INTRODUCTION

Candida auris is a recently recognised opportunistic, nosocomial yeast pathogen that was first reported in a patient treated for an ear infection at a Japanese hospital in 2009.\(^1\) The earliest known isolate was retrospectively identified from an archived blood culture sample collected in 1996 in South Korea,\(^2\) and the earliest European isolate dates to 2007 in France.\(^3\) The mortality rate due to C. auris infection remains significant, up to 60\% depending on the patient’s underlying conditions and the therapeutic management of infection.\(^4\) It significantly affects

Emergence of Candida auris in intensive care units in Algeria

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**Abstract**

**Background:** Currently, Candida auris is among the most serious emerging pathogens that can be associated with nosocomial infections and outbreaks in intensive care units. Clinicians must be able to identify and manage it quickly.  

**Objective:** Here, we report for the first time in Algeria seven cases of C. auris infection or colonisation.

**Methods and Results:** The strains were isolated from clinical sites including bronchial aspirates (n = 4), wound swabs (n = 1), urine sample (n = 1) and peritoneal fluid (n = 1), in patients admitted to the intensive care unit. Candida auris was identified both by MALDI-TOF and by sequencing the ITS region and the D1/D2 domain. Antifungal susceptibility testing was performed using the E-test method. Non-wildtype susceptibility was observed for five strains against fluconazole, itraconazole, voriconazole and caspofungin. Genotyping showed the presence of four clades (I–IV) in one hospital.

**Conclusions:** Appropriate antifungal treatments with rapid and accurate microbial identification are the cornerstone for the management and control of C. auris infections.

**KEYWORDS**  
Algeria, Candida auris, clades (I–IV), emergence, ICU

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immunocompromised patients and is associated with many comorbid conditions such as diabetes, pneumonia, organ dysfunction, kidney diseases and malignancies. This yeast can easily cause nosocomial infections and can be transmitted in healthcare settings due to its capacity to persist on inanimate surfaces for weeks, form biofilms, and colonise skin and other anatomical sites. Moreover, it is a thermo-resistant, halotolerant and multidrug-resistant fungus; many studies show that it can grow at temperatures up to 42°C with a salinity tolerance of up to 10%. In addition, C. auris strains may show high resistance levels to several clinically used antifungal agents such as fluconazole, amphotericin B and echinocandins. Diagnosis and treatment of this newly described, multi-resistant species remains a challenge, especially in developing countries. Recently, new molecular and phenotypic techniques to identify C. auris have been developed. Despite this, the identification and detection of this yeast still presents difficulties in many countries and research laboratories, especially those using commercial methods that misidentify C. auris, such as VITEK, BD Phoenix, API20C-AUX, MicroScan and Chromagar Candida culture medium.

Since its emergence, C. auris has been identified in invasive infections and nosocomial outbreaks reported from various countries across six continents. Whole-genome sequencing has divided C. auris isolates in five distinct clonal lineages, commonly referred to as clade I (South Asian), clade II (East Asian), clade III (African), clade IV (South American) and clade V (Iranian). Clades I and III are the most widespread, with numerous reported cases and a broad geographic distribution. To date, C. auris is poorly documented in Africa and has only been reported in Egypt, Kenya, Nigeria, South Africa and Sudan, with the prevalence of clades I, III and IV. In Algeria, C. tropicalis has been the most prevalent isolate from candidemia with no published data on C. auris. Here, we describe a series of C. auris infection and colonisation at a hospital in Tlemcen, Algeria, with clade distribution and antifungal susceptibility profiles.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethical issues

All rules of confidentiality and ethics as prescribed in the Helsinki Declaration have been respected. Informed consent is not required given that the patients’ samples were not collected for research purposes but were already ordered by medical doctors to be processed as part of standard laboratory practice (i.e., for routine diagnostic testing). Only residual samples were used for this study. Patient data were collected by the medical staff and were anonymised before further use. The Local Ethics Committee of Tlemcen University approved this survey study.

