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Outbreak of *Candida auris* in Spain: A comparison of antifungal activity by three methods with published data

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ABSTRACT

Candida auris is an emerging pathogen causing candidaemia outbreaks in several countries for which azole, amphotericin B (AmB) and echinocandin resistance has been reported. In this study, the antifungal susceptibilities of 73 Spanish *C. auris* isolates (56 bloodstream and 17 urine) to eight antifungal agents were determined using three methods. Isolates were identified by internal transcribed spacer (ITS) sequencing, and minimum inhibitory concentrations (MICs) of fluconazole, isavuconazole, itraconazole, posaconazole, voriconazole, anidulafungin, micafungin and AmB were determined by EUCAST methodology and Sensititre® YeastOne® (SYO) (bloodstream isolates) and Liofilchem® MIC Test Strip (all isolates). Agreement between the methods was analysed and the MICs (ours and published data) were categorised using recently proposed epidemiological cut-off values (ECVs). Fluconazole MICs were >64 mg/L, whilst >60% of voriconazole MICs were >1 mg/L by the three methods. Posaconazole was the most active azole (EUCAST geometric mean MIC, 0.053 mg/L), followed by isavuconazole (0.066 mg/L) and itraconazole (0.157 mg/L). Echinocandins MICs were ≤0.5 mg/L by SYO and EUCAST. The overall lowest AmB MICs (≤0.25 mg/L) were obtained by EUCAST. Essential agreement (±2 dilutions) between EUCAST and SYO was >93% for the eight antifungals. For this new *C. auris* clade, all isolates were resistant to fluconazole, and MICs for anidulafungin, micafungin and AmB were ≤1 mg/L using dilution methods. Voriconazole MICs were method-dependent. The number of non-wild-type (non-WT) isolates depended on the ECV applied; by the 97.5% ECV all isolates were WT except for isavuconazole (1.8% non-WT). Good essential agreement (>93%) was observed between EUCAST and SYO.

1. Introduction

There has been an increase in reports of hospital outbreaks of *Candida auris* candidaemia in several countries [1] and recently from a Spanish hospital [2]. The Spanish isolates belong to a clade genotypically distinct from those previously reported from India, Oman, the UK and Venezuela but with some connections with South African isolates [3]. This relationship has also been determined through whole-genome sequencing of 18 blood culture isolates (unpublished data). Management of *C. auris* candidaemia infection is similar to that caused by other *Candida* spp.; however, establishment of an appropriate treatment is difficult due to the fact that *C. auris* is closely related to *Candida krusei, Candida lusitaniae* and *Candida haemulonii*, which belong to a clade resistant to fluconazole (FLU) and amphotericin B (AmB) [1,4].

The susceptibility of *C. auris* to various antifungal agents has been determined by Clinical and Laboratory Standards Institute (CLSI) methodology for a limited number of isolates, mostly from India and the UK [1,5–8]. However, a report by Chowdhary et al. included CLSI minimum inhibitory concentration (MIC) data for 350 *C. auris* isolates from India (72.2% from blood) [9], but limited data have been reported on the widely used commercial Sensititre® YeastOne® (SYO) and Etest or gradient strip methods. Although both methods have demonstrated good essential agreement with CLSI data when testing AmB and echinocandins, agreement is lower with Etest, especially for the azoles [10]. Also, low agreement and misleading elevated AmB MICs are reported with another commercial method, VITEK® AST-YS07 [6,11]. As breakpoints for *C. auris* are not yet available for any method/agent, the US Centers for Disease Control and Prevention (CDC) has recommended the use of available breakpoints for *Candida* spp.

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Table 1
In vitro antifungal susceptibility profile (MIC in mg/L) of 73 *Candida auris* isolates by three susceptibility testing methods.

