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LETTER TO THE EDITOR

Rapid Detection of *Candida auris* Based on Loop-Mediated Isothermal Amplification (LAMP)

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Candida auris is an emerging, multidrug-resistant pathogen associated with a high mortality rate. Since this yeast's first identification and classification by our research group in 2009 (1), there have been several outbreaks linked to this pathogen in health care facilities around the world (2–10). It has been reported that most clinical isolates are resistant to azoles, and about half of the isolates also are resistant to more than one class of antifungal agent, limiting the therapeutic options (2–8, 10). Moreover, the pathogen can persist on environmental surfaces for weeks, resulting in the yeast's spread among patients in health care facilities (11). Therefore, accurate identification of *C. auris* is critical for controlling this pathogen's prevalence around the globe and preventing further outbreaks.

Traditional methods have proven to be unsuitable for accurate identification of *C. auris*. Automated identification systems popularly used in clinical laboratories, like the Vitek 2 YST card (bioMérieux, Marcy l'Etoile, France) or API20C AUX (bioMérieux), commonly misidentify *C. auris* as *Candida haemulonii* or *Rhodotorula glutinis*, respectively (2, 4–7, 12), and MicroScan misidentifies *C. auris* as any of several different *Candida* species (12). On the other hand, specialized methods can provide accurate identification. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is useful for identifying *C. auris*, if a proper reference database is available (13–15). Moreover, sequencing of the genes for the D1/D2 region of large subunit ribosomal DNA (rDNA) or of the internal transcribed spacer (ITS) region of rDNA is a reliable option. Real-time PCR assays also are useful for detection of *C. auris* (16, 17). However, these methods may not be suitable for local or small clinical settings due to financial and technical issues.

As shown in the present study, we have successfully devised and assessed the reliability of a loop-mediated isothermal amplification (LAMP)-based identification approach specific to *C. auris*, enabling distinction of the pathogen from closely related species and other fungi.

To design the LAMP primers, the genome sequences of four *Candida* species, *C. auris* (PRJNA342691), *C. tropicalis* (GCF_000006335.2), *C. albicans* (GCA_000182965.3), and *C. lusitaniae* (LYUB00000000.2), were aligned and compared using Mauve (version 20150226) (18). An 869-bp DNA fragment of the *C. auris* genome (accession no. XM_018317007) that encodes a pyruvate:ferredoxin oxidoreductase domain (19) was identified as sharing low similarity with other *Candida* species. This DNA fragment was amplified using EmeraldAmp PCR master mix (TaKaRa Bio, Inc., Shiga, Japan) in combination with *C. auris* JCM15448^T as a template and a pair of primers, AurisF (5′-GCT

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TABLE 1 LAMP oligonucleotide primer sequences specific to *Candida auris*

Primer	Sequence (5′→3′)
AurisFIP	AGGCTACTGAGCTTGCTGGTGTAACCAAACCAACAGGAGAGG
AurisBIP	ACGGTTTCAGGGTTAGCATGGCTCAACAAAGTCGCTGGTACA
AurisLoop-F	CATCTCGAAGGCCTCGGT
AurisLoop-B	CACATACTCGAACGGAGTC
AurisF3	GGGAAAGGAACCCTGACCT
AurisB3	GGACACAGCATTCGAAGTGT

ATGCCGCTAGCAACG-3′) and AurisR (5′-CACTACAGCAGGATCAACGG-3′). The resulting amplicon was purified with a QIAquick PCR purification kit (Qiagen, Venlo, The Netherlands), cloned into the pTAC-2 vector using a DynaExpress TA PCR cloning kit (BioDynamics Laboratory, Inc., Tokyo, Japan) to create plasmid pTAC-2Auris, and subsequently sequenced using an ABI PRISM 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). A candidate LAMP primer set (LAMPAuris) (Table 1) was designed using the sequence of this DNA fragment and PrimerExplorer V5 software (https://primerexplorer.jp/lampv5e/index.html), specifically targeting a 192-bp fragment (corresponding to bp 774 to 965 of the XM_018317007 sequence).

LAMP amplification reactions were run at 56°C for 90 min using a Loopamp turbidimeter RT-160C (Eiken Chemical, Co., Ltd., Tochigi, Japan). Reactions were terminated by deactivating the DNA polymerase at 80°C for 5 min. Each 25-μl reaction mixture consisted of 12.5 μl of 2× reaction mix (Eiken Chemical Co., Ltd.), 1 μl of each primer (40 μM FIP, 40 μM BIP, 20 μM Loop-F, 20 μM Loop-B, 5 μM F3, and 5 μM B3), 1 μl of *Bst* DNA polymerase, 2 μl of sample DNA solution, and 3.5 μl distilled water.

