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Candida auris Clinical Isolates from South Korea: Identification, Antifungal Susceptibility, and Genotyping

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ABSTRACT *Candida auris* is an emerging worldwide fungal pathogen. Over the past 20 years, 61 patient isolates of *C. auris* (4 blood and 57 ear) have been obtained from 13 hospitals in Korea. Here, we reanalyzed those molecularly identified isolates using two matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems, including Biotyper and Vitek MS, followed by antifungal susceptibility testing, sequencing of the *ERG11* gene, and genotyping. With a research-use-only (RUO) library, 83.6% and 93.4% of the isolates were correctly identified by Biotyper and Vitek MS, respectively. Using an *in vitro* diagnostic (IVD) library of Vitek MS, 96.7% of the isolates were correctly identified. Fluconazole-resistant isolates made up 62.3% of the isolates, while echinocandin- or multidrug-resistant isolates were not found. Excellent essential (within two dilutions, 96.7%) and categorical agreements (93.4%) between the Clinical and Laboratory Standards Institute (CLSI) and Vitek 2 (AST-YS07 card) methods were observed for fluconazole. Sequencing *ERG11* for all 61 isolates revealed that only 3 fluconazole-resistant isolates showed the Erg11p amino acid substitution K143R. All 61 isolates showed identical multilocus sequence typing (MLST). Pulsed-field gel electrophoresis (PFGE) analyses revealed that both blood and ear isolates had the same or similar patterns. These results show that MALDI-TOF MS and Vitek 2 antifungal susceptibility systems can be reliable diagnostic tools for testing *C. auris* isolates from Korean hospitals. The Erg11p mutation was seldom found among Korean isolates of *C. auris*, and multidrug resistance was not found. Both MLST and PFGE analyses suggest that these isolates are genetically similar.

KEYWORDS *Candida auris*, MALDI-TOF MS, antifungal susceptibility testing, genotyping

Candida auris is an emerging worldwide health care-associated pathogen associated with high mortality (1–3). It has a low susceptibility to azole antifungal agents and often shows multidrug resistance (2–6). The rapid and accurate identification of *C. auris* and detection of its antifungal resistance are important for determining treatment strategies and preventing nosocomial transmission of *C. auris* (5, 7, 8). However, this poses a challenge to routine microbiology laboratories (5, 6, 8), as *C. auris* can be misidentified using commercial assimilation identification methods (5, 9–11), and biochemical profiles of *C. auris* differ according to geographical origin (9, 12). Incorporation of a research-use-only (RUO) library containing *C. auris* has enabled the correct identification of *C. auris* by commercially available matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) systems (5, 8, 13), although some isolates have not been identified (10). Moreover, misleadingly high MICs of amphotericin B have been reported using a commercially available automated system (5).

So far, evaluation of the performance of commercially available methods for the

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identification of *C. auris* and detection of its antifungal resistance is limited (5, 8, 10). In the past 20 years, we have identified 61 patient isolates of *C. auris* from 13 hospitals in Korea by DNA sequence-based methods. This study evaluated the performance of two commercially available MALDI-TOF MS systems for identification of *C. auris* and compared the Clinical and Laboratory Standards Institute (CLSI) and Vitek 2 (bioMérieux, Marcy d'Etoile, France) antifungal susceptibility testing methods by testing the above clinical isolates of *C. auris*. In addition, the *ERG11* gene encoding the azole target was sequenced to study the mechanism of resistance to azoles (2, 6), and multilocus sequencing typing (MLST) and pulsed-field gel electrophoresis (PFGE) were performed to investigate the genetic relationships among the *C. auris* isolates from Korea.

MATERIALS AND METHODS

Fungal isolates. The Korean collection consisted of 61 patient isolates of *C. auris* submitted from 1996 to 2018 to the Chonnam National University Hospital (CNUH), Gwangju, South Korea, from 13 Korean hospitals located throughout Korea (1, 11). The isolate number from 13 hospitals (A through M) were as follows: A, Gwangju, 35 isolates; B, Seoul, 3 isolates; C, Daegu, 3 isolates; D, Seoul, 5 isolates; E, Suwon, 1 isolate; F, Wonju, 1 isolate; G, Busan, 3 isolates; H, Seoul, 1 isolate; I, Seoul, 1 isolate; J, Jeonju, 5 isolates; K, Seoul, 1 isolate; L, Daejeon, 1 isolate; and M, Yangsan, 1 isolate. All isolates were identified by sequencing the internal transcribed spacer (ITS) region and/or D1/D2 regions of the 26S ribosomal DNA of their rRNA genes (11). A total of 61 nonduplicate isolates of *C. auris* recovered from blood (4 patient isolates) and ear (57 patient isolates) cultures were assessed for identification using MALDI-TOF MS, antifungal susceptibility testing, sequence analyses of the *ERG11* gene and multilocus sequence typing (MLST) analyses. Duplicate *C. auris* isolates from the same patient were excluded, but two sequential blood isolates from three patients with the first reported *C. auris* fungemia (1) were included for pulsed-field gel electrophoresis (PFGE) analyses. PFGE was performed to compare blood and ear isolates (7 and 19 isolates from 4 and 19 patients, respectively) from Korean hospitals. In addition, a panel of ten *C. auris* isolates with representatives from each of four clades (*C. auris* AR0381 to AR0390, provided by the U.S. Centers for Disease Control and Prevention [CDC]) was used for a comparison study among *ERG11* gene sequence, MLST, and PFGE analyses. The Food and Drug Administration (FDA)-CDC Antimicrobial Resistance (AR) Bank numbers for the ten *C. auris* strains used this study are *C. auris* AR0382, *C. auris* AR0387, *C. auris* AR0388, *C. auris* AR0389, and *C. auris* AR0390 (South Asia, clade I); *C. auris* AR0381 (East Asia, clade II); *C. auris* AR0383 and *C. auris* AR0384 (Africa, clade III); and *C. auris* AR0385 and *C. auris* AR0386 (South America, clade IV).

