Failed identification of *Candida vulturna* using the updated Vitek 2 yeast identification system, version 9.02 and CHROMagar Candida Plus

J. Zurita[1,2], A. Paz y Miño[1], M. B. Solís[1] and G. Sevillano[1,3]

1) *Unidad de Investigaciones en Biomedicina. Zurita &Zurita Laboratorios,* 2) *Facultad de Medicina. Pontificia Universidad Católica Del Ecuador and* 3) *Maestría de Biomedicina, Facultad de Ciencias de La Salud, Universidad Internacional SEK, Quito, Ecuador*

Keywords: Candida species, Gold standard test,, Identification techniques, Misidentify, Sequencing
Original Submission: 29 June 2022; **Accepted:** 5 July 2022
Article published online: 9 July 2022

Corresponding author. J. Zurita, Unidad de Investigaciones en Biomedicina.Zurita & Zurita Laboratorios, Ecuador.
E-mail: jzurita@zuritalaboratorios.com

Dear Editor,

Candida vulturna was described as a member of the *Candida haemulonii* species complex that comprises nine species, four are known as human pathogens: *C. haemulonii*, *C. pseudohaemulonii, C. duobushaemulonii,* and *C. auris*. *Candida vulturna* was isolated from blood, endothelial secretion, wound, gastric acid in different countries [1].

We describe a case of 69-year-old man that is diabetic. On August 25, 2021, we received in our laboratory a sample from foot wound for culture. The sample was placed on CHROMagar Candida Plus, Sabouraud agar, and Mycosel agar. The Vitek 2 automated identification system (bioMérieux) was used to identification and was performed according to the manufacturer's instructions, using the software of the Vitek 2 identification system version 09.01.

Low-discrimination (LD) results were obtained with an inability to distinguish between *C. auris, C. duobushaemulonii*, with a 50% identity percentage. The isolated was again analyzed using the Vitek system on two occasions with 93% and 91% of identity for *C. auris*, respectively. This yeast was placed to CHROMagar Candida Plus, specific medium to differentiate *C. auris* from other *Candida* species, with a specificity and sensitivity of 100% [2]. The typical appearance of *C. auris* was present (Fig. 1) [2].

The isolate was identified using large-subunit RNA gene (D1-D2 region) and ITS region. The maximum likelihood model was used for phylogenetic analysis with the D1/D2 sequences using MEGAX. The sequencing result showed an identity percentage of 99.87% and 100% using ITS and D1/D2 for *C. vulturna*, respectively. Phylogenetic analysis showed a monophyletic clade composed of *Candida vulturna* that includes the sequence obtained from the patient (ZZ3048), with a divergence percentage of 0%, indicating that the *C. vulturna* sequences and the analyzed sample are identical, and was considered as a species under the monophyletic association. *C. haemulonis, C. duobushaemulinii,* and *C. pseudohaemulonii* are close relatives of *C. vulturna*, and more distant relatives of *C. auris.*

Antimicrobial susceptibility testing was performed by broth microdilution method Sensititre™ Yeast One (ThermoScientific, USA). Minimal inhibitory concentrations (MICs) were determined after incubation at 33°C for two days. The break points used were according to CLSI for *C. albicans* (Table 1). Previous studies reported similar results for *C. vulturna* isolated from plants and clinical isolates where this was more sensitive than strains of the *C. haemulonis* species complex (Fig. 2).

Therefore, the correct identification of species belonging to the *C. haemulonii* group has a great importance due to cause life-threatening infections with high rates of clinical treatment failure. Especially *C. auris* is characterized by its ability to acquire resistance to multiple antifungals, as well as its propensity for transmission within hospital facilities with high morbidity and mortality [3].

C. auris has been described in Latin America such as Venezuela, Brazil, and Colombia. So, the Pan American Health Organization recommended that correct early detection and notification are necessary to allow the implementation of adequate measures to prevent and control the spread in communities and health services in the Americas [1].