To determine the detection limit of the LAMPAuris primer set, pTAC-2Auris was serially diluted (1×10^0 to 1×10^{10} copies/μl) and used as the template in triplicate reactions. LAMPAuris was able to detect pTAC-2Auris when the plasmid was present at concentrations as low as 2×10^1 copies per reaction (Fig. 1), demonstrating the high sensitivity of the reaction.

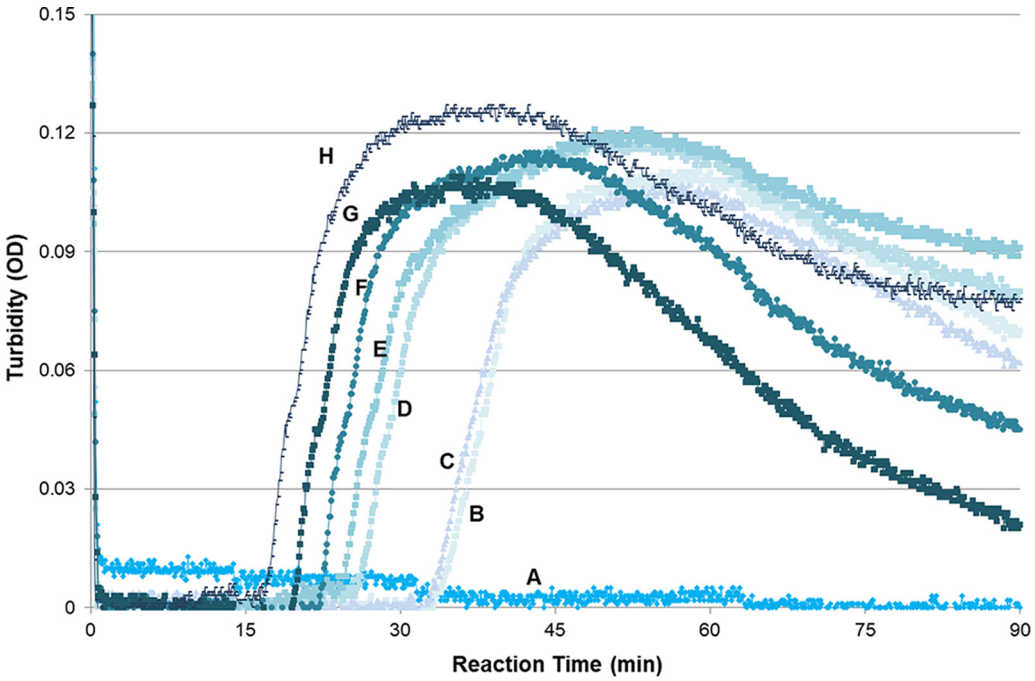

FIG 1 Sensitivity of the LAMPAuris approach. The reaction was run at 56°C for 90 min; the sensitivity of 2×10^1 copies per reaction was confirmed in triplicate reactions. A, negative control (no reaction); B, 2×10^0 copies/reaction (34 min); C, 2×10^1 copies/reaction (33 min); D, 2×10^2 copies/reaction (26 min); E, 2×10^3 copies/reaction (24 min); F, 2×10^4 copies/reaction (24 min); G, 2×10^6 copies/reaction (22 min); H, 2×10^8 copies/reaction (17 min).

TABLE 2 *Candida auris* strains and their origins tested in this study

Country of isolation	Clade[a]	Strain
Japan		JCM15448^T, LSEM52-3449, LSEM53-3540, LSEM53-3541
South Korea		CBS12372, CBS12373
India		CBS12766, CBS12767, CBS12768, CBS12769, CBS12770, CBS12771, CBS12772, CBS12773, CBS12774, CBS12775
United Kingdom	Japan/Korea	NCPF8984
	India/Kuwait/Malaysia	NCPF8971, NCPF8985
	South Africa	NCPF8977

[a]Clade as specified by Borman et al. (9).