Identification using MALDI-TOF MS. Each isolate was cultured on Trypticase soy agar with 5% (vol/vol) sheep blood at 35°C for 48 h and tested using Biotyper (Bruker Daltonics, Billerica, MA, USA) and Vitek MS (bioMérieux, Manchester, UK) instruments in accordance with the manufacturers' recommendations. The Biotyper employed a full-tube extraction method using formic acid plus acetonitrile (FA/ACN) (8, 14). We used Flex Control version 3.4 (Bruker Daltonics) and Bruker Biotyper 3.1 software, including RUO library version 3.3.1.0, which comprises 6,903 mean spectra (MSP) and 2,461 species. The "correct identification" category included correct identification of *C. auris* with cutoff scores of $\geq$1.7, whereas the "incomplete" category included correct identification of *C. auris* with cutoff scores of $<$1.7 (14). Isolates with results of "incomplete identification" or "no identification" were retested by repeating the full-tube extraction method.

For identification using the Vitek MS, the extraction was performed using the direct on-plate method, using formic acid (FA) for all isolates (14). The spectrum of identification results was obtained using the RUO library (Spectral ARchive And Microbial Identification System [SARAMIS] version 4.14 database), simultaneously with the *in vitro* diagnostics (IVD) library version 3.2. Confidence values of $\geq$60% and $\geq$75% with a unique spectrum of a single organism (*C. auris*) indicated good species-level identification (correct identification) for IVD and RUO, respectively, whereas a determination of *C. auris* with "low discrimination" (confidence values of $<$60% and $<$75% for IVD and RUO, respectively) indicated incomplete identification. If no unique identification pattern was found ("bad spectrum"), or the strain was determined to be outside the scope of the database ("no identification") (14, 15), the result was considered an incorrect identification.

Antifungal susceptibility testing. The *in vitro* antifungal tests for susceptibility to fluconazole, voriconazole, amphotericin B, caspofungin, and micafungin were performed using the CLSI M27-A3 broth microdilution (BMD) method and Vitek 2 system (AST-YS07 card: bioMérieux, Hazelwood, MO) (5, 16). Two reference strains, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258, were included in each antifungal susceptibility test as control isolates. Essential agreement was defined as the MIC results from both methods being within two dilutions (two wells) of each other. Categorical results were obtained according to the following tentative MIC breakpoints for *C. auris* published by the CDC: fluconazole, 32 μg/ml; voriconazole, not available; amphotericin B, 2 μg/ml; caspofungin, 2 μg/ml; and micafungin, 4 μg/ml (https://www.cdc.gov/fungal/candida-auris/recommendations.html).

Sequence analyses of *ERG11*. *Candida* genomic DNA was extracted as described previously (17). The *ERG11* gene of each of the 61 *C. auris* isolates was sequenced using a procedure described previously (6). The genomic DNA was amplified and sequenced using primers (CGUERG_F, 5′-CGCTCGGTTATCTGC TGACT-3′; CGUERG_R, 5′-GTTCTGCTCCATCACCTTCGT-3′; and CGUERGseq145F, 5′-CCCTTGGTGTTTCACT GGGT-3′) based on the *C. auris ERG11* gene sequence (GenBank accession no. XM_018315289.1) from the NCBI database. Each PCR mixture contained 100 ng genomic DNA, 2.5 U *Taq* polymerase (Genetbio), 5 μl

TABLE 1 Identifications of 61 *Candida auris* isolates from Korean hospitals by two MALDI-TOF MS systems[d]

System	Database[c]	Extraction method	No. (%) of isolates			
			Correct ID[a]	Incomplete ID[a]	No ID	Mis-ID
Biotyper	RUO library version 3.3.1.0	Initial full-tube extraction	46 (75.4)	8 (13.1)	7 (11.5)	0 (0)
		Additive full-tube extraction[b]	51 (83.6)	9 (14.8)	1 (1.6)	0 (0)
Vitek MS	RUO library version 4.14	On-plate extraction	57 (93.4)	4 (6.6)	0 (0)	0 (0)
	IVD library version 3.2	On-plate extraction	59 (96.7)	0 (0)	2 (3.3)	0 (0)

[a]The "correct ID" category includes correct identifications (IDs) of *C. auris* with cutoff scores of ≥1.7 by Biotyper or a confidence value of ≥75.0 by the Vitek MS with RUO library and ≥60.0 by Vitek MS with IVD library, whereas the "incomplete" category includes correct IDs of *C. auris* with cutoff scores of <1.7 by Biotyper or with a confidence value of <75.0 by the Vitek MS with RUO library and <60.0 by Vitek MS with IVD library.

[b]The isolates with results of "incomplete identification" or "no identification" following initial full-tube extraction were retested using the same full-tube extraction method.