Method (no. isolates)		FLU	ITR	ISA	PSC	VRC	AFG	MFG	AmB
EUCAST (56)[a]	MIC range	>64	0.06–0.5	0.03–2	0.03–0.12	0.5 to >8	0.016–0.5	0.03–0.12	0.06–0.25
	MIC_{50}	>64	0.12	0.06	0.06	2	0.03	0.06	0.12
	MIC_{90}	>64	0.25	0.12	0.12	4	0.06	0.06	0.25
	GM MIC	>64	0.157	0.066	0.053	2	0.035	0.052	0.136
SYO (56)[a,b]	MIC range	>256	0.06–0.5	ND	0.015–0.25	0.5 to >8	0.06–0.5	0.03–0.5	0.25–1
	MIC_{50}	>256	0.125	ND	0.06	2	0.125	0.06	0.5
	MIC_{90}	>256	0.5	ND	0.125	4	0.25	0.06	0.5
	GM MIC	>256	0.149	ND	0.057	1.7	0.121	0.057	0.38
MTS (73)	MIC range	256 to >256	ND	0.016–1	0.016–0.5	8–64	0.008–1	0.008–1	0.016–2
	MIC_{50}	>256	ND	0.125	0.25	64	0.064	0.032	1
	MIC_{90}	>256	ND	0.25	0.5	64	0.125	0.064	2
	GM MIC	256	ND	0.117	0.201	52.43	0.051	0.031	0.407

FLU, fluconazole; ITR, itraconazole; ISA, isavuconazole; PSC, posaconazole; VRC, voriconazole; AFG, anidulafungin; MFG, micafungin; AmB amphotericin B; EUCAST, European Committee on Antimicrobial Susceptibility Testing; SYO, Sensititre® YeastOne®; MTS, MIC Test Strip; MIC, minimum inhibitory concentration; $MIC_{50/90}$, MIC at which 50% and 90% of the test isolates were inhibited, respectively; GM, geometric mean; ND, not determined.

[a] Blood isolates only.

[b] SYO MIC data of 42/56 isolates have been published previously [3].

However, only a few CLSI breakpoints are available for *C. krusei* and *C. lusitaniae*, which are the closest species [12]. Arendrup et al. have proposed tentative CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off values (ECVs) for *C. auris* [5] using only isolates from India. However, these need to be evaluated in multicentre studies with MIC data from different countries. The aim of the current study was (i) to determine the susceptibility of a clade of *C. auris* by two commercial methods, namely SYO and Liofilchem® MIC Test Strip (MTS), (ii) to correlate the MIC results with those obtained by the reference EUCAST methodology, and (iii) to apply the recently reported ECVs for this species to our and published MIC data.

2. Materials and methods

2.1. Isolates

A total of 73 *C. auris* isolates were analysed in this study, including 56 individual bloodstream isolates from patients with candidaemia and 17 urine isolates from 10 patients with persistent candiduria. All isolates were recovered from La Fe University Hospital (Valencia, Spain). Isolates were identified by VITEK® MS-RUO/SARAMIS databases (bioMérieux, Marcy-l'Étoile, France) [13] and sequencing of the internal transcribed spacer (ITS) region of the ribosomal subunit as described previously [14]. Sequencing was performed using a GenomeLab GeXP™ Genetic Analysis System (Beckman Coulter Inc., Brea, CA) and the sequences obtained were compared with those in online databases (http://www.ncbi.nlm.nih.gov/guide/sequence-analysis/; http://www.westerdijkinstitute.nl/collections/).

2.2. In vitro antifungal susceptibility testing

Antifungal susceptibilities to FLU, isavuconazole (ISA), itraconazole (ITR), posaconazole (PSC), voriconazole (VRC), anidulafungin (AFG), micafungin (MFG) and AmB were determined according to the EUCAST E.Def 7.2 guidelines [15] and by SYO (SYO-09; TREK Diagnostic Systems, East Grinstead, UK) and Liofilchem® MTS (Liofilchem, Roseto degli Abruzzi, Italy) following the manufacturers' instructions. The concentration ranges tested were 0.008–4 mg/L for AmB, ITR, ISA, PSC, VRC, AFG and MFG and 0.125–64 mg/L for FLU as recommended by EUCAST.

MICs for the bloodstream isolates were determined by all three methods, whereas for urine isolates determination was only done by MTS. All MICs were determined after 24 h, and *C. krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality control strains.

