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## ***Candida auris* in contemporary mycology labs: A few practical tricks to identify it reliably according to one recent French experience**

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*Haemulonii* complex  
*Candida auris*

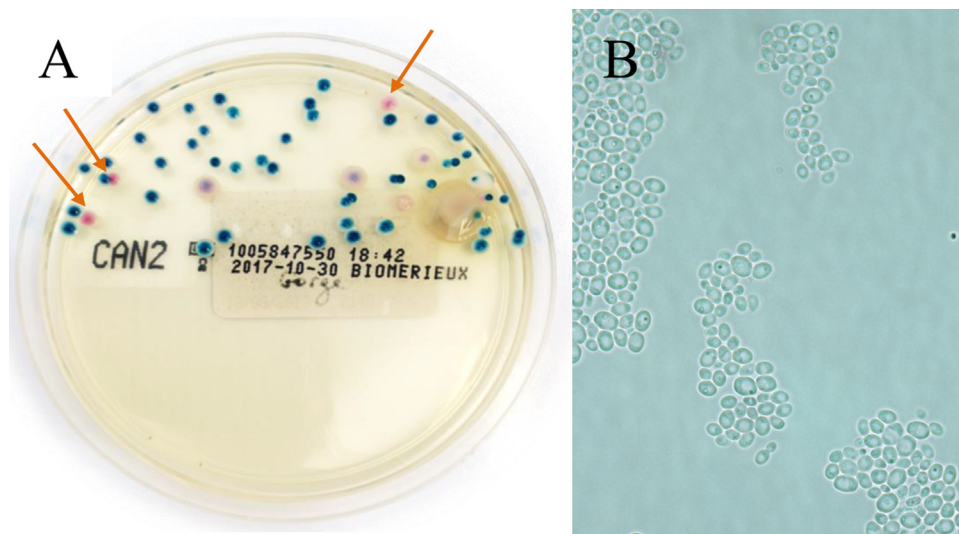
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*Candida auris* is an emerging pathogenic yeast which can be potentially multidrug-resistant and responsible for invasive infections with treatment failure. This species was described in 2009 based on sequencing of ITS (nuclear ribosomal DNA internal transcribed spacer) and D1/D2 (26S ribosomal DNA large subunit) regions. Actually, *C. auris* is phylogenetically related to the *haemulonii* complex (*Metchnikowiaceae* family), which also includes *C. haemulonii stricto sensu*, *C. ruelliae*, *C. duobushaemulonii*, *C. heveicola*, and *C. pseudohaemulonii* [1]. All these *Candida* species are phenotypically very similar, so that they are sometimes designated as cryptic species, i.e. based on traditional biochemical methods only, it is particularly challenging for mycologists to correctly distinguish one species from the others of this complex [2]. Furthermore, isolates of *C. auris* were also frequently misidentified with other distinct yeast species such as *C. lusitaniae* and *C. famata*.

Recently, several *C. auris* outbreaks have been described worldwide in hospital settings, including both cases of casual colonization and cases of true infection. For instance, 50 patients were detected to host this yeast within a single cardio-thoracic surgery center in England (April 2015–July 2016), including 22 with probable or proven infection [3]; 33 cases of *C. auris* fungemia were notified in a surgical intensive care unit in Spain (April 2016–November 2016) [4], but many other outbreaks were also reported in U.S., India, South America [4]. All these clustered isolates suggested nosocomial transmission and potential risk of emergence. Since the first description of *C. auris* in the external ear canal of a Japanese inpatient in 2009 [1], 90 publications have been indexed to the PubMed database about this hot topic in January 2018. The trend is continuously increasing, e.g. no fewer than 46 articles were published for the year 2017 alone [4], but hopefully no cases had been reported in French laboratories so far. We describe herein how we practically proceeded to definitively identify the first *C. auris* strains isolated in France

from a 58 year-old-Lebanese man who was hospitalized for liver transplantation because of severe cirrhosis, and how we implemented adequate means for controlling its widespreading.