### 2.2 | Sample collection, strain characterisation and antifungal susceptibility tests

For microbiological diagnostic purposes, 87 samples including bronchial aspirates (n = 20), peritoneal fluid (n = 4), urine (n = 23), wounds and bedsores swabs (n = 40) were collected from the intensive care unit at the University Hospital of Tlemcen in Algeria between 1 October 2017 and 1 June 2019. Firstly, to search for C. auris strains, each sample was enriched in Sabouraud liquid medium (Oxoid™, Dardilly, France) for 3 days at 37°C. For DNA extraction, we then used, for each enrichment broth, the EZ1 biorobot (Qiagen BioRobot EZ1) with the EZ1 DNA tissue kit (EZ1 DNA Qiagen). Subsequently, for each DNA sample, a specific real-time PCR test for C. auris detection was performed, exactly as described by Ibrahim et al.

In addition, 100 μl of each sample was cultured on CHROMID® Candida (bioMérieux) and SCA (Specific C. auris) medium and then incubated at 37°C for 48 h. For microbiological identification, each isolated colony was identified using Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS) (Microflex™; Bruker Daltonik, Bremen, Germany) as described by L’Ollivier et al.

To be more specific, we performed an additional molecular test on each colony identified as C. auris. DNA was extracted, and PCR amplifications of the ITS region and the D1/D2 domain were carried out to confirm its identity. This was followed by Sanger sequencing of the obtained amplicons. The sequences obtained from all tested strains were analysed by NCBI BLASTn against the nr database and deposited in GenBank with the following numbers: OM403669 to OM403675 for the ITS region and OM434430 to OM434436 for the D1/D2 domain. The strains were also submitted to the CSUR collection (Collection de Souches de l’Unite des Rickettsies) with the following numbers: L0048 to L0054.

Antifungal susceptibility tests were carried out using the E-test method on RPMI 1640–2% glucose agar (bioMérieux, Marcy-l’Étoile, France) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Nine antifungal agents were tested, including amphotericin B, fluconazole, isavuconazole, itraconazole, posaconazole, voriconazole, anidulafungin, caspofungin and micafungin (bioMérieux) and incubated for 24 h at 37°C. The susceptibility breakpoints were used as suggested previously and those proposed by the US Centers for Disease Control and Prevention (CDC) in October 2017 and modified in April 2019.

2.3 | Molecular fingerprinting of C. auris isolates

The genotypic relationship between the seven C. auris isolates from Algeria and those obtained from other geographical locations, including the major C. auris clades, was determined by 12-loci-based short tandem repeat (STR) typing, which was performed as previously described. The phylogenetic relationship between C. auris isolates was analysed using the BioNumerics v.7.6.1 software (Applied Maths), by employing the unweighted pair group method with arithmetic mean averages (UPGMA).

# References

[1] Ibrahim, A. et al. (2021). Molecular fingerprinting of C. auris isolates from Algeria and those obtained from other geographical locations, including the major C. auris clades, was determined by 12-loci-based short tandem repeat (STR) typing, which was performed as previously described. The phylogenetic relationship between C. auris isolates was analysed using the BioNumerics v.7.6.1 software (Applied Maths), by employing the unweighted pair group method with arithmetic mean averages (UPGMA).
| Strain | Sex/Age | Hospitalisation date | Cause of admission to ICU | Co-morbidity | Duration of ICU stay | Antimicrobial treatment | Site of *C. auris* isolation |
|--------|---------|----------------------|---------------------------|-------------|---------------------|------------------------|--------------------------|
| L0048  | M/52 years - 01/09/2017 | Head trauma: Meningeal haemorrhage + cerebral oedema | Dialysis | 330 days | Broad-spectrum antibiotics / Amphotericin B | Urine |
| L0049  | M/30 years - 26/05/2017 | Multiple trauma: Discomfort + chest pulmonary contusion | / | 112 days | Broad-spectrum antibiotics / Amphotericin B | Bronchial aspiration |
| L0050  | F/76 years - 04/08/2018 | Postoperative peritonitis | Diabetes insipidus Dyslipidaemia / Heart disease | 2 days | Broad-spectrum antibiotics | Peritoneal fluid |
| L0051  | M/67 years - 26/07/2019 | Polytrauma: operated for intraparenchymal haematoma | Diabetes | 19 days | Broad-spectrum antibiotics / Fluconazole | Wound swabs |
| L0052  | M/55 years - 08/08/2019 | Haemorrhagic stroke + ventricular flood | Diabetes | 277 days | Broad-spectrum antibiotics / Fluconazole / Caspofungin | Bronchial aspiration |
| L0053  | M/50 years - 02/08/2019 | Postoperative respiratory failure: Pituitary Adenoma Surgery | Pituitary adenoma / Diabetes / Heart disease | 9 days | Broad-spectrum antibiotics / Fluconazole | Bronchial aspiration |
| L0054  | F/72 years - 14/01/2018 | Pneumothorax disease | Arterial hypertension | 15 days | Broad-spectrum antibiotics | Bronchial aspiration |
RESULTS