2.3. Data analysis

For calculations, off-scale MICs were left unchanged. The geometric mean (GM) MIC as well as the MIC_{50} and MIC_{90} values (MICs at which 50% and 90% of the isolates were inhibited, respectively) were calculated. For comparison, MTS MICs were increased to the next two-fold dilution drug concentration matching the EUCAST scale. Significant differences in MICs among methods and clinical source were determined by analysis of variance (ANOVA) followed by *t*-test for each agent. A *P*-value of ≤0.05 was considered statistically significant. Essential agreement (±1 and ±2 two-fold dilutions) between methods and categorical agreement, applying the recently tentative published EUCAST ECVs [5], were estimated to classify the isolates as either wild-type (WT) or non-WT (harbouring mechanisms of resistance).

3. Results

MICs for all of the antifungal agents are listed in Table 1. All EUCAST FLU MICs were >64 mg/L, whilst VRC MICs were >1 mg/L for 64.3% of bloodstream isolates. ITR and PSC MICs for all bloodstream isolates were ≤0.5 mg/L and ≤0.12 mg/L, respectively, and ISA MICs were 2 mg/L for 1.8% (1/56) of the isolates. All isolates were inhibited by ≤0.5 mg/L and ≤0.12 mg/L of AFG and MFG, respectively, and by ≤0.25 mg/L of AmB.

By SYO, FLU MICs were >256 mg/L for all isolates, above the ECV (non-WT), and 60.7% exhibited VRC MICs > 1 mg/L. ITR MICs were in general one dilution lower than that of PSC, with 100% of isolates being inhibited by ≤0.5 mg/L and ≤0.25 mg/L, respectively. AFG MICs for bloodstream isolates were higher than those of MFG (GM MICs of 0.121 mg/L and 0.057 mg/L, respectively), and AmB inhibited 100% of isolates at ≤1 mg/L.

High FLU and VRC MICs by MTS were observed both for bloodstream and urine isolates (FLU MICs >256 mg/L and VRC MICs ≥8 mg/L), values that are considered resistant for *Candida albicans*. On the other hand, ISA and PSC showed good activity (GM MICs of 0.117 mg/L and 0.201 mg/L, respectively); however, ISA MICs were ≤1 mg/L for 89% of bloodstream isolates and ≤0.125 mg/L for all urine isolates. Among the echinocandins, both for bloodstream and urine isolates MFG showed the best activity, followed by AFG (GM MICs of 0.031 mg/L and 0.051 mg/L, respectively). AmB MICs were ≤1 mg/L for 85.7% of bloodstream isolates and ≤0.125 mg/L for all urine isolates.

Table 2
Percentage agreement between minimum inhibitory concentration (MIC) results obtained by three methods for 56 bloodstream isolates of *Candida auris*.

Antifungal agent	EUCAST vs. SYO		EUCAST vs. MTS	
	±1	±2	±1	±2
Isavuconazole	ND	ND	69.4	87.8
Itraconazole	85.7	95.9	ND	ND
Posaconazole	73.5	98.0	36.7	73.5
Voriconazole	87.8	95.9	0.0	0.0
Anidulafungin	73.5	98.0	61.2	83.7
Micafungin	98.0	98.0	83.7	93.9
Amphotericin B	44.9	93.9	8.2	38.8

EUCAST, European Committee on Antimicrobial Susceptibility Testing; SYO, Sensititre® YeastOne®; MTS, MIC Test Strip; ND, not determined.

For blood isolates, when comparing MICs obtained by the three methods, significant differences were found among the methods. SYO AmB and PSC MICs were significantly lower than those obtained by MTS ($P < 0.05$). Comparing the source of isolates (determined only for MTS), significant differences were observed, with urine isolate MICs being 10 times lower (0.07 mg/L vs. 0.7 mg/L). Using MTS, the GM MICs of AmB for bloodstream and urine isolates were two times higher than those determined by SYO (0.7 mg/L vs. 0.4 mg/L; not an important difference, still all under 1 mg/L).