[c]RUO, research use only; IVD, *in vitro* diagnostics.

[d]MALDI-TOF MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry.

10× buffer, 10 mM deoxynucleoside triphosphates (dNTPs), and 25 μM primer pairs to make a total volume of 50 μl. The reaction was carried out at 94°C for 5 min, 94°C for 40 s, 50°C for 1 min, and 72°C for 50 s for 30 cycles, followed by 74°C for 10 min. Then the amplification products were purified using a PCR purification kit (GeneAll Biotechnology, Seoul, South Korea). The purified products were analyzed bidirectionally using the same primer pairs as those used for the PCR, and sequencing data were obtained using an ABI PRISM 3730XL analyzer (Applied Biosystems, Foster City, CA). The mutations in each isolate were compared and analyzed based on the reference *ERG11* sequence of *C. auris* (GenBank accession no. MK059959) using MegAlign (Lasergene; DNAStar Inc., Madison, WI).

MLST analyses. MLST was performed using a previously described procedure (12). *Candida* DNA was extracted as described previously (17). The four genes selected for MLST analyses were *RPB1*, *RPB2*, and those encoding the ITS and D1/D2 regions. Amplification products were purified using a PCR purification kit (GeneAll Biotechnology). The purified reagents were analyzed using an ABI PRISM 3730XL analyzer (Applied Biosystems) with the same primers as the primers used for the PCR. The two *C. auris* strains (KCTC 17809 and KCTC 17810) in cluster 2 in Prakash et al. (12) were used as control strains.

PFGE analyses. PFGE typing consisted of electrophoretic karyotyping (EK) and restriction endonuclease analyses of genomic DNA using *Not*I (REAG-N). The PFGE methods for DNA preparation, REAG digestion, and electrophoresis have previously been reported (18). For EK, isolates that differed with respect to one or more bands were considered to have different karyotypes. For REAG-N, strains with banding patterns of identical size and number of bands were assigned to the same type, while strains with banding patterns that differed by three or fewer bands were considered closely related or genetically similar, and strains with banding patterns that differed by four or more bands were considered different (19).

Data availability. Sequences were deposited in GenBank under accession numbers MK294563 to MK294576, MK294578 to MK294591, MK294593 to MK294606, MK294608 to MK294621, MK294623 to MK294636, MK308726 to MK308745, MK308747 to MK308770, MK308772 to MK308795, MK308797 to MK308820, MK308822 to MK308845, and MK308847 to MK308850 (Tables 3 and 4).

RESULTS

Identification using MALDI-TOF MS. Table 1 shows the identification results. With an RUO library, 75.4% (46/61) of the isolates were correctly identified by Biotyper (with a cutoff score of ≥1.7) after initial full-tube extraction. When isolates with results of "incomplete identification" (8 isolates) or "no identification" (7 isolates) following initial full-tube extraction were retested using the same full-tube extraction method, 83.6% (51/61) of the isolates were correctly identified by Biotyper (cutoff score, ≥1.7). The Vitek MS system (with a confidence value of ≥75.0%) after direct on-plate extraction correctly identified 93.4% (57/61) of the *C. auris* isolates. The IVD library and the RUO library both yielded comparable percentages of correct identifications when used with the Vitek MS system (IVD library, 96.7%; RUO library, 93.4%), although the IVD library yielded a lower percentage of isolates that were incompletely identified (0% versus 6.6%). No isolates were misidentified using either of the two MALDI-TOF MS systems.

Antifungal susceptibility. The Vitek 2 yeast susceptibility test was compared to the CLSI reference BMD method against fluconazole, voriconazole, amphotericin B, and two echinocandins (Table 2). The ranges of MICs for fluconazole, voriconazole, amphotericin B, caspofungin, and micafungin were 2 to ≥64 μg/ml, ≤0.03 to 4 μg/ml, 0.25 to 1 μg/ml, 0.06 to 0.25 μg/ml, and ≤0.03 to 0.25 μg/ml, respectively, using the CLSI-BMD method. Based on tentative MIC breakpoints, the percentage of isolates showing fluconazole resistance (MIC, ≥32 μg/ml) was 62.3% (38/61) using both methods. Nei-

TABLE 2 *In vitro* susceptibilities of 61 *Candida auris* isolates to five antifungal agents, as determined by the CLSI and Vitek 2 yeast susceptibility test methods

Antifungal agent	Test method[c]	No. of occurrences at an MIC (μg/ml) of:												Essential agreement (%)[a]	Tentative MIC breakpoint (μg/ml)[b]	Resistance (%)[b]	Categorical agreement (%)[b]
		≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64				
Fluconazole	CLSI							4	6	7	6	10	28	96.7	≥32	62.3	93.4
	Vitek 2						1	13	1	7	1	12	26			62.3	
Voriconazole	CLSI	8	8	7	7	13	12	5	1					88.5	NA[d]	NA	NA
	Vitek 2			30	26	5										NA	
Amphotericin B	CLSI				2	29	30							100	≥2	0	100
	Vitek 2				56	5										0	
Caspofungin	CLSI		1	9	51									100	≥2	0	100
	Vitek 2				61											0	
Micafungin	CLSI	11	14	32	4									100	≥4	0	100
	Vitek 2		59	2												0	