TABLE 1. Antimicrobial susceptibility testing of *C. vulturna* (Z&Z3048)

Antimicrobics	MIC	Interpretation
Anidulafungin	<0.015	S
Amphotericin B	0.5	S
Micafungin	0.015	S
Caspofungin	0.008	S
5-Flucytosine	2	S
Posaconazole	0.008	S
Voriconazole	0.015	S
Itraconazole	0.015	S
Fluconazole	1	S

MIC: minimal inhibitory concentration, S: sensible.

© 2022 The Author(s). Published by Elsevier Ltd
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
https://doi.org/10.1016/j.nmni.2022.101012

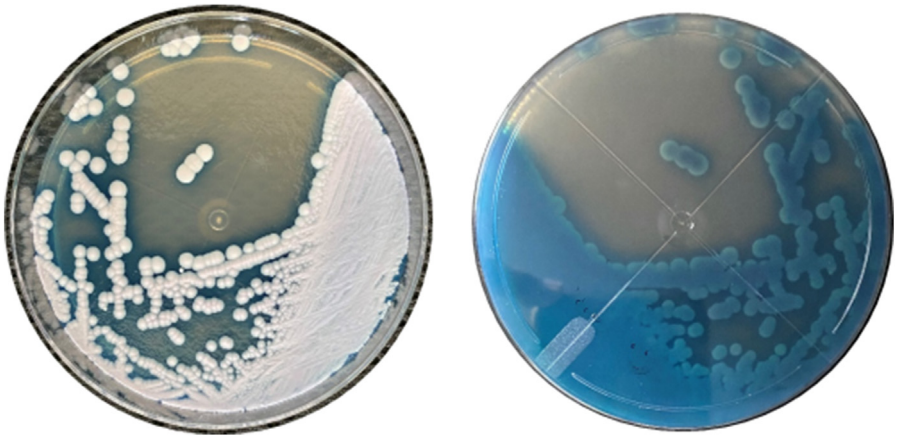

FIG. 1. *Candida vulturna* growing in a very specific morphology as *Candida auris*: light blue colonies with halo and blue from the back of the plate.

