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Candida auris arriving on our shores: an Australian microbiology laboratory's experience

Sir,

Nosocomial outbreaks of *Candida auris* have been reported globally, predominantly from India, the United States and the UK. Although first reported from Japan in 2009, a retrospective analysis discovered a misidentified isolate from a case of fungaemia from 1996 in South Korea.[1,2] The identification of *C. auris* is challenging for routine laboratories, particularly where matrix assisted laser desorption/ionisation–time of flight mass spectrometry (MALDI-TOF MS) technology is not available, as biochemical methods are often unable to differentiate *C. auris* from other closely related *Candida* species.

We wish to share our experience in the recent identification of *C. auris* isolated from a urine specimen. It was initially presumptively identified by MALDI-TOF MS (Bruker Daltonics, USA), and subsequently confirmed by sequencing of the internal transcribed spacer (ITS) regions of the ribosomal DNA. Additionally, Vitek 2 YST (software version 8.01) identified it as *C. auris* with 99% probability.

In July 2018, a urine specimen was submitted for microscopy, culture and sensitivities; no clinical information was provided on the request form. Cell count results were: leukocytes 2×10^6/L, erythrocytes 2×10^6/L, 0 epithelial cells; culture on Columbia HBA and MacConkey agar (aerobic, 35°C) had 10^9 CFU/L pure growth of yeast after 24 h. This was presumptively identified by the MALDI-TOF MS Biotyper 3.1 using the Research Use Only (RUO) database as *C. auris* with a log score of 1.75 by direct formic acid extraction, which improved to 1.86 when the ethanol formic acid extraction method was used. Three *C. auris* isolates are represented in the RUO database, and all appeared in the top three identifications with no other *Candida* spp. in the top ten scores.

The isolate was referred to the National Mycology Reference Centre (NMRC, Adelaide, SA) for confirmation of identification and susceptibility testing. MALDI-TOF MS Bruker identification using a supplemented database of known *C. auris* isolates (log score 1.85 by direct formic acid extraction), coupled with ITS sequencing (100% sequence identity to curated sequences from confirmed *C. auris* isolates; http://www.westerdijkinstitute.nl and http://www.fungalbarcoding.org), confirmed the identification as *C. auris*. The in-house Bruker database of the NMRC contains the spectra of three additional *C. auris* strains obtained from international laboratories and definitively identified by ITS sequencing. The ITS sequence obtained from this isolate was submitted to Genbank (accession no. MK367811).

A review of the literature showed the identification of *C. auris* isolates using the Bruker RUO library occasionally yielded log scores of $\leq$1.8 with the direct formic acid extraction method but improved to $\geq$2.0 using the full in tube extraction method.[3,4] When we performed the full ethanol formic acid extraction method the log score improved only to 1.86. This may be explained by the limited representation of *C. auris* spectra in the database in comparison to clinical isolates. Indeed, when the isolate was re-identified after a recent manufacturer update of the RUO database with six additional C. *auris* reference spectra, a log score match of $>$2.0 to all six new spectral profiles was obtained by the direct formic acid extraction method. A MALDI-TOF MS log score of $\geq$1.7 is sufficient for species level identification of common *Candida* spp. as reported in several validation studies.[5,6] There is less experience with the score threshold required for an acceptable identification of *C. auris,* but the absence of reports of other yeasts being misidentified as *C. auris* is reassuring in this respect. The TGA registered Bruker IVD library for clinical use now has the same nine strains of *C. auris* included in the database (MBT IVD Library DB-7712, April 2018). If required, CDC's RUO Bruker database which is accessible on MicrobeNet has four reference spectra of *C. auris*, one from each of the four phylogenetic clades.

For users of the Vitek MALDI-TOF MS system (bioMérieux, France), the routine clinical use database (Version 3.2.0) has been updated to include *C. auris* as of June 2018; its performance in the Australian clinical setting remains to be determined.

Vitek 2 YST (software version 8.01) identification was performed from day 1 and day 2 Sabouraud's dextrose agar (SAB) subcultures, both with identification of *C. auris* at 99% probability. Much of the literature warns of misidentifications of *C. auris* as *Candida haemulonii* complex by Vitek 2 YST, as *C. auris* is not represented in previous database versions. Almost all Vitek 2 YST users in Australia have now been upgraded to software version 8.01 with the inclusion of *C. auris* (personal correspondence, bioMérieux, Australia). Despite this improvement, any identification of *Candida haemulonii* or *Candida duobushaemulonii* by Vitek 2 YST must still prompt the exclusion of *C. auris* by a more reliable method of identification. With the exception of Vitek 2 YST, none of the other phenotypic identification systems have included *C. auris* in their databases to date, and therefore cannot be used to identify or exclude *C. auris*.