[a]Essential agreement (±2 $\log_2$ dilutions) between the CLSI and Vitek 2 yeast susceptibility tests.
[b]Results of resistance and categorical agreement were analyzed using the tentative CLSI MIC breakpoints for *C. auris* published by the Centers for Disease Control (CDC) (https://www.cdc.gov/fungal/candida-auris/recommendations.html).
[c]CLSI test method refers to the CLSI M27-3A broth microdilution method after a 24-h incubation.
[d]NA, not available.

ther method indicated that any isolate showed resistance to amphotericin B, echinocandin, or multidrug resistance. The percentages of isolates showing essential agreement (within 2 dilutions) between the CLSI-BMD method and Vitek 2 system were 96.7%, 88.5%, 100%, 100%, and 100% for fluconazole, voriconazole, amphotericin B, caspofungin, and micafungin, respectively. Similarly, the percentages of categorical agreement were 93.4%, 100%, 100%, and 100% for fluconazole, amphotericin B, caspofungin, and micafungin, respectively.

***ERG11* sequence analysis.** The molecular characteristics of all 61 isolates from Korean hospitals are summarized in Table 3. Among the 61 isolates obtained from 13 Korean hospitals (A through M) collected during 1996 to 2018, only four (B1 to B4) were recovered from blood cultures of four patients at four different hospitals (hospitals A, E, F, and K). Of 57 ear isolates, 35 were from hospital A, and 1 to 5 isolates were recovered from each of other 12 hospitals. In hospital A, the isolates of one to eight patients were recovered from ear specimens each year during 2006 to 2018; however, apparent nosocomial clusters were not detected during this period. When the *ERG11* sequences of all 61 isolates were compared to that of the *C. auris* reference strain (GenBank accession no. MK059959), only 3 ear isolates with an MIC of $\geq$32 μg/ml for fluconazole harbored the amino acid substitution K143R (two from hospital A in 2014 and 2015 and one from hospital J in 2017). Additionally, two isolates harbored additional amino acid substitutions in Erg11p, L43H (one from hospital A in 2016) and Q357K (one from hospital J in 2017). However, amino acid substitutions F126L and Y132F were absent from all 61 isolates from Korean hospitals. Of ten CDC isolates from four geographic clades, seven that had an MIC of $\geq$32 μg/ml for fluconazole showed amino acid substitutions F126L (two isolates of clade III, AR0383 and AR0384), Y132F (two isolates of clade IV, AR0385 and AR0386; one isolate of clade 1, AR0389), and K143R (two isolates of clade I, AR0388 and AR0390) in Erg11p (Table 4).

MLST and PFGE analyses. Sequences of the internal regions of four housekeeping genes (*RPB1*, *RPB2*, ITS, and D1/D2) were the same for all 61 *C. auris* isolates from the Korean collection, including the two control isolates, KCTC 17809 and KCTC 17810, which were classified as cluster 2 (Table 3). However, MLST differentiated ten CDC isolates of the four clades into four clusters (clusters 1 to 4) (Table 4). Among ten CDC isolates from four different geographic clades, one isolate of clade II (*C. auris* AR0381) showed 100% homology with *C. auris* isolates from the Korean collection. PFGE typing determined that all *C. auris* isolates from the Korean hospitals tested shared a common EK pattern, which was quite different from those of ten isolates from other areas (Fig. 1). Two sequential blood isolates from each of three patients (isolates B1 from patient 1, isolates B2 from patient 2, and isolates B3 from patient 3) had identical EK and REAG-N patterns. For all *C. auris* isolates from the Korean collection tested, the isolates from both blood and ear shared a common EK pattern (K1) and showed only minor genetic differences (one to three bands) in REAG-N analyses, which belonged to N1 subgroups (N1a to N1l) (Fig. 1 and Table 3). Each of the ten CDC isolates produced a unique EK pattern (K2 to K11) (Fig. 1 and Table 4). REAG-N analyses of ten CDC isolates showed four different REAG-N patterns (N1 to N4) according to their clades. Only one isolate of clade II (AR0381) showed a REAG-N pattern (N1m) similar to those of the Korean isolates. Five isolates of clade I (AR0382 and AR0387 to AR0390) showed the similar REAG-N patterns (N2a-c) that are identical or differ by three or fewer bands.

DISCUSSION

To date, only one study has compared the performances of two commercial MALDI-TOF MS systems equipped with an RUO library of *C. auris* entries for the identification of *C. auris* isolates (8). In a study using the CDC panel of ten *C. auris* isolates, all ten *C. auris* isolates were identified correctly by the Vitek MS system after the direct extraction method, while 50% and 100% of *C. auris* isolates were correctly identified by the Biotyper after direct on-plate extraction and after full-tube extraction, respectively, indicating that the Biotyper performs better following the full-tube extraction method (8). Although direct on-plate FA extraction is simpler, Bruker Biotyper

TABLE 3 Molecular characterization of 61 isolates of *Candida auris* from Korean hospitals