Quantitative essential agreement (±2 dilutions) between EUCAST and SYO was >93% for all antifungal agents and >83% between EUCAST and MTS for ISA, AFG and MFG (Table 2).

Applying the statistical EUCAST 95% and 99% ECVs to ISA (0.12 mg/L and 0.25 mg/L, respectively), VRC (4 mg/L and 8 mg/L) and AFG (0.25 mg/L and 0.5 mg/L) proposed by Arendrup et al. [5], the percentage of non-WT isolates by EUCAST was 3.6% for the three agents by applying the 95% ECV, whereas only 1.8% of the isolates were classified as ISA non-WT applying the 99% ECV. Using the derivative ECV (dECV), the percentages of non-WT were greater (Table 3). Applying the ECV for EUCAST to SYO, the number of non-WT isolates was lower in general (Table 3).

4. Discussion

Until now, the majority of studies on *C. auris* susceptibility have been carried out using a few isolates (≤20 isolates) from only ten countries, with MICs determined by reference methods (CLSI M27-A and/or EUCAST). Despite SYO and Etest/MTS being the most frequently used methods in clinical laboratories, data are scarce and furthermore they are not compared with the reference methods, which precludes comparisons. Only Kathuria et al. compared Etest results with CLSI reference microdilution [6] (Table 4). In the current study, we compared for the first time MIC data for 73 Spanish *C. auris* isolates obtained by SYO and MTS methods with the reference EUCAST method. The isolates were also categorised as non-WT and WT applying the newly proposed tentative ECVs [5] to our results and those published by other authors.

Although some authors have reported low FLU MICs for 2–11.8% of their isolates [8,9,21–23], in the current study all FLU MICs were >64 mg/L, independent of source or susceptibility method (Table 1). On the other hand, VRC MICs were dependent on method and source of the isolates. Whilst by MTS all VRC MICs were ≥8 mg/L, by SYO and EUCAST only 1.8% (1/56) and 3.5% (2/56) of bloodstream isolates, respectively, had MICs ≥ 8 mg/L in contrast to the 14.6% reported by Arendrup et al. by EUCAST [5]. Kathuria reported the lowest percentage of VRC MICs ≥8 mg/L by Etest and CLSI (11.11% and 16%, respectively) [6]. Using MTS, ISA MICs for all urine isolates were 0.125 mg/L, but only 59% of bloodstream isolates were inhibited at this concentration. Although the ISA GM MIC was the lowest by EUCAST, one bloodstream isolate (1.8%) was inhibited by 2 mg/L compared with 4.1% reported by Arendrup et al. by the same method [5]. In the current study, both EUCAST and SYO ITR MIC ranges (0.06–0.5 mg/L for both methods) and GM MICs (0.157 mg/L and 0.149 mg/L, respectively) are similar to those reported by Arendrup et al. [5]. The PSC MIC_{90} reported by other authors using the CLSI method ranged from 0.06–2 mg/L and the maximum PSC MIC reported was 8 mg/L [5,6,9]; in the present study the maximum PSC MIC (0.12 mg/L by EUCAST, 0.25 mg/L by SYO and 0.5 mg/L by MTS) was lower than that reported by Arendrup et al. by the EUCAST method (0.5 mg/L) [5] (Table 4).

MICs of the two echinocandins evaluated were ≤1 mg/L by the three methods; however, AFG and MFG MICs ≥8 mg/L have been reported [1,5,6,9] (Table 4).

Although SYO and EUCAST AmB MICs were ≤1 mg/L, the MTS MICs for 14.3% (8/56) of bloodstream isolates were 2 mg/L, higher than the expected serum levels. Variable AmB MICs > 1 mg/L has been published, ranging from 0 to 23.5% by Etest and from 0 to 75% by CLSI; however, by EUCAST no AmB MIC > 1 mg/L has been reported (Table 4). In general, the percentage of isolates inhibited by >1 mg/L is highest by CLSI methodology (e.g. Kathuria et al. report AmB MICs > 1 mg/L for 15.5% of *C. auris* isolates by CLSI and 1.1% by Etest), whereas in our case it was the contrary.