Additional phenotypic investigations were performed retrospectively. Macroscopically, colonies were smooth and white-cream on SAB, beige on Brilliance Candida Agar

(Oxoid, UK), pale pink on Candida CHROMagar (Oxoid), and pink on CHROMID Candida (bioMérieux). The isolate produced no germ tubes in horse serum after 2.5 hours (35°C, aerobic), and grew equally well at 30°C, 35°C and 42°C, consistent with other international reports. However, in contrast with many laboratory guides, this isolate formed pseudohyphae on Cornmeal Tween 80 agar (30°C, aerobic) at 5 days (Fig. 1). *Candida auris* isolates from the UK have been reported to form rudimentary pseudohyphae in contrast to isolates from other countries.[1]

An isolate of *C. auris* was included in the RCPA Microbiology Quality Assurance Program in December 2017. The QAP data extracted for Australia/New Zealand participants only (unpublished data) had 64.7% of 71 participating Australasian laboratories submitting the correct species identification. The majority of correct identifications were made by Bruker MALDI-TOF MS (28/29 users correct), Vitek 2 YST (12/18 users correct) and DNA sequencing (2/2 users correct). Incorrect or incomplete identifications were predominantly among participants using the Vitek MALDI-TOF MS (5/7 users incorrect), and commercial biochemical methods API 20C/ID 32C (4/4 users incorrect); this can largely be attributed to the absence of *C. auris* in the databases of these systems.

Multi-azole and amphotericin B resistance, either alone or in combination, is common of *C. auris* isolates.[1,7] Susceptibility testing performed using the Sensititre YeastOne YO10 (ThermoScientific, USA) microbroth dilution plate yielded MICs (mg/L) of fluconazole >256, voriconazole 1.0, itraconazole 0.25, posaconazole 0.12, anidulafungin 0.12, micafungin 0.06, and amphotericin B 4.0. There are no endorsed clinical breakpoints for *C. auris* as yet. However, if using the CDC tentative breakpoints,[7] this isolate may be considered resistant to both fluconazole and amphotericin B.

A review of the patient's history revealed that he had been heavily treated for relapsed and refractory myeloma since initial diagnosis at a UK hospital in 2017. At the time of isolation of the *C. auris*, he was well and afebrile; this was in context of a urinary catheter that had been removed 2 days prior to this urine specimen. The local Infection Prevention Unit was notified when we obtained the initial presumptive MALDI-TOF MS identification of *C. auris*. The patient was already in a single room with standard precautions since hospital admission. This was stepped up to contact precautions with contact tracing undertaken by the Infection Prevention Unit. A look back at this patient's records in our laboratory information system identified multiple previous specimens sent for culture including urine (12), blood cultures (30), stool (4), sputum (4), central line tips (2), and VRE surveillance screen (2). Although all yeasts are identified to species level within our laboratory, we cannot exclude the possibility that a *C. auris* isolate might have gone undetected in the presence of mixed culture in urine or stool specimens.

This is the second known isolation of *C. auris* in Australia. Heath *et al.* reported a case of *C. auris* sternal osteomyelitis from a traveller to Perth, WA, in 2015, who succumbed to complications from his co-morbidities despite initial successful treatment.[8] A review of seven stored isolates previously identified as *C. haemulonii* using biochemical methods at the National Mycology Reference Centre (2004−2010), found that none were *C. auris*. However, since many laboratories do not identify *Candida* to species level from non-sterile sites and there is currently no recommendation for *C. auris* screening in Australia, it is quite plausible that other Australian patients are colonised by this fungal 'superbug' in the context of international travel. In fact, we are aware that other sporadic cases of *C. auris* colonisation in relation to overseas travel have been detected since the isolation we report here.

In summary, the ability to correctly identify *C. auris* in a tertiary microbiology laboratory is critical for rapid response regarding treatment and infection control. *Candida auris* no longer appears to be as difficult to identify as previous reports have warned. This can be attributed to advances in MALDI-TOF MS and Vitek 2 YST databases, and increased education and awareness of this pathogen. Other phenotypic identification methods are unable to identify *C. auris*; and when using Vitek 2 YST, microbiologists should still be cautious of the possibility of *C. auris* being misidentified as *C. haemulonii* complex and perform further identification using another reliable method. When in doubt, laboratories should liaise with manufacturers regarding the representation of *C. auris* in their identification system databases.

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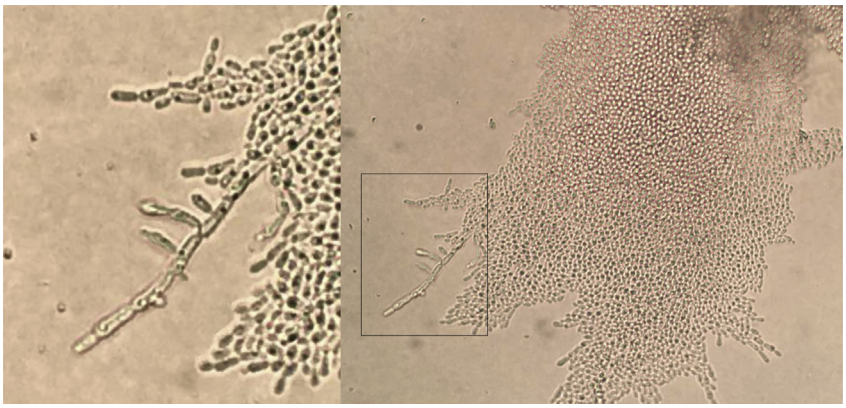

Fig. 1 *Candida auris*, day 5 on CMT80 showing occasional pseudohyphae formation.