Year	Source/ sample no.	Hospital (no. of isolates)	FR[a]	Erg11p[b] AAS	Erg11p[b] GenBank accession no. or similar isolate	MLST results for 4 alleles (ITS-*RPB1*-*RPB2*-D1/D2)[e] Allele profile	MLST results for 4 alleles (ITS-*RPB1*-*RPB2*-D1/D2)[e] GenBank accession no. or similar isolate	MLST results for 4 alleles (ITS-*RPB1*-*RPB2*-D1/D2)[e] ST cluster	PFGE type[c] EK	PFGE type[c] REAG-N
1996	Blood/B1	A (1)	R	None	MK294623	a-a-a-a	MK294578-MK294608-MK294593-MK294563	2	K1	N1a
2006	Ear/E4	A (1)	S	None	MK308826	a-a-a-a	MK308751-MK308776-MK308801-MK308726	2	K1	N1a
	Ear/E5	A (1)	R	None	MK308828	a-a-a-a	MK308753-MK308778-MK308803-MK308728	2	K1	N1b
	Ear/E6	A (1)	R	None	MK308829	a-a-a-a	MK308754-MK308779-MK308804-MK308729	2	K1	N1c
	Ear/E7	A (1)	R	None	MK308830	a-a-a-a	MK308755-MK308780-MK308805-MK308730	2	K1	N1d
	Ear/E8	A (1)	R	None	MK308831	a-a-a-a	MK308756-MK308781-MK308806-MK308731	2	K1	N1c
	Ear	A (1)	S	None	MK308827	a-a-a-a	MK308752-MK308777-MK308802-MK308727	2	K1	N1e
	Ear	A (1)	R	None	MK308832	a-a-a-a	MK308757-MK308782-MK308807-MK308732	2	K1	N1f
	Ear	A (1)	R	None	MK308833	a-a-a-a	MK308758-MK308783-MK308808-MK308733	2	K1	N1g
2007	Ear	B (1)	S	None	MK308834	a-a-a-a	MK308759-MK308784-MK308809-MK308734	2	K1	N1h
	Ear	B (1)	S	None	MK308835	a-a-a-a	MK308760-MK308785-MK308810-MK308735	2	K1	N1c
	Ear	B (1)	S	None	MK308836	a-a-a-a	MK308761-MK308786-MK308811-MK308736	2	K1	N1i
	Ear	C (1)	S	None	MK308837	a-a-a-a	MK308762-MK308787-MK308812-MK308737	2	K1	N1c
	Ear	C (1)	S	None	MK308838	a-a-a-a	MK308763-MK308788-MK308813-MK308738	2	K1	N1d
	Ear	C (1)	R	None	MK308839	a-a-a-a	MK308764-MK308789-MK308814-MK308739	2	K1	N1j
2008	Ear/E1	D (1)	S	None	MK308840	a-a-a-a	MK308765-MK308790-MK308815-MK308740	2	K1	N1c
	Ear/E2	D (1)	R	None	MK308841	a-a-a-a	MK308766-MK308791-MK308816-MK308741	2	K1	N1k
	Ear/E3	D (1)	R	None	MK308842	a-a-a-a	MK308767-MK308792-MK308817-MK308742	2	K1	N1a
2009	Blood/B2	E (1)	S	None	MK294624	a-a-a-a	MK294579-MK294609-MK294594-MK294564	2	K1	N1l
	Blood/B3	F (1)	S	None	MK294625	a-a-a-a	MK294580-MK294610-MK294595-MK294565	2	K1	N1c
2012	Ear	D (1), G (2), H (1)	S	None	B1-100%[d]	a-a-a-a	B1-100%	2	NT[f]	NT
	Ear	D (1), G (1), I (1)	R	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT
2014	Ear	A (1)	R	K143R	MK308843	a-a-a-a	MK308768-MK308793-MK308818-MK308743	2	NT	NT
	Ear	A (5)	R	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT
2015	Ear	A (1)	R	K143R	MK308844	a-a-a-a	MK308769-MK308794-MK308819-MK308744	2	NT	NT
	Ear	A (1), J (1)	S	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT
	Ear	A (2)	R	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT
2016	Ear	A (1)	R	L43H	MK308845	a-a-a-a	MK308770-MK308795-MK308820-MK308745	2	NT	NT
	Ear	A (2), J (1)	S	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT
	Ear	A (3)	R	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT
2017	Blood/B4	K (1)	R	None	MK294626	a-a-a-a	MK294581-MK294611-MK294596-MK294566	2	K1	N1c
	Ear/E9	A (1)	R	None	MK308847	a-a-a-a	MK308772-MK308797-MK308822-MK308747	2	K1	N1c
	Ear	J (1)	R	K143R	MK308848	a-a-a-a	MK308773-MK308798-MK308823-MK308748	2	NT	NT
	Ear	J (1)	R	Q357K	MK308849	a-a-a-a	MK308774-MK308799-MK308824-MK308749	2	K1	N1c
	Ear	A (1), J (1), L (1)	S	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT
	Ear	A (5), M (1)	R	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT
2018	Ear	A (1)	S	None	MK308850	a-a-a-a	MK308775-MK308800-MK308825-MK308750	2	NT	NT
	Ear	A (2)	R	None	B1-100%	a-a-a-a	B1-100%	2	NT	NT

[a]Fluconazole resistance (FR) was categorized as resistant (R) or susceptible (S) according to the tentative fluconazole MIC breakpoints (32 μg/ml) for *C. auris* published by the U.S. Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/fungal/candida-auris/recommendations.html).
[b]The amino acid substitutions (AAS) in each isolate were analyzed based on the reference *ERG11* sequence of *C. auris* (GenBank accession no. MK059959).
[c]Pulsed-field gel electrophoresis (PFGE) typing consisted of electrophoretic karyotyping (EK) and restriction endonuclease analyses of genomic DNA using *Not*I (REAG-N). Lower case letters (a to l) in REAG-N analysis indicate subgroups of a given (N1) type with banding patterns that are identical or differ by three or fewer bands.
[d]B1-100%, all isolates in the same row ($n = 2$ to 6 isolates) were identical at the DNA sequence level to isolate B1.
[e]MLST, multilocus sequence typing; ST, sequence type.
[f]NT, not tested.