To evaluate the specificity of the LAMPAuris primer set toward *C. auris*, a panel of 63 strains consisting of 39 species, including 21 filamentous fungi and 18 yeasts, were tested (Tables 2 and 3). For each of the filamentous fungi, total DNA was extracted and purified as described previously (20). For each of the yeast strains, small portions of colonies grown on Sabouraud dextrose agar (SDA) plates were suspended in 25 μl distilled water, heated at 100°C for 15 min, and briefly centrifuged. For each LAMPAuris reaction, an aliquot of 2 μl of the purified DNA (filamentous fungi) or heated supernatants (yeasts) was used as a template. All of the 20 *C. auris* strains yielded amplifi-

TABLE 3 Strains tested in this study and LAMPAuris results

Species[a]	Strain	LAMPAuris result
Acremonium curvulum	NBRC32242	−
Aspergillus fumigatus	TIMM0108	−
Aspergillus niger	TIMM0115	−
Candida albicans	LSEM11-828	−
Candida auris	20 strains as described in Table 2	+
*Candida duobushaemulonii**	CBS7799	−
*Candida famata**	NBRC0083, NBRC0623	−
Candida glabrata	CBS138, NBRC0005	−
*Candida guilliermondii**	TIMM0257	−
*Candida haemulonii**	JCM3762	−
Candida krusei	TIMM3378	−
*Candida lusitaniae**	NBRC1019, NBRC10059	−
*Candida parapsilosis**	ATCC 22019	−
Candida pseudohaemulonii	JCM12453	−
*Candida sake**	NBRC0435	−
Candida tropicalis	ATCC 750, TIMM0313	−
Chaetomium globosum	TSY-0369	−
Cladosporium carrionii	TIMM3048	−
Cunninghamella bertholletiae	TIMM3392	−
Exophiala jeanselmei	TSY-0396	−
Fusarium oxysporum	TSY-0351	−
Fusarium solani	TSY-0403	−
Malassezia furfur	CBS1878, LSEM51-3422	−
Malassezia restricta	CBS7877	−
Microsporum gypseum	NBRC5948	−
Mucor circinelloides	TIMM3177	−
Paecilomyces variotii	NBRC4855	−
Penicillium citrinum	LSEM34-2305	−
Pseudallescheria boydii	TIMM0886	−
*Rhodotorula glutinis**	LSEM 20-1447	−
Rhodotorula minuta	TIMM6222	−
Saccharomyces cervisiae	LSEM 14-1013	−
Scopulariopsis brevicaulis	NBRC4843	−
Scopulariopsis brumptii	NBRC6441	−
Scytalidium lignicola	NBRC104988	−
Trichophyton benhamiae	SM103	−
Trichophyton mentagrophytes	TIMM2789	−
Trichophyton rubrum	TIMM2659	−
Trichophyton tonsurans	NBRC5928	−

[a]Asterisks indicate species that *C. auris* has been commonly misidentified as.

TABLE 4 Reaction time of LAMPAuris under mock environmental conditions

No. of *C. auris* cells in mock sample	No. of *C. auris* cells/reaction (2 μl)	Mean reaction time in min (SD) in:		
		Saline	Set A[a]	Set B[b]
1×10^1	1×10^{-1}	NR[c]	NR	NR
1×10^2	1×10^0	NR	NR	NR
1×10^3	1×10^1	29 (2.8)	38 (17)	30 (2.9)
1×10^4	1×10^2	22 (1.6)	21 (0.8)	22 (0)
1×10^5	1×10^3	19 (1.4)	20 (0.6)	20 (0)

[a]In mock sample set A, *Penicillium citrinum* and *Malassezia furfur* cells were on the order of 10^2 cells, and *Staphylococcus aureus* and *Bacillus subtilis* cells were on the order of 10^3 cells.
[b]In mock sample set B, *P. citrinum* and *M. furfur* cells were on the order of 10^3 cells, and *S. aureus* and *B. subtilis* cells were on the order of 10^4 cells.
[c]NR, no reaction.

cation signals using the LAMPAuris primer set. In contrast, none of the filamentous fungi or the other yeast species, including those for which *C. auris* is commonly misidentified, yielded any amplification signal. To validate the quality of the DNA templates used in the LAMPAuris reactions, LAMP reactions using a panfungal LAMP primer set (20) were run separately; amplifications were detected with templates from each of the tested species (data not shown).

