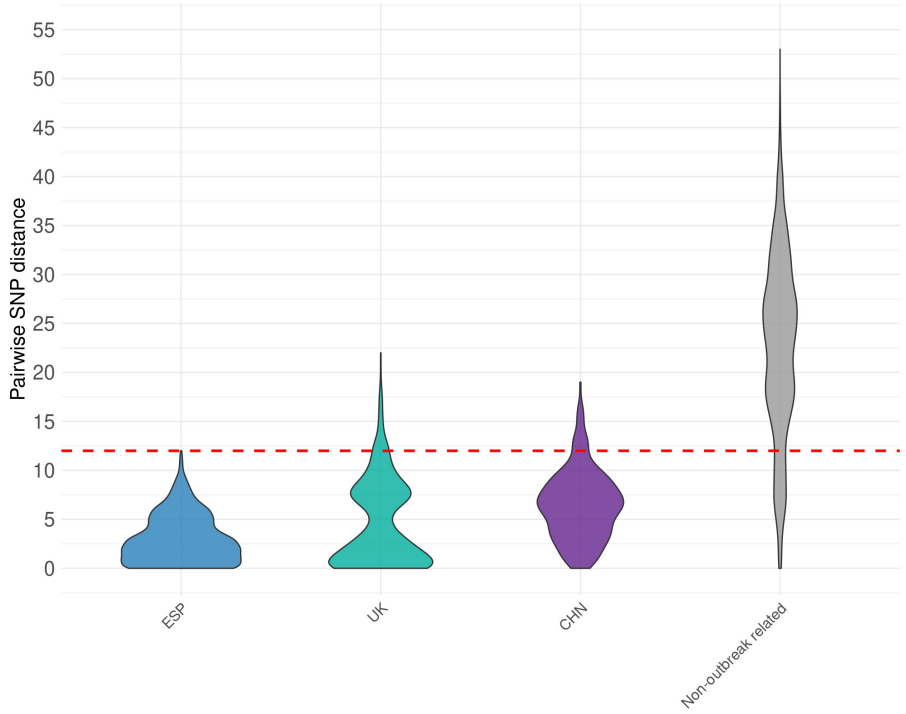

FIGURE 5 Pairwise genetic distances between *Candida auris* clade III samples belonging to the same nosocomial outbreak (intra-outbreak) and those that are not considered to be from the same outbreak. The red dashed line indicates the threshold marker of recent transmission (12 SNPs).

would have had multiple introductions from different clades, as it occurred in the United States[22] or even in single institutions.[24] The maximum genetic distance among Spanish isolates is 12 SNPs, suggesting a recent transmission event within a healthcare facility.[22] Nevertheless, this is not necessarily a universal threshold, applicable in all cases. By analysing the genetic diversity among samples from other well-defined nosocomial outbreaks, we observed larger differences (range 0–22 SNPs, Figure 5). On the contrary, epidemiologically unrelated isolates show genetic distances compatible with recent transmission (range 0–53 SNPs). This, along with the fact that the first reported case was a patient transferred from another hospital, suggests that the threshold for recent transmission would have to be redefined. This variation may be due to the pathogen not evolving at the same rate in all cases, or to slight differences in the pattern of transmission among settings. For instance, the establishment of single strains in different locations in the same facility might lead to sustained divergence among them. This phenomenon apparently occurred in a nosocomial outbreak in China, where the generation and adaptation of different clades can be observed depending on the hospital area where the isolates were identified.[15] In addition, factors such as antifungal selective pressure within patients and site of infection might influence the rate of evolution of the pathogen. A recent study has mentioned that the maximum number of genetic differences between samples from the same patient may vary according to time and site of infection.[25] Over a 10-month period of infection, the authors describe 11 SNPs of difference between the initial sensitive sample and the resistant sample from the same patient. More detailed information about the time and location of the isolates within the hospital along with matching environmental samples would be necessary to test this hypothesis.[25]