3.1 Detection of Candida auris strains

All seven samples were positive for the C. auris specific GPI-encoding gene, as tested by real-time PCR. Moreover, cultures on the SCA medium were suggestive of C. auris and pink colonies were observed on the CHROMagar Candida medium. According to the MALDI-TOF-MS identification, all these colonies belong to C. auris, with score >2.0. In addition to the MALDI-TOF-MS results, PCR and Sanger sequencing of the ITS region and D1/D2 domains of each isolated C. auris were performed and all sequences belong to C. auris species according to the BLASTn NCBI database.

Positive samples were obtained from bronchial aspirates (n = 4), urine (n = 1), peritoneal fluid (n = 1) and wound swabs (n = 1). Of the seven patients, five were male and the mean age was 57 (ranging from 30 to 76 years). The mean duration of stay in the ICU was 109 days (ranging from 2 to 330 days). Of these patients, four underwent recent surgery and all patients had indwelling devices including central venous catheters, arterial catheters, nasopharyngeal tubes and urinary catheters. All seven patients received mechanical ventilation and broad-spectrum antibiotics. Antifungal drugs including amphotericin B, fluconazole and caspofungin were prescribed for five of the patients. Here, Table 1 shows the demographic and clinical characteristics of patients. It is important to note that, in addition to the C. auris strains, we cultured different Candida species such as C. albicans, C. glabrata, C. parapsilosis, C. rugosa and C. tropicalis.

All isolates had a similar susceptibility profile, being wildtype susceptible to isavuconazole. Posaconazole, micafungin and anidulafungin exhibited reduced susceptibility, while non-wildtype susceptibility was observed against itraconazole (MICs ranging from 0.09 to 6 μg/ml) and voriconazole (MICs ranging from 0.064 to 32 μg/ml). Five strains showed high MICs of fluconazole (>256 μg/ml). Only one strain was found with high amphotericin MIC (12 μg/ml) and another with caspofungin (MIC >32 μg/ml). Table 2 presents the MIC values of the C. auris isolates.

3.2 Fingerprinting of Candida auris isolates by STR typing

DNA fingerprinting with STR analysis of seven isolates from seven patients showed that there were C. auris isolates from the four clades involved. Three patients had genetically identical isolates involving the South American clade IV, with genotype 9 (L0049, L0050 and L0051 isolates). Similarly, two patients also had a similar genotype 35 in the South African clade III (L0052 and L0053 isolates). Patients L0048 (clade II) and L0054 (clade I) both had a single genotype (Figure 1).

DISCUSSION

Candida auris is an emerging multidrug-resistant yeast that has caused nosocomial outbreaks in multiple countries. In 2016, the
FIGURE 1 UPGMA dendrogram of STR of seven *Candida auris* isolates from this study and 67 strains originating from various countries and clades. The Algerian isolates clustered in clade I with isolates from India, in clade II with isolates from East Asia, in clade III with isolates from South Africa and in clade IV with isolates from South America.
Centre for Disease Control and Prevention (CDC) issued a clinical alert on the international emergence of C. auris infections and released recommendations for disinfection and treatment.\(^2\) In this study, we report the first isolation of C. auris in Algeria consisting of different clades and antifungal susceptibility.