We also tested the LAMPAuris method on a clinical sample. An ear swab specimen obtained from otitis caused by *C. auris* LC318417 (21) was tested. The swab was placed in a 2-ml microtube containing 1 ml of saline supplemented with 0.05% Tween 80 and then shaken for 10 min. The resulting suspension was centrifuged at 20,000 $\times$ *g* for 10 min. The generated pellet was washed with 100 μl saline and then subjected to total DNA extraction using the Kaneka Easy DNA extraction kit version 2 (Kaneka Co., Hyogo, Japan) according to the manufacturer's instructions. An aliquot of 2 μl of the extracted DNA was used as a template for the LAMPAuris reaction, yielding a LAMP-positive signal. The entire process of identifying this clinical sample required approximately 1 h, including the direct extraction of total DNA. Prior to use for DNA isolation, the swab was rubbed across the surface of an SDA plate, and this plate then was incubated at 37°C; the resulting small creamy colonies also were identified as *C. auris* by MALDI-TOF MS (Bruker Daltonics K.K., Kanagawa, Japan) and sequencing of rDNA.

The application of the LAMPAuris method to environmental surveillance was also assessed using mock environmental samples. The samples were prepared by mixing suspensions of *Penicillium citrinum* (LSEM34-2305), *Malassezia furfur* (LSEM51-3422), *Staphylococcus aureus* (ATCC 25923), and *Bacillus subtilis* (NBRC14132) as background species with *C. auris* (JCM15448^T). First, the concentration of each species was adjusted to 10^6 cells/ml for fungi and 10^8 cells/ml for bacteria using a McFarland no. 1 turbidity standard, with additional cell counting for *P. citrinum* and *C. auris*. Each solution was then diluted and mixed to create two sets of environmental conditions with different concentrations of microbial cells; set A contained approximately 1×10^2 cells of each background fungal species (*P. citrinum* and *M. furfur*) and approximately 1×10^3 of each bacterial species (*S. aureus* and *B. subtilis*), and set B contained approximately 1×10^3 cells of each background fungal species and approximately 1×10^4 cells of each bacterial species. A dilution series (1×10^1 to 1×10^5 cells) of *C. auris* was added to the background solutions, and the mixtures were subjected to total DNA extraction using a Kaneka Easy DNA extraction kit as described above. The LAMPAuris method was tested in triplicate for each condition. As shown in Table 4, there was no apparent interference due to the presence of common skin flora or organisms common to the hospital environment.

Our LAMP approach was proven to reliably identify all of the tested *C. auris* strains, distinguishing these isolates from other strains (even very closely related species) with a specificity of 100%. Our assay was able to detect a template provided at concentrations as low as 2×10^1 copies of target DNA per reaction. Moreover, the results were obtained within a short time, without any technical complications regarding the use of the amplification instrument. Direct LAMP from a clinical specimen was demonstrated;

thus, this technique is expected to save clinicians the time required for cultivation and DNA extraction, allowing an early diagnosis. Recently, portable LAMP amplification equipment has been made commercially available. This availability is expected to facilitate the use of the LAMP assay, enabling large-scale and field surveillance detection. However, care should be taken when handling LAMPAuris product as opening the reaction tube could result in considerable contamination, as we mentioned previously (22). Overall, this assay should be particularly valuable for *C. auris*, a pathogen that is an important target of environmental control in health care facilities.