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First two confirmed cases of *Dibothriocephalus nihonkaiensis* in Southeast Asia (Singapore)

Sir,

Recent reports suggest that there has been an increase of diphyllobothriosis globally, particularly in developed countries with high standards of sanitation.[1–5] Diphyllobothriosis is an intestinal parasitosis acquired by consumption of raw or undercooked fish harbouring the infective plerocercoid larvae of *Dibothriocephalus* and *Diphyllobothrium* species. More than 50 species of broad fish tapeworm have been described, of which *Dibothriocephalus latus* (previously known as *Diphyllobothrium latum*) and *Dibothriocephalus nihonkaiensis* (previously known as *Diphyllobothrium nihonkaiense*[6] have been considered the principal human pathogens in Europe and Northeast Asia, respectively. *Dibothriocephalus latus* uses freshwater fish such as pike, perch, burbot and char as the second intermediate hosts, while *D. nihonkaiensis* exploits anadromous Pacific salmons.[3] The consumption of raw Pacific salmon may be a risk factor for *D. nihonkaiensis* infection.[2]

Dibothriocephalus nihonkaiensis is almost exclusively found in Japan; however, clinical cases have been subsequently reported in South Korea, China, European countries and the North Pacific coast of North America in the past decade, possibly due to the globalisation of the fresh or frozen Pacific salmon trade and also due to awareness and better diagnostic techniques. Singapore imports over 90% of the fish consumed in the country.[7] All salmon products consumed in Singapore are imported. Atlantic (Norwegian) salmon is widely available in local supermarkets. Pacific salmon sourced from various countries is also available in specialty stores and food outlets locally.

We present the first two cases of *D. nihonkaiensis* diagnosed and reported from Singapore. To our knowledge, these are the first cases of *D. nihonkaiensis* confirmed in Southeast Asia based on the DNA sequences of mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*).

The first case was a 46-year-old asymptomatic woman, who sought medical attention after she had passed a worm per rectum. In the laboratory it was found to be a tapeworm strobili, which measured approximately 2.85 m long. The scolex was not found. There were 835 segments and the proglottids measured 2.0–2.5 mm long × 5.0–7.5 mm wide after fixation in 10% neutral buffered formalin.

Two weeks later, a 29-year-old woman similarly sought medical attention after passing a worm. Specimen submitted (Fig. 1A) was again identified to be a section of strobila

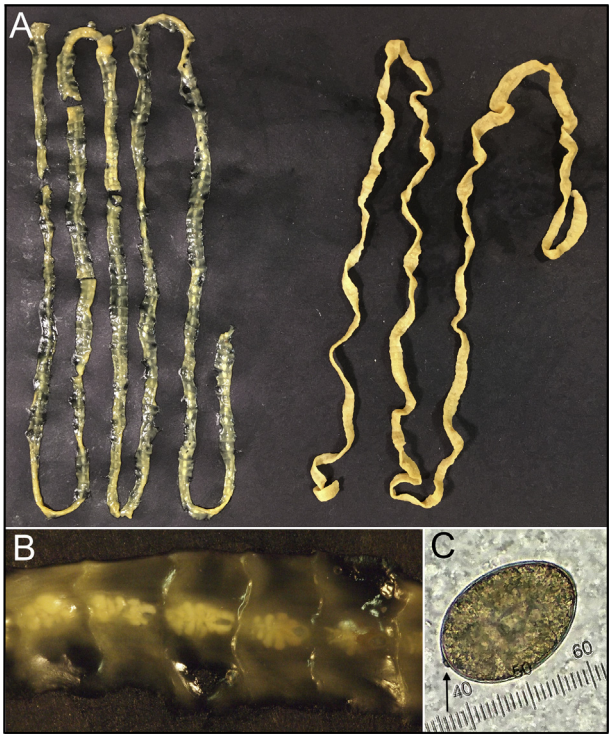

Fig. 1 (A) The specimen was a section of strobila without scolex. The strobila was approximately 1.5 m long. There were 748 segments and the proglottids measured 2.0–2.5 mm long × 5.0–7.5 mm wide after fixation. The segment on the right was soaked in formalin. (B) Close up view of proglottids showing rosette-shaped uterus in the centre of each. (C) *Dibothriocephalus nihonkaiensis* egg in an unstained wet mount. The egg measured approximately 55 µm × 90 µm. Note the apical knob at the abopercular end (arrow).