Two months before arriving in France, the patient travelled in India and Iran for medical purposes. He probably acquired there several highly-resistant microorganisms, e.g. azole-resistant *C. glabrata*, fluconazole – and echinocandin-resistant *Geotrichum fragrans*, carbapenem-resistant *Escherichia coli*, vancomycin-resistant *Enterococcus faecium* – all isolated from digestive samples –, and a *E. coli* strain with extended spectrum  $\beta$ -lactamase – in ascites fluid. In addition, a *C. auris* strain was iteratively isolated 11 times in our patient over a 52-day-long period of hospitalization (eight times in throat, one time in rectum, in urines and in peritoneal fluid). On CAN2<sup>®</sup> chromogenic culture medium (BioMérieux, Marcy-L'Étoile, France), colonies grew quite slowly at 30–37°C. They appeared initially whitish, and then displayed a light reddish-pink color, very close to this of *C. kefyr* or *C. tropicalis* (Fig. 1A). However, the strain was still able to readily grow at 37–42°C, and in contrast to the two aforementioned species and to *C. haemulonii*, its microscopic observation did not show filamentous or pseudo-filamentous hyphae (Fig. 2B). Thus, we went ahead with identification testing through MALDI-TOF (matrix associated laser desorption ionization–time of flight) mass spectrometry (MS) [5]. Using the Microflex LT<sup>®</sup> instrument (Bruker Daltonics, Bremen, Germany) and the software FlexControl<sup>®</sup> v3.4 with default settings, our MALDI Biotyper Compass IVD<sup>®</sup> database v4.2.50 was able to suggest *C. auris* as possible species name for 64.71% attempts (i.e. each isolate was tested in duplicate, but around one third were left unidentified) (Fig. 2). When present in the top-ten identification listing, *C. auris* was proposed 81.81% ranked in first position and 72.72% repeated at least a second time in this listing (mostly in second and third position). However, the log-score values were constantly low, i.e. the mean value was only  $1.59 \pm 0.18/3$ , and the most elevated score was 1.89 which theoretically allows only mild reliability, according to the manufacturer's recommendations. Instead, a lot of bacteria names were suggested among the identification listing, like those belonging to the Actinomycetales order (27.03% of the top-ten ranking, including *Arthrobacter*, *Agromyces*, *Corynebacterium*, ... species) or the Lactobacillales (13.51%, e.g. species of the *Lactobacillus* and *Streptococcus* genera), Burkholderiales (13.51%, mostly *Burkholderia* species), and Pseudomonales (11.71%, mostly *Pseudomonas* species) orders. In only 7.20% cases, alternative fungal names were proposed, including 2.70% *Cryptococcus* species which are nonetheless phylogenetically kind very different from *Candida* species. *A posteriori*, we thought that these poor results were maybe due to the limited number of validated main mass spectra (MSP) that are referenced in the MALDI Biotyper Compass IVD<sup>®</sup> database for *C. auris*: only three that come from Japanese (DSM 21092T CBS) and Korean (KCTC\_17809 CBS and KCTC\_17810 CBS) isolates. Besides according to our experience, the manual full-tube extraction for MS did not better than the direct



**Fig. 1.** Phenotypic features of *Candida auris* in vitro cultures. A. Macroscopic observation after 72 h-incubation at 37°C on CAN2<sup>®</sup> chromogenic culture medium (*BioMérieux*, Marcy-L'Étoile, France). *C. auris* colonies are small and usually develop a reddish-pink color after  $\geq 48$  h culture (red arrows). On non-chromogenic Sabouraud conventional culture plate, colonies usually stay creamy white, whereas they rather display a light purple color on BBL CHROMagar *Candida*<sup>®</sup> medium (Becton Dickinson, Le-Pont-de-Claix, France). B. Microscopic observation after 72h-incubation at 37°C (fresh mounting, magnification  $\times 1000$ ). *C. auris* is small yeast that does not produce filamentous or pseudo-filamentous hyphae, but it can form budding.