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REFERENCES

1. Satoh K, Makimura K, Hasui Y, Nishiyama Y, Uchida K, Yamaguchi H. 2009. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. Microbiol Immunol 53:41–44. https://doi.org/10.1111/j.1348-0421.2008.00083.x.
2. Lee WG, Shin JH, Uh Y, Kang MG, Kim SH, Park KH, Jang HC. 2011. First three reported cases of nosocomial fungemia caused by *Candida auris*. J Clin Microbiol 49:3139–3142. https://doi.org/10.1128/JCM.00319-11.
3. Chowdhary A, Sharma C, Duggal S, Agarwal K, Prakash A, Singh PK, Jain S, Kathuria S, Randhawa HS, Hagen F, Meis JF. 2013. New clonal strain of *Candida auris*, Delhi, India. Emerg Infect Dis 19:1670–1673. https://doi.org/10.3201/eid1910.130393.
4. Sarma S, Kumar N, Sharma S, Govil D, Ali T, Mehta Y, Rattan A. 2013. Candidemia caused by amphotericin B and fluconazole resistant *Candida auris*. Indian J Med Microbiol 31:90–91. https://doi.org/10.4103/0255-0857.108746.
5. Chowdhary A, Anil Kumar V, Sharma C, Prakash A, Agarwal K, Babu R, Dinesh KR, Karim S, Singh SK, Hagen F, Meis JF. 2014. Multidrug-resistant endemic clonal strain of *Candida auris* in India. Eur J Clin Microbiol Infect Dis 33:919–926. https://doi.org/10.1007/s10096-013-2027-1.
6. Magobo RE, Corcoran C, Seetharam S, Govender NP. 2014. *Candida auris*-associated candidemia, South Africa. Emerg Infect Dis 20:1250–1251. https://doi.org/10.3201/eid2007.131765.
7. Calvo B, Melo AS, Perozo-Mena A, Hernandez M, Francisco EC, Hagen F, Meis JF, Colombo AL. 2016. First report of *Candida auris* in America: clinical and microbiological aspects of 18 episodes of candidemia. J Infect 73:369–374. https://doi.org/10.1016/j.jinf.2016.07.008.
8. Borman AM, Szekely A, Johnson EM. 2016. Comparative pathogenicity of United Kingdom isolates of the emerging pathogen *Candida auris* and other key pathogenic *Candida* species. mSphere 1:e00189-16. https://doi.org/10.1128/mSphere.00189-16.
9. Borman AM, Szekely A, Johnson EM. 2017. Isolates of the emerging pathogen *Candida auris* present in the UK have several geographic origins. Med Mycol 55:563–567. https://doi.org/10.1093/mmy/myw147.
10. 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.
11. Welsh RM, Bentz ML, Shams A, Houston H, Lyons A, Rose LJ, Litvintseva AP. 2017. Survival, persistence, and isolation of the emerging multidrug-resistant pathogenic yeast *Candida auris* on a plastic health care surface. J Clin Microbiol 55:2996–3005. https://doi.org/10.1128/JCM.00921-17.
12. Mizusawa M, Miller H, Green R, Lee R, Durante M, Perkins R, Hewitt C, Simner PJ, Carroll KC, Hayden RT, Zhang SX. 2017. Can multidrug-resistant *Candida auris* be reliably identified in clinical microbiology laboratories? J Clin Microbiol 55:638–640. https://doi.org/10.1128/JCM.02202-16.
13. 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.
14. Ghosh AK, Paul S, Sood P, Rudramurthy SM, Rajbanshi A, Jillwin TJ, Chakrabarti A. 2015. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeasts causing bloodstream infections. Clin Microbiol Infect 21:372–378. https://doi.org/10.1016/j.cmi.2014.11.009.
15. Girard V, Mailler S, Chetry M, Vidal C, Durand G, van Belkum A, Colombo AL, Hagen F, Meis JF, Chowdhary A. 2016. Identification and typing of the emerging pathogen *Candida auris* by matrix-assisted laser desorption ionisation time of flight mass spectrometry. Mycoses 59:535–538. https://doi.org/10.1111/myc.12519.
16. Kordalewska M, Zhao Y, Lockhart SR, Chowdhary A, Berrio I, Perlin DS. 2017. Rapid and accurate molecular identification of the emerging multidrug-resistant pathogen *Candida auris*. J Clin Microbiol 55:2445–2452. https://doi.org/10.1128/JCM.00630-17.
17. Leach L, Zhu Y, Chaturvedi S. 2018. Development and validation of a real-time PCR assay for rapid detection of *Candida auris* from surveillance samples. J Clin Microbiol 56:e01223-17. https://doi.org/10.1128/JCM.01223-17.
18. Darling ACE, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14:1394–1403. https://doi.org/10.1101/gr.2289704.
19. Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY, Bryant SH. 2017. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. Nucleic Acids Res 45:D200–D203. https://doi.org/10.1093/nar/gkw1129.
20. Nakayama T, Yamazaki T, Yo A, Tone K, Mahdi Alshahni M, Fujisaki R, Makimura K. 2017. Detection of fungi from an indoor environment using loop-mediated isothermal amplification (LAMP) method. Biocontrol Sci 22:97–104. https://doi.org/10.4265/bio.22.97.
21. Iguchi S, Mizushima R, Kamada K, Itakura Y, Yoshida A, Uzawa Y, Arai Y, Takaoka M, Sato S, Goto A, Karasawa T, Tsuruoka N, Totsuka D, Ono E, Nonaka M, Makimura K, Kikuchi K. 2018. The second *Candida auris* isolate from aural discharge in Japan. Jpn J Infect Dis 71:174–175. https://doi.org/10.7883/yoken.JJID.2017.466.
22. Uemura N, Makimura K, Onozaki M, Otsuka Y, Shibuya Y, Yazaki H, Kikuchi Y, Abe S, Kudoh S. 2008. Development of a loop-mediated isothermal amplification method for diagnosing Pneumocystis pneumonia. J Med Microbiol 57:50–57. https://doi.org/10.1099/jmm.0.47216-0.