MS instructions recommend full-tube FA/ACN extraction for yeast identification (10, 14). The reasons for the low rate of correct *C. auris* identification by Biotyper after direct on-plate extraction are poorly understood, but may be due to the characteristics of this pathogen, which renders solubilization of proteins difficult by simple extraction, or due

TABLE 4 Molecular characterization of ten isolates of *Candida auris* with representatives from each of the four clades

Isolate no.	FDA-CDC AR no. (clade)[a]	FR[b]	Erg11p[c] AAS	Erg11p[c] GenBank accession no.	MLST results for 4 alleles (ITS-*RPB1*-*RPB2*-D1/D2)[e] Allele profile	MLST results for 4 alleles (ITS-*RPB1*-*RPB2*-D1/D2)[e] GenBank accession no.	MLST results for 4 alleles (ITS-*RPB1*-*RPB2*-D1/D2)[e] ST cluster	PFGE type[d] EK	PFGE type[d] REAG-N
A1	AR0381 (II)	S	None	MK294627	a-a-a-a	MK294582-MK294612-MK294597-MK294567	2	K2	N1m
A2	AR0382 (I)	S	None	MK294628	b-b-a-b	MK294583-MK294613-MK294598-MK294568	1	K3	N2a
A3	AR0383 (III)	R	F126L, V125A	MK294629	b-b-a-a	MK294584-MK294614-MK294599-MK294569	3	K4	N3
A4	AR0384 (III)	R	F126L, V125A	MK294630	b-b-a-a	MK294585-MK294615-MK294600-MK294570	3	K5	N3
A5	AR0385 (IV)	R	Y132F, K177R, N335S, E343D	MK294631	c-c-c-c	MK294586-MK294616-MK294601-MK294571	4	K6	N4
A6	AR0386 (IV)	R	Y132F, K177R, N335S, E343D	MK294632	c-c-c-c	MK294587-MK294617-MK294602-MK294572	4	K7	N4
A7	AR0387 (I)	S	None	MK294633	b-b-a-b	MK294588-MK294618-MK294603-MK294573	1	K8	N2b
A8	AR0388 (I)	R	K143R	MK294634	b-b-a-b	MK294589-MK294619-MK294604-MK294574	1	K9	N2c
A9	AR0389 (I)	R	Y132F	MK294635	b-b-a-b	MK294590-MK294620-MK294605-MK294575	1	K10	N2c
A10	AR0390 (I)	R	K143R	MK294636	b-b-a-b	MK294591-MK294621-MK294606-MK294576	1	K11	N2c

[a]Food and Drug Administration (FDA) and U.S. Centers for Disease Control and Prevention (CDC) Antimicrobial Resistance (AR) Bank numbers.
[b]Fluconazole resistance (FR) was categorized as resistant (R) or susceptible (S) according to the tentative fluconazole MIC breakpoints (32 μg/ml) for *C. auris* published by the CDC (https://www.cdc.gov/fungal/candida-auris/recommendations.html).
[c]The amino acid substitutions (AAS) in each isolate were analyzed based on the reference *ERG11* sequence of *C. auris* (GenBank accession no. MK059959).
[d]Pulsed-field gel electrophoresis (PFGE) typing consisted of electrophoretic karyotyping (EK) and restriction endonuclease analyses of genomic DNA using *Not*I (REAG-N). Lower case letters (m, a, b, and c) indicate subgroups of a given REAG-N type (N1 or N2).
[e]MLST, multilocus sequence typing; ST, sequence type.

to insufficient database entries to enable spectral matches because the *C. auris* database is designed for isolates after in-tube FA/ACN extraction.

The identification of *Candida* species by MALDI-TOF systems depends on the database, the age or growth phase of the culture, and the extraction methods used in sample preparation (8, 10, 14). RUO libraries of two MALDI-TOF systems, Biotyper and