As breakpoints for *C. auris* have not yet been established by any method for any antifungal agent, the tentative dECV reported for *C. auris* was applied to categorise isolates as WT or non-WT, both for our results and for those previously reported (although this dECV has been calculated with data from one laboratory). For ISA, 1.8% of isolates in the current study were non-WT (MIC > 1 mg/L) in contrast to the reported 4.1% and 11% by EUCAST and CLSI, respectively [5,6] (Table 4). Applying the PSC dECV of 0.12 mg/L, 8.9–100% isolates previously reported would be categorised as non-WT

Table 3
Percentage of non-wild-type (non-WT) blood isolates by European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology and Sensititre® YeastOne® (SYO) according to statistical epidemiologic cut-off values (ECVs) and derivative ECV (dECV).

Antifungal agent	Statistical 95% ECV	% non-WT isolates		Statistical 97.5% ECV	% non-WT isolates		Statistical 99% ECV	% non-WT isolates		dECV	% non-WT isolates	
		EUCAST	SYO		EUCAST	SYO		EUCAST	SYO		EUCAST	SYO
Fluconazole	NR	NR	NR	NR	NR	NR	NR	NR	NR	128	100	100
Itraconazole	1	0	0	1	0	0	2	0	0	1	0	0
Isavuconazole	0.12	3.6	NR	0.25	1.8	NA	0.25	1.8	ND	1	1.8	ND
Posaconazole	0.125	0	7.2	0.25	0	0	0.25	0	0	0.125	0	0
Voriconazole	4	3.6	1.8	4	3.6	17.8	8	0	0	2	17.8	17.8
Anidulafungin	0.25	3.6	1.8	0.25	3.6	1.8	0.5	0	0	0.25	3.6	1.8
Micafungin	0.25	0	1.8	0.25	0	1.8	0.25	0	1.8	0.25	0	1.8
Amphotericin B	NR	NR	NR	NR	NR	NR	2	0	0	2	0	0

NR, not reported; ND, no data.

Table 4
Published antifungal susceptibility data for *Candida auris* with more than ten isolates.

