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Viability of *Candida auris* and Other *Candida* Species after Various Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) Mass Spectrometry-Based Extraction Protocols

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Candida spp. are the most common cause of fungal infections worldwide and the fifth most common cause of nosocomial infections (1). *Candida auris* has recently emerged as a global public health threat. *C. auris* is capable of causing outbreaks (2, 3), resists disinfection with cleaning agents widely used in hospitals and long-term care facilities (4, 5), is frequently drug resistant, and can persist in the environment for months (6). Therefore, identification of *C. auris* is important for patient treatment, infection control, and public health response (7, 8).

Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry can accurately identify *C. auris* (2, 9, 10), but processing isolates can expose laboratory workers to infectious agents. To inform biosafety risk assessments, we evaluated the viability of various *Candida* species after MALDI-TOF extraction protocols.

Ten isolates of *C. auris* and 15 isolates of other *Candida* spp. were obtained from the CDC and ATCC and tested using three extraction methods: on-plate extraction, quick-tube extraction, and extended tube extraction. For on-plate extraction, colonies were spotted to a target and overlaid with 70% formic acid (Fluka) followed by matrix (α-cyano-4-hydroxycinnamic acid) (Bruker). The quick-tube extraction used a 1-μl loopful of colonies suspended in 50 μl water followed by 50 μl of pure ethanol (Fisher), mixed with a pipette, spotted onto a target, and overlaid with formic acid and matrix. In the extended tube extraction, 3 to 5 colonies were suspended in 900 μl water. Three hundred microliters of pure ethanol was added, tubes were vortexed and centrifuged (12,000 rpm, 2 min), the supernatant was discarded, and residual liquid was allowed to air dry. The pellet was vortexed in 50 μl formic acid followed by 50 μl of acetonitrile (Honeywell) and centrifuged (maximum speed, 2 min), and supernatant was spotted onto the target and overlaid with matrix. Viability during the extended tube extraction was evaluated by removing an aliquot after each major step. Growth controls were spotted, swabbed off the target, and then diluted 1:100 before plating.

All samples were spotted to a Bruker MALDI target in duplicate and allowed to air dry between steps. Both spots were collected from the target with a FLOQswab (Copan) dampened with RPMI broth (made in-house). Additional spots were analyzed by MALDI. Swabs or aliquots were vortexed in 0.5 ml RPMI broth, and 50 μl of the suspension was plated to Sabouraud dextrose (Remel) for CFU enumeration. Broths and plates were incubated at 37°C for 48 h.

All three methods effectively killed *Candida*. The on-plate extraction resulted in viable colonies from only 2/3 *Candida albicans* isolates, and there was >99% reduction in growth. No viable yeast was recovered after the quick-tube extraction or any step of the extended tube extraction. Both tube extraction methods had better MALDI-TOF

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TABLE 1 Comparison of extraction methods[a]

Species (no. of isolates)	% reduction in CFU vs control by method[b]:					Mean MALDI score by method:		
			Extended tube extraction					
	On-plate	Quick tube	33% EtOH	Acid	Target	On-plate	Quick tube	Extended tube
Candida albicans (3)	99.9	100	100	100	100	1.943	2.236	2.026
Candida auris (10)	100	100	100	100	100	NT	2.107	2.090
Candida dubliniensis (1)	100	100	100	100	100	1.904	2.153	2.044
Candida glabrata (2)	100	100	100	100	100	2.010	2.149	2.103
Candida kefyr (1)	100	100	100	100	100	NP	2.194	1.892
Candida krusei (2)	100	100	100	100	100	2.190	2.099	2.249
Candida lusitaniae (2)	100	100	100	100	100	NP	2.064	2.200
Candida parapsilosis (2)	100	100	100	100	100	NP	2.046	1.972
Candida tropicalis (2)	100	100	100	100	100	2.226	2.089	2.114

[a]Data represent an average for each species and of duplicate spots for MALDI. MALDI scores above 2.0 are considered good quality. MALDI scores for *C. auris* are from the MicrobeNet database (751 spectra); all others are from the Bruker RUO library (6,903 spectra). Abbreviations: EtOH, ethanol; NP, no peaks; NT, not tested.
[b]Percent killing compared to untreated spots.

confidence scores than the on-plate method and were not significantly different from each other (Table 1). The extended extraction is widely used, but the shorter quick-tube extraction is easier. These findings support the use of the quick-tube extraction method for MALDI-TOF analysis of *Candida* spp. to improve the safety of laboratory staff without compromising the quality of results.