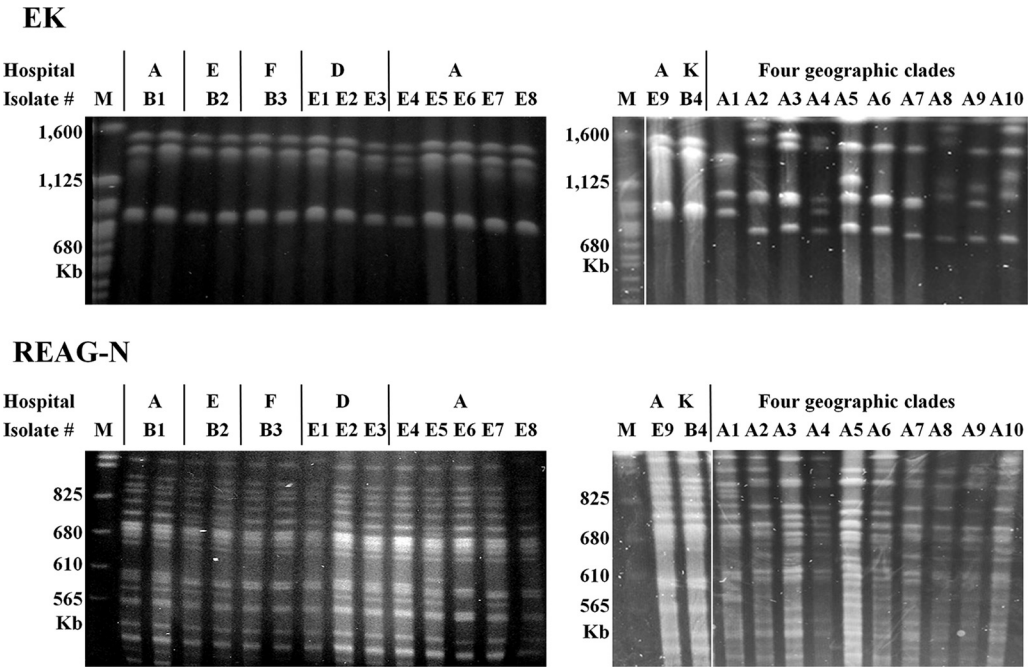

FIG 1 Representative PFGE patterns of *Candida auris* obtained by electrophoretic karyotyping (EK) and restriction endonuclease analyses of genomic DNA using *Not*I (REAG-N) for blood (isolates B1 to B4) and ear (isolates E1 to E9) isolates of *C. auris* from Korean hospitals, and ten *C. auris* isolates with representatives from each of the four clades (A1 to A10, *C. auris* AR0381 to *C. auris* AR0390, respectively) provided by the U.S. Centers for Disease Control and Prevention (CDC). See Tables 3 and 4 for detailed information on each isolate. Two sequential blood isolates each from the same patient (isolates B1 from patient 1, isolates B2 from patient 2, and isolates B3 from patient 3) had the same EK and the same REAG-N patterns. All isolates from Korean hospitals exhibited the same EK pattern and showed the same or similar REAG-N patterns, which were different from those of ten CDC *C. auris* isolates. M, *Saccharomyces cerevisiae* DNA concatemers that served as a molecular size marker.

Vitek MS, as well as a new IVD library (Vitek IVD 3.2) of Vitek MS can differentiate *C. auris* from other closely related *Candida* species, such as *C. duobushaemulonii* and *C. haemulonii*; however, not all of the reference databases included in MALDI-TOF devices allow for their detection (8). The Biotyper RUO library has a database of only three strains of *C. auris*, two from Korea (KCTC 17809 and KCTC 17810) and one type strain from Japan (DSM 21092T), and all ten CDC isolates of *C. auris* with representatives from each of the four clades were correctly identified with this library (8). However, a more recent study showed that Biotyper with an RUO database identified only 39% (13/33) of *C. auris* isolates after full-tube extraction, suggesting that the low identification rate from an RUO database could be caused by the loss of proteins during full-tube extraction database creation (10). In the present study, Biotyper with an RUO library containing *C. auris* entries correctly identified 75.4% of the 61 *C. auris* Korean isolates after initial full-tube extraction, and it correctly identified 83.6% of isolates after additive full-tube extraction. These results indicate that repeat Biotyper testing for isolates with "incomplete or no identification" results after initial full-tube extraction may be required, and careful handling considerations may be necessary during the *C. auris* extraction process.

A recent study by Bao et al. (10) showed that Biotyper with the CMdb database, which was created using internationally collected yeasts, identified 100% of *C. auris* isolates after direct on-plate extraction. This finding indicates that database expansion may address identification challenges by providing consistently higher MALDI identification scores. Although the use of in-house databases may have limitations in that they are not easily accessible and results may vary according to culture conditions, sample preparation method, and extraction procedure, the study by Bao et al. (10) provides an example of an online MALDI database that provides users access to additional *C. auris* MALDI spectral libraries, as well as to Bruker's most up-to-date database; these resources can be used to improve *C. auris* identification.

The Vitek MS clinical database was created using 12 *C. auris* reference strains that facilitated the successful identification and typing of *C. auris* by the Vitek MS (20). In the present study, the Vitek MS system with the RUO library correctly identified 93.4% of isolates after direct on-plate extraction, whereas the Vitek MS system equipped with a new IVD library (Vitek IVD 3.2) correctly identified 96.7% of the isolates after direct on-plate extraction, with a lower rate of incomplete identification than that of the Vitek MS with an RUO library (IVD, 0% versus RUO, 6.6%). The Vitek MS with the IVD library also correctly identified all ten CDC isolates of *C. auris* from four clades (data not shown). These data show for the first time that *C. auris* can be reliably identified by the Vitek MS system with the new IVD library (Vitek IVD 3.2).

Several surveillance programs of *C. auris* isolates have documented consistently high fluconazole MICs and variable rates of resistance to amphotericin B and the echinocandins (2–6, 9). In recent years, multidrug-resistant *C. auris* strains have emerged in Asia, Africa, Europe, and the Americas, resulting in several cases of fungemia (2, 4, 7, 9). Due to the limited available treatment choices and high rate of therapeutic failure, *in vitro* interactions between echinocandins and azoles against multidrug-resistant *C. auris* strains have been determined using a microdilution checkerboard technique (21). In the present study, the percentage of isolates showing fluconazole resistance was 62.3%, but amphotericin B-, echinocandin-, or multidrug-resistant isolates were not found, demonstrating that Korean isolates of *C. auris* have relatively lower resistance to antifungal agents than do isolates from other geographical areas.