*Candida auris* can colonise multiple sites on the body.\(^30\) In the intensive care unit, the massive use of broad-spectrum antibiotics and antifungals has resulted in the commensal microflora elimination and provided favourable ground for the proliferation of this pathogen.\(^31\) In addition, prolonged stay in the ICU and indwelling devices can predispose patients to the transmission of this yeast through caregivers, the environment and patients themselves.\(^32\) We isolated *C. auris* strains from seven patients that spent variable periods of time in the ICU. None of these patients had a recent travel history and all were previously hospitalised in the same university hospital. The infected/colonised patients in this study had similar risk factors as reported in other studies, including immunosuppression, ICU admission, diabetes, dialysis, broad-spectrum antibiotic therapy, recent surgery, and urinary and central venous catheters.\(^31\)

Biochemical methods and phenotypic commercial identification kits often confuse *C. auris* with other yeasts. Laboratories in developing countries which lack molecular identification techniques fail to identify *C. auris*, leading to potentially inappropriate treatment.\(^29\) We previously developed a SCA medium for the selective isolation of *C. auris* yeasts\(^13\) and a RT-PCR system for the specific and rapid detection of this pathogen directly from clinical and environmental samples.\(^14\) This helped with the fast, precise, and low-cost diagnosis of *C. auris* strains, improving infection management and epidemiological surveillance in our healthcare facility. The real prevalence of *C. auris* across the African continent is still unknown due to the limited availability of accurate diagnostic tools. So far, *C. auris* infection/colonisation has only been reported in five African countries, namely Egypt,\(^18\) Kenya,\(^19,20\) Nigeria,\(^21\) South Africa\(^35\) and Sudan.\(^22\)

*Candida auris* infections are among the most reported nosocomial infections worldwide, and there are no accurate criteria to distinguish colonisation from infection in *C. auris* cases.\(^34\) We recovered *C. auris* from different human sites, including wounds, urine, peritoneal fluid and bronchial aspirations, which is rarely described. Most described strains are isolated from the blood and the ear canal.\(^33\) It is essential to highlight that the presence of *C. auris* in bronchial aspirations, wounds and urine was often interpreted as colonisation rather than infection.\(^33,35\)

The collection of *C. auris* strains had non-wildtype susceptibility to fluconazole, itraconazole, voriconazole, with low MICs to posaconazole, isavuconazole, micafungin and anidulafungin. One isolate (clade I) was resistant to amphotericin B (MIC 12μg/ml). Moreover, MICs of antifungal drugs varied among cases and geographical locations, but many studies have described *C. auris* as a multidrug-resistant yeast.\(^36,37\) It is interesting to note that three clade IV isolates from three patients (L0049, L0050, L0051) were genotypically the same, but included one with low fluconazole MIC. Genotyping suggests cross-infection between patients L0049-L0051 and patients L0052-L0053.

Although there are no susceptibility breakpoints for *C. auris* strains,\(^38\) echinocandins are the first-line treatment.\(^39\) However, antifungal susceptibility testing is still recommended for the optimal management of infections and outbreaks.\(^40\) We reported for the first time an outbreak of *C. auris* in Algeria. Different epidemiological studies have previously reported the presence of different *C. auris* clades in a single country, such as the presence of four different clades (I, II, III and IV) in the United States\(^41\) and Canada,\(^42\) three clades (I, III and IV) in South Africa,\(^16\) and two clades (I and III) in Germany\(^33\) and Iran (clades I and V),\(^44\) which shows the dissemination of *C. auris* clades between countries (except for the fifth clade). Only in Kenya were *C. auris* isolates from two clades (I and III) reported simultaneously in the same hospital.\(^23\) It is important to note that this is the first report of four different clades of *C. auris* in a single hospital, which has never been described before, especially since the patients reported no recent travel history. Therefore, it would be prudent to routinely test the clade origin of future *C. auris* isolates to trace this yeast in the African continent.

**AUTHOR CONTRIBUTIONS**

Conceptualisation: J.F.M., J.-M.R. and F.B.; methodology: H.Z., A.I. and T. d.-G.; data collection: S.-A.R., Y.E. and D.-E.B.; writing original draft preparation: H.Z., A.I., J.F.M. and F.B.; writing review and editing: J.F.M., J.-M.R. and F.B.; supervision: J.F.M., J.-M.R. and F.B. All authors have read and agreed to the published version of the manuscript.

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None.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are openly available in GenBank at [https://www.ncbi.nlm.nih.gov/genbank/](https://www.ncbi.nlm.nih.gov/genbank/), reference number OM403669 to OM403675 and OM434430 to OM434436.

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