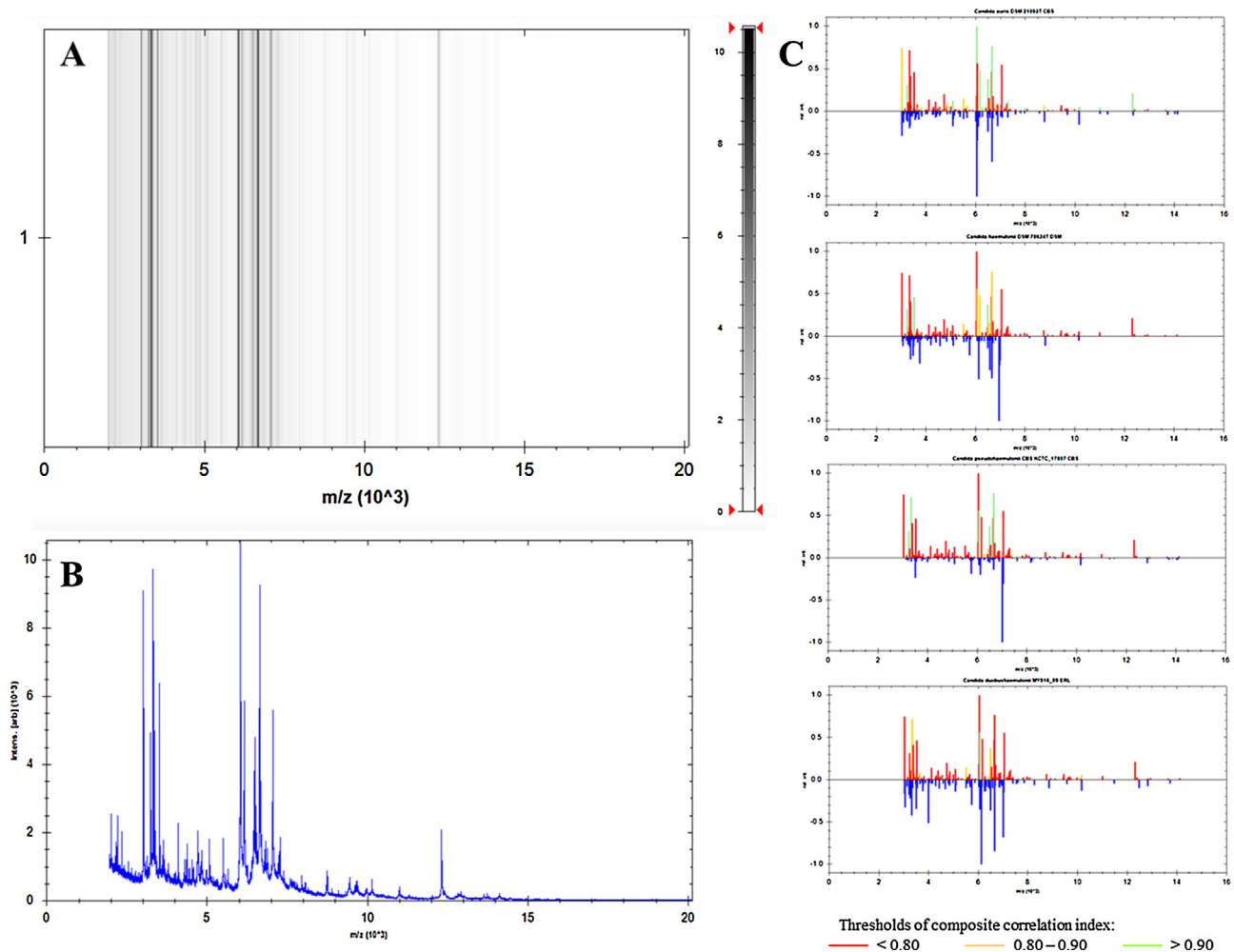
on-plate extraction. This finding was contradictory with previous reports [6]. Anyway, strain identification was finally unambiguously confirmed by DNA sequencing of the D1/D2 (query cover = 100%; identity = 99% of similarity with sequence of UZ1447\_15 type strain for 326 bp sequence) and ITS (query cover = 100%; 99% of similarity with sequence of CBS 10913 type strain for 400 bp sequence) regions. GenBank accession No. MG736297 was granted to the D1/D2 strain sequence. Concomitantly, correct identification was not achievable through auxanogram galleries: API20C<sup>®</sup> system (*BioMérieux*, Marcy-L'Étoile, France) provided *Rhodotorula glutinis* (percentage of identification (%id) = 98.3%; T-index = 0.54) as alternative name (but urease test in tube was of course negative), and ID32C<sup>®</sup> biochemical platform (*BioMérieux*, Marcy-L'Étoile, France) gave *Candida sake* (%id = 98.1%; T-index = 0.64), but noteworthy both biochemical galleries don't have *C. auris* in their respective libraries. Thus, we suggest that identification of *R. glutinis* by auxanogram system should trigger additional investigation if fungal colonies are not naturally pink-colored on conventional non-chromogenic culture plates. Besides, Vitek MS<sup>®</sup> system (*BioMérieux*, Marcy-L'Étoile, France) didn't succeed in identifying *C. auris* when the test was performed with the current IVD (in vitro diagnosis) v3.2 database, probably because the latter includes no reference MSP for this species yet (same comment with the current Andromas<sup>®</sup> [Paris, France] MS database). Likewise, Vitek-2<sup>®</sup> automated instrument (*BioMérieux*, Marcy-L'Étoile, France) was shown to commonly misdiagnose *C. auris* as other yeast [2,5]; besides, an Indian study found that 88.2% of the 102 clinical isolates, previously identified as *C. haemulonii* or *C. famata* with this system, were actually *C. auris* [5].

Susceptibility of our *C. auris* strain to antifungal drugs was in vitro assessed according to the E-test<sup>®</sup> (*BioMérieux*, Marcy-L'Étoile, France) and EUCAST (European committee for antimicrobial susceptibility testing) methods [7]; elevated minimal inhibition concentrations (MICs) were consistently observed for flucytosine and fluconazole ( $> 32$  mg/L) with both techniques. Anyway, determination of MICs seems critical in light of the high probability of resistance acquisition in *C. auris*, e.g. 90% clinical strains display high MIC to fluconazole and 35% to amphotericin B (herein, MIC for amphotericin B was estimated at 0.5 mg/L) [8]. Very recently, some

authors proposed specific guidelines defining the MIC breakpoints and the epidemiological cut-off for *C. auris* species [9].