REFERENCES

1. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K, EPIC II Group of Investigators. 2009. International study of the prevalence and outcomes of infection in intensive care units. JAMA 302:2323–2329. https://doi.org/10.1001/jama.2009.1754.
2. Chowdhary A, Sharma C, Meis JF. 2017. Candida auris: a rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathog 13:e1006290. https://doi.org/10.1371/journal.ppat.1006290.
3. Schelenz S, Hagen F, Rhodes JL, Abdolrasouli A, Chowdhary A, Hall A, Ryan L, Shackleton J, Trimlett R, Meis JF, Armstrong-James D, Fisher MC. 2016. First hospital outbreak of the globally emerging Candida auris in a European hospital. Antimicrob Resist Infect Control 5:35. https://doi.org/10.1186/s13756-016-0132-5.
4. Cadnum JL, Shaikh AA, Piedrahita CT, Sankar T, Jencson AL, Larkin EL, Ghannoum MA, Donskey CJ. 2017. Effectiveness of disinfectants against Candida auris and other Candida species. Infect Control Hosp Epidemiol 38:1240–1243. https://doi.org/10.1017/ice.2017.162.
5. Kean R, Sherry L, Townsend E, McKloud E, Short B, Akinbobola A, Mackay WG, Williams C, Jones BL, Ramage G. 2018. Surface disinfection challenges for Candida auris: an in-vitro study. J Hosp Infect 98:433–436. https://doi.org/10.1016/j.jhin.2017.11.015.
6. Vallabhaneni S, Kallen A, Tsay S, Chow N, Welsh R, Kerins J, Kemble SK, Pacilli M, Black SR, Landon E, Ridgway J, Palmore TN, Zelzany A, Adams EH, Quinn M, Chaturvedi S, Greenko J, Fernandez R, Southwick K, Furuya EY, Calfee DP, Hamula C, Patel G, Barrett P, Lafaro P, Berkow EL, Moulton-Meissner H, Noble-Wang J, Fagan RP, Jackson BR, Lockhart SR, Litvintseva AP, Chiller TM. 2017. Investigation of the first seven reported cases of Candida auris, a globally emerging invasive, multidrug-resistant fungus-United States, May 2013-August 2016. Am J Transplant 17:296–299. https://doi.org/10.1111/ajt.14121.
7. Lockhart SR, Jackson BR, Vallabhaneni S, Ostrosky-Zeichner L, Pappas PG, Chiller T. 2017. Thinking beyond the common Candida species: need for species-level identification of Candida due to the emergence of multidrug-resistant Candida auris. J Clin Microbiol 55:3324–3327. https://doi.org/10.1128/JCM.01355-17.
8. Lepak AJ, Zhao M, Berkow EL, Lockhart SR, Andes DR. 2017. Pharmacodynamic optimization for treatment of invasive Candida auris infection. Antimicrob Agents Chemother 61:e00791-17. https://doi.org/10.1128/AAC.00791-17.
9. Kathuria S, Singh PK, Sharma C, Prakash A, Masih A, Kumar A, Meis JF, Chowdhary A. 2015. Multidrug-resistant Candida auris misidentified as Candida haemulonii: characterization by matrix-assisted laser desorption ionization-time of flight mass spectrometry and DNA sequencing and its antifungal susceptibility profile variability by Vitek 2, CLSI broth microdilution, and Etest method. J Clin Microbiol 53:1823–1830. https://doi.org/10.1128/JCM.00367-15.
10. Lockhart SR, Berkow EL, Chow N, Welsh RM. 2017. Candida auris for the clinical microbiology laboratory: not your grandfather's Candida species. Clin Microbiol Newsl 39:99–103. https://doi.org/10.1016/j.clinmicnews.2017.06.003.