In two previous studies, the *ERG11* gene encoding the azole target was sequenced to study the mechanism of *C. auris* resistance to azoles (2, 6). Those studies showed that three hot-spot amino acid substitutions in the *ERG11* gene, including F126L, Y132F, and K143R, were found only in fluconazole-resistant *C. auris*, suggesting that these substitutions confer a phenotype of fluconazole resistance similar to that described for *Candida albicans* (2, 6). These amino acid substitutions were found in almost all fluconazole-resistant isolates of *C. auris* from Pakistan, India, South Africa, and Venezuela (2), and in 77% (34/44) of fluconazole-resistant isolates of *C. auris* from India (6). In the present study, seven of ten CDC isolates from four geographic clades showed amino acid substitutions F126L, Y132F,

and K143R in Erg11p. However, amino acid substitutions F126L and Y132F were absent from all 61 isolates from the Korean collection, and of 38 fluconazole-resistant isolates, only 3 harbored the amino acid substitution K143R, suggesting that other resistance mechanisms, such as an efflux pump, may contribute to fluconazole resistance in *C. auris* isolates from our Korean collection (3, 4, 6).

A comparative study of data from CLSI and Vitek 2 yeast susceptibility tests of 90 *C. auris* isolates from India for amphotericin B, voriconazole, and echinocandins showed misleadingly high MICs of amphotericin B using the Vitek 2 and very low essential agreement (10%) between Vitek 2 and the CLSI-BMD method for amphotericin B (5). However, both the essential and categorical agreement of amphotericin B between the CLSI and Vitek 2 methods were 100% in the present study, which is in agreement with our previous report (22). Considering that 15.5% of Indian *C. auris* isolates exhibited elevated amphotericin B MICs ($\geq$2 μg/ml) by CLSI-BMD (5), while none of the *C. auris* isolates from our Korean collection were resistant to amphotericin B, it becomes evident that the comparability of CLSI and Vitek 2 is limited to the susceptible isolates examined in this study. Notably, the present study shows excellent essential (96.7%) and categorical agreement (93.4%) between the CLSI and Vitek 2 methods for fluconazole susceptibility, suggesting that the two methods are comparable for fluconazole and the *C. auris* isolates included in this study.

The genetic similarity of ear isolates of *C. auris* from Japan ($n = 1$) and Korea ($n = 2$, KCTC 17809 and KCTC 17810) was demonstrated using amplified fragment-length polymorphism, MLST, and MALDI-TOF MS (12). In the present study, all 61 isolates from blood (4 isolates) and ear (57 isolates) cultures had the same multilocus sequence type as the two isolates KCTC 17809 and KCTC 17810, which were classified as cluster 2. Among ten CDC isolates, an isolate of East Asian clade II (AR0381) revealed the same multilocus sequence type as the Korean isolates, but other isolates showed different multilocus sequence types according to their clades. Our PFGE results show that all isolates from Korean hospitals had the same EK pattern and the same or similar REAG-N patterns, as reported previously (18). The present study showed for the first time that Korean isolates of *C. auris* had quite different EK patterns from those of ten CDC *C. auris* isolates, and the EK patterns of the ten CDC isolates were more diverse (ten EK types) than those of REAG-N (four different genotypes).

Candida auris was first reported in 2009 after isolation from ear cultures of 1 Japanese patient and 15 Korean patients (11, 23), rapidly followed by isolation from the blood cultures of 3 patients from three hospitals in Korea (1). The latter study reported that the earliest isolate of *C. auris* was found in 1996 in the Korean isolate collection (1). The Korean collection used in the present study showed that since the first three reported cases of *C. auris* fungemia (1), only one patient isolate from blood cultures was obtained in 2017. The isolation of this organism from ear cultures has been observed continually from several hospitals in Korea (11, 24). To date, no genotyping of the blood isolates of *C. auris* from the first three cases of fungemia in Korea has been reported. In the present study, we showed that blood isolates of *C. auris* from the first three cases of fungemia in Korea exhibited the same multilocus sequence type (cluster 2) and had the same or similar PFGE pattern as other ear isolates, which suggests that *C. auris* isolates collected in Korea from both blood and ear since 2009 are genetically similar. However, a more detailed analysis, such as whole-genome sequencing, would be needed to confirm the common clonal origin of the isolates included in this study.

Candida auris virulence is comparable to that reported for *C. albicans* in a murine model (25); however, *C. auris* isolates from different geographic clades may have different virulence traits. In contrast to isolates from other geographic areas, the propensity to cause nosocomial outbreaks of fungemia within the same hospital has not been reported among *C. auris* isolates from Korea, and almost all *C. auris* isolates have been recovered from ear specimens, suggesting that *C. auris* isolates from Korean hospitals have different clinical and epidemiological characteristics from isolates from other geographic areas. *C. auris* isolates from Korea do not assimilate *N*-acetylglucosamine (NAG), in contrast to isolates from India that assimilated NAG (1, 26). In the

current study, we found that all isolates from Korean hospitals had quite different EK and REAG-N patterns from CDC *C. auris* isolates of the other three clades (clades I, III, and IV). Overall, this report highlights the differences in *C. auris* isolates from our Korean collection with respect to lower rates of antifungal susceptibilities, lower rates of *ERG11* amino acid substitutions in association with fluconazole resistance, and their unique genotypes, as revealed by MLST and PFGE analyses.

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We declare no conflicts of interest.

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