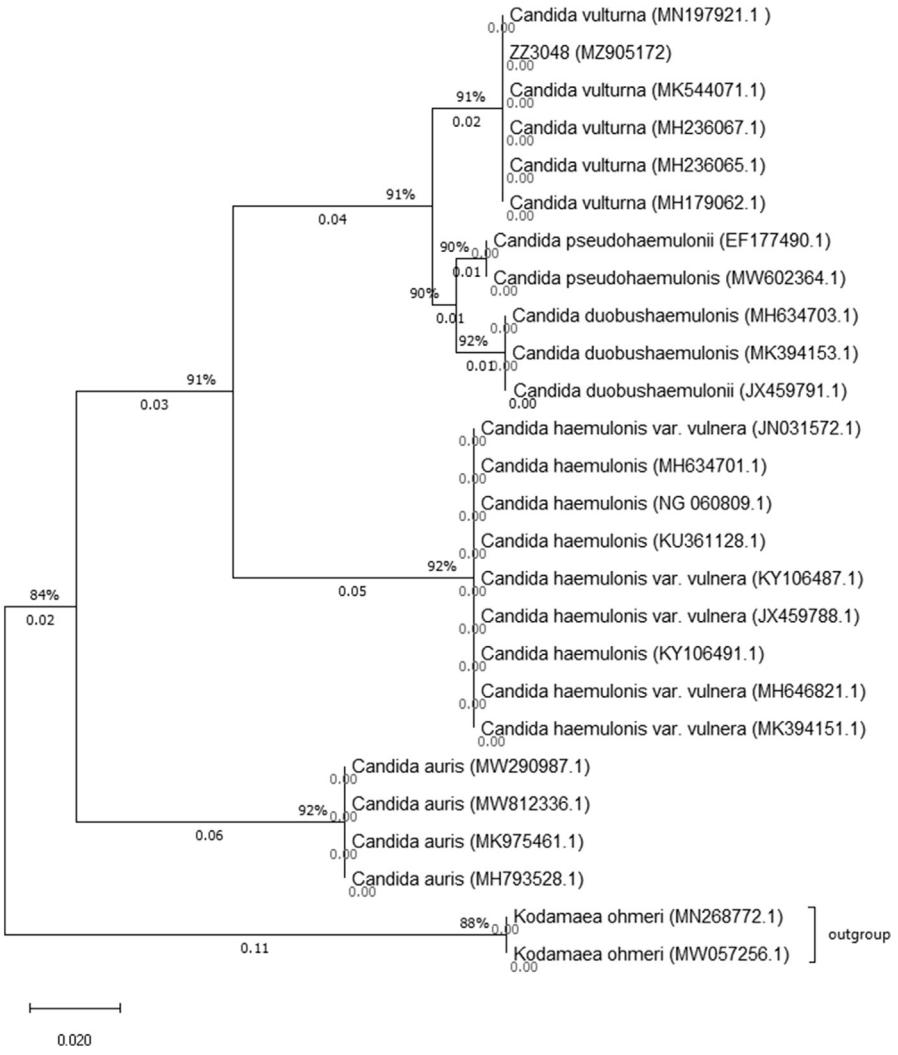

FIG. 2. Phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the maximum likelihood method and Kimura 2-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 26 nucleotide sequences.

© 2022 The Author(s). Published by Elsevier Ltd, *NMNI*, **48**, 101012
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Finally, common phenotypic biochemical identification methods, including VITEK 2 YST, API 20C, the BD Phoenix yeast identification system, and MicroScan can misidentify *C. auris*, and even proteomic systems. It is important for microbiologists and physicians to be aware of the possibility of inaccurate identification when using older versions of the MALDI-TOF MS database. The Centers for Disease Control and Prevention (CDC) website maintains an up-to-date list of database library versions that can accurately identify *C. auris* [4], and Public Health Ontario has also indicated which database versions provide accurate identification in its January 2019 statement [5]. Therefore, the gold standard of diagnosis remains molecular identification by sequencing.

The identification of these species continues to be a challenge for microbiology laboratories, due to the flaws in the techniques used. We suggest that the identification of non-albicans *Candida* species should be carried out by sequencing and if this is not possible in clinical laboratories, these isolates should be sent to a reference center for correct identification. Up to this moment, *C. auris* has not been reported in Ecuador. This is the first case reported in Ecuador of *C. vulturna* in a clinical sample.

Author contributions

J.Z: Conceptualization, data curation; formal analysis; methodology; writing—original draft; writing—review and editing. A. PyM: Formal analysis; methodology; acquisition of data, writing—original draft; MB. S: Formal analysis; methodology; acquisition of data, writing—original draft. G. S: Formal analysis; methodology; acquisition of data, writing—original draft.

Conflict of interest

None

Acknowledgments

We thank Cecibel Gonzalez and Carolina Alulema for their technical support.

This work was presented as an abstract at the Infocus 2021 in Cordova, Argentina.

References

[1] Pan American Health Organization/World Health Organization. Epidemiological Alert: Candida auris outbreaks in health care services. Washington, D.C. 2016.

[2] Borman AM, Fraser M, Johnson EM. CHROMagar TM Candida Plus: a novel chromogenic agar that permits the rapid identification of Candida auris. Med Mycol 2020:1–6. 0.

[3] Simon L, Ughetto E, Gaudart A, Degand N, Lotte R, Ruimy R. Direct identification of 80 percent of bacteria from blood culture bottles by matrix-assisted laser desorption ionization-time of flight mass spectrometry using a 10-minute extraction protocol. J Clin Microbiol 2019;57(2):1–47.

[4] Centers for Disease Control and Prevention. Identification of Candida auris | Candida auris | fungal diseases | CDC [Internet]. 2020. Available from: https://www.cdc.gov/fungal/candida-auris/identification.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Ffungal%2Fcandida-auris%2Frecommendations.html.

[5] Public Health Ontario. Candida auris reference identification and susceptibility testing. 2019.

© 2022 The Author(s). Published by Elsevier Ltd, *NMNI*, **48**, 101012
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).