Following the first detection of *C. auris* in our department, we decided to apply very intense means of decontamination [3,8], so that to avoid selection of this strain and its widespreading in our mycology laboratory [10,11]. The Center for Diseases Control and prevention (Atlanta CDC) specifically recommends use of a Environmental Protection Agency (EPA)-registered hospital-grade disinfectant (List K) effective against *Clostridium difficile* spores (<https://www.cdc.gov/fungal/diseases/candidiasis/c-auris-infection-control.html>) [12]. Indeed, experimental works recently showed significant higher length of *C. auris* survival on health care surfaces: at day 28, 3.0 Log<sub>10</sub> colony forming units (CFU) viable cells vs. 2.6 CFU for *C. parapsilosis* ( $P < 0.05$ ) [10]. Likewise, quaternary ammonium products, e.g. Surfanios Premium<sup>®</sup> (*Laboratoire Anios*, Lille – Hellemmes, France), exhibited poor activity against *C. auris* (in average, reduction of only 1.0 Log<sub>10</sub> CFU vs. 6.0 for *Staphylococcus aureus*), whereas chlorine, peracetic acid and hydrogen peroxide remained quite effective (respective mean reduction of 6.0, 5.0, and 5.5 Log<sub>10</sub> CFU, like for *C. albicans*, *C. glabrata* and *S. aureus*;  $P > 0.05$ ) [11]. Herein, every *C. auris* isolate was confined in a sealed plastic bag and moved to the fridge at +4°C. Horizontal surfaces, plus all items and furniture that may have come into contact with the strain, were daily cleaned by thorough wet wiping and by application of bleach, 2.6% chlorine in cold water (Avix Javel<sup>®</sup> pH12, Coldis, Entraigues-sur-la-Sorgue, France). Floor was similarly treated thrice weekly. Besides, UV treatment was implemented for the microbiological flow hoods during 30 min, instead of 5 min, because data are limited about hands-free disinfection methods. All these efforts were sustained for four weeks following the last isolate [10].

The patient finally died 52 days after hospital admission. Meanwhile, contact individuals were screened thrice by sampling urines, rectum, axillae and throat. Detection was also carried out in exposed patients weekly by cutaneous swabbing of the groins and the axillae. Altogether, no proofs occurred neither for *C. auris* fungemia nor transmission of the strain to other patients of our facility.



**Fig. 2.** Example of mass spectra for one *Candida auris* isolate based on analysis with the Microflex LT<sup>®</sup> instrument (Bruker Daltonics, Bremen, Germany) and with the MALDI Biotyper Compass Explorer v4.1.80.7 (Build [80])<sup>®</sup> software (Bruker Daltonics, Bremen, Germany). A. Normalized spectrum (in-gel view). B. Normalized spectrum (classic view). Sixteen MSP peaks were theoretically expected to be present (frequency = 100%). C. Comparison of spectra with various strains of the database (from top to bottom): *C. auris* DSM 21092 T CBS (log-score value = 1.81), *C. haemulonii* 70624 T DSM (log-score value = 0.98), *C. pseudohaemulonii* KCTC 17807 CBS (log-score value = 0.82), and *C. duobushaemulonii* MY 916\_09 ERL (log-score value = 0.87), according to the MALDI Biotyper<sup>®</sup> MSP identification standard method v1.1 (frequency threshold for spectra adjusting = 50%, frequency threshold for score calculation = 5%, max mass error of the raw spectrum = 2000 Da, desorption mass tolerance of the adjusted spectrum = 250 Da, furthermore accepted mass tolerance for a peak = 600 Da). Our isolate is represented in the upper part of each panel, the reference spectra of the database appear in blue in the lower part of each (thresholds of composite correlation index: < 0.80, red–low matching; 0.80–0.90, yellow–moderate matching; > 0.90, green–high matching). arb: Arbitrary; intens.: Intensity; m: Mass; rel. int.: Relative intensity; z: ionic charge.

In conclusion, mycologists have to stay vigilant when patients come back from tropical countries, especially from Asia but also from Middle East, because they are likely to vehicle unusual and emerging highly-resistant microorganisms, including fungal species like *C. auris*. Although this yeast species remains rare in Western countries, its real prevalence in healthcare settings may have been perhaps underestimated so far, because of unreliable methods of identification [2]. However, it seems critical for clinical mycology laboratories to accurately and rapidly identify this microorganism to aid in preventing healthcare-associated outbreaks. For such a purpose, updating the reference databases with well-identified isolates is imperative.

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### Disclosure of interest

The authors declare that they have no competing interest. They have submitted the ICMJE form.

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