Genome studies showed that several known antifungal drug targets for *C. albicans* are conserved in *C. auris*, including the azole target lanosterol 14 α-demethylase (ERG11) and the echinocandin target 1,3-beta-glucan synthase (FKS1), and several mutations in their genes have been described and linked to drug resistance, both in *C. albicans* and *C. auris*.[4,26] The mutation VF125AL (also referred to as F126) in ERG11 gene was present in all isolates from our setting, conferring them resistance to azole drugs, which was consistent with their phenotypic behaviour. This mutation is strongly associated with the geographic clade III, as it has been reported in clonal clade III isolates from South Africa.[26] Our isolates belong to group G3 of clade III, only integrated by fluconazole-resistant isolates with the mentioned mutation, while groups G1 and G2 are integrated by isolates without fluconazole-resistant associated mutations. With respect to echinocandins, several hot-spot mutations in FKS1 gene have been reported to cause resistance to these antifungals (mainly in hot-spot regions HS1 and HS2, resulting in several alterations of FKS1, such as F635Y, F635L, S639F, F639P, S639Y, R1354S, R1354H, D642Y or W691L).[18,26,27] Mutation S639Y was found in the echinocandin-resistant isolate S67, which was isolated from a patient carrying a long-term peripherally inserted central catheter line and who was previously treated with echinocandins, probably selecting the raise of resistance mutants (this case was previously reported in Mulet-Bayona et al.[28]). The S639Y mutation has been reported

to cause echinocandin resistance in some *C. auris* isolates from clades I and III.[29]

With respect to AFST methodology, it must be mentioned that microdilution CLSI and EUCAST methods are the reference methods, although they are time-consuming and not usually suitable for a daily routine in clinical settings. Indeed, the commercial method Sensititre® YeastOne was used in this work, because it has shown to correlate better than other commercial methods with reference methods,[30] although it has been reported that it also runs the risk of overestimating amphotericin B resistance.[31] However, it is noteworthy that the AFST profile of these samples correlated well with the WGS analysis, and this fact validates the performance of Sensititre® YeastOne in these cases.

Two different phenotypes have been described for *C. auris*: the aggregating and the non-aggregating phenotypes, the latter having been linked to a greater virulence of the strains.[6] Unfortunately, the genetic regulation of the *C. auris* morphogenesis and its in vivo significance remains largely uncharacterized, although some insights into the *C. auris* genome have been provided.[32] The crude mortality rate for candidaemia cases in our setting (32.6%) is in line with the mortality reported from other outbreaks, from 20% to 60%, so it is difficult to evaluate if the non-aggregating phenotype of our isolates has contributed to a higher mortality. Instead, Sherry et al. found that the non-aggregating phenotype strains produced more biofilm,[33] so this fact might explain the high prevalence of invasive infections (candidaemia) and the difficulty in ending the outbreak in our setting, as biofilm-producing bacteria have a better adherence to medical devices and surfaces. In a previous study, isolates belonging to different clades showed different phenotypic traits, including the aggregating/non-aggregating phenotype, being therefore possible that isolates from every clade had an associated phenotypic behaviour.[11] Interestingly, all our clade III isolates exhibited a non-aggregating phenotype, differing from the clade III isolates from Szekely et al., which were all strongly-aggregating isolates.[11] Therefore, it seems that phenotypic traits are not uniform within a clade, but they are strain-dependent and remain constant over the years. More isolates from different clades and countries should be tested in order to better assess the heterogeneity and the clinical significance of the phenotype.

A limitation of this study is that only a small subset of isolates among all positive cultures has been characterised, so a different strain might have been missed. However, the random selection from several days apart over 4 years should minimise that risk. The molecular epidemiological investigation of *C. auris* outbreaks from different locations is clearly needed to understand the population structure and transmission dynamics of this novel pathogen. Our results provide evidence that nosocomial outbreaks are usually originated from a single origin. Patient environments serve as reservoirs of the pathogen and this results in a cross-transmission among patients within the hospital setting, usually in ICUs. This fact should stress the importance of implementing infection control practices as soon as the first case is detected or when a patient is transferred from a setting with known cases.

AUTHOR CONTRIBUTIONS

Juan Vicente Mulet-Bayona: Conceptualization; investigation; writing – original draft; methodology; formal analysis; data curation. **Irving Cancino-Muñoz:** Conceptualization; investigation; writing – original draft; methodology; formal analysis; data curation. **Carme Salvador-García:** Conceptualization; methodology; supervision. **Nuria Tormo-Palop:** Conceptualization; methodology; supervision. **María del Remedio Guna-Serrano:** Investigation. **Carolina Ferrer-Gómez:** Investigation. **Mercedes Melero-García:** Investigation. **Fernando González-Candelas:** Conceptualization; methodology; investigation; supervision; writing – review and editing; resources. **Concepción Gimeno-Cardona:** Conceptualization; investigation; methodology; writing – review and editing; supervision; resources.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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