Reference	Total no. of isolates	Country (no.)	Source (no.)	Drug	Method	MIC (mg/L)				dECV	No. (% R or non-WT)
						Range	MIC_{50}	MIC_{90}	GM		
Kim et al. [16]	15	Korea (15)	Ear (15)	FLU	CLSI	2–128	NR	NR	NR	>4[a]	9 (60.0)
				ITR	CLSI	0.12–2	NR	NR	NR	0.25	8 (53.3)
				VRC	CLSI	0.03–2	NR	NR	NR	1	2 (13.3)
				MFG	CLSI	0.03–0.06	NR	NR	NR	0.25	0 (0)
				AmB	Etest	0.5–2	NR	NR	NA	NA	NA
Shin et al. [17]	20	Korea (20)	Ear (17)	AmB	Etest	0.25–1	0.5	1	NR	NA	NA
			Blood (3)	AmB	VITEK®2	0.25–1	0.5	0.5	NR	NA	NA
				AmB	CLSI	0.25–1	0.5	1	NR	1	0 (0)
				AmB	EUCAST	0.25–1	0.5	1	NR	1	0 (0)
Chowdhary et al. [11]	15	India (15)	Blood (7)	FLU	CLSI	64	64	64		64	15 (100)
			OS (8)	ISA	CLSI	0.06–0.5	0.25	0.425	0.23	0.5	0 (0)
				PSC	CLSI	0.016–0.12	0.06	0.06	0.03	0.12	0 (0)
				VRC	CLSI	0.5–4	1	2	1.16	>0.5[a]	11 (73)
				AFG	CLSI	0.12–0.25	0.12	0.25	0.14	0.25	0 (0)
				MFG	CLSI	0.06–0.12	0.12	0.12	0.1	0.25	0 (0)
				AmB	CLSI	0.25–1	1	1	0.64	1	0 (0)
Chowdhary et al. [9][b]	350	India (350)	Blood (267)	FLU	CLSI	1 to >64	64	64	43.2	64	255 (72.9)
				ISA	CLSI	0.016–4	0.03	0.5	0.07	0.5	21 (6)
				ITR	CLSI	0.03–16	0.12	0.5	0.12	0.25	44 (12.6)
				PSC	CLSI	0.016–8	0.03	0.12	0.05	0.12	37 (10.6)
				VRC	CLSI	0.03–16	0.25	2	0.31	1	52 (14.9)
				AFG	CLSI	0.016–8	0.25	1	0.27	0.25	149 (42.6)
				MFG	CLSI	0.016–16	0.12	0.25	0.11	0.25	19 (5.4)
				AmB	CLSI	0.12–8	1	1	0.74	1	27 (7.7)
Kathuria et al. [6]	90	India (90)	Blood (78)	FLU	CLSI	4 to >64	64	64	36	>8[a]	80 (89)
			OS (12)	ISA	CLSI	0.016–4	0.25	2	0.18	≥1[a]	10 (11)
				ITR	CLSI	<0.03–2	0.12	0.5	0.15	0.25	NR
				PSC	CLSI	<0.016–8	0.06	2	0.06	≥1[a]	10 (11)
				VRC	CLSI	<0.03–16	1	8	1.01	1	35 (38.9)
				VRC	Etest	<0.03 to >16	1	16	NR	NA	NA
				VRC	VITEK®2	0.06–8	1	4	NR	NA	NA
				AFG	CLSI	<0.016–8	0.12	0.5	0.23	>2[a]	7 (8)
				MFG	CLSI	<0.016–8	0.12	0.25	0.11	>2[a]	7 (8)
				AmB	CLSI	0.12–8	1	4	0.8	1	14 (15.6)
				AmB	Etest	<0.03–2	0.5	1	NR	NA	NA
				AmB	VITEK®2	2–16	8	16	NR	NA	NA
Schelenz et al. [7]	50	UK (50)	Blood (9)	FLU	SYO	>256	>256	>256	>256	NA	NA
			OS (41)	Echino.	SYO	0.06–0.25	NR	NR	NR	NA	NA
				AmB	SYO	0.5–2	NR	NR	NR	NA	NA
Calvo et al. [18]	18	Venezuela (18)	Blood (18)	FLU	CLSI	>64	>64	>64	>64	64	18 (100)
				VRC	CLSI	4	4	4	4	1	18 (100)
				AFG	CLSI	0.06–0.12	0.12	0.12	0.12	0.25	0 (0)
				AmB	CLSI	1–2	1	2	1.41	1	9 (50)
Lockhart et al. [1]	54	Pakistan (18)	NR	FLU	CLSI	4–256	128	256	NR	64	43 (79.6)
		India (19)		ITR	CLSI	0.12–2	0.5	1	NR	0.25	45 (83.3)
		South Africa (10)		PSC	CLSI	0.06 –1	0.5	1	NR	0.12	47 (87.0)
		Venezuela (5)		VRC	CLSI	0.03 –16	2	8	NR	1	29 (53.7)
		Unknown (2)		AFG	CLSI	0.12 –16	0.5	1	NR	0.25	44 (81.5)
				MFG	CLSI	0.06–4	0.25	1	NR	0.25	21 (38.9)
				AmB	CLSI	0.5–4	1	2	NR	1	19 (35.2)
Larkin et al. [21]	16	Germany (2)	Blood (15)	FLU	CLSI	1 to >64	16	>64	NR	64	5 (31.3)
		Japan (1)	Ear (1)	ISA	CLSI	0.004–0.25	0.63	0.125	NR	0.5	0 (0)
		Korea (2)		ITR	CLSI	<0.06–1	0.5	1	NR	0.25	15 (93.8)
		India (11)		PSC	CLSI	0.25–1	0.25	0.5	NR	0.12	16 (100)
				VRC	CLSI	<0.06–1	0.5	1	NR	1	0 (0)
				AFG	CLSI	0.12–0.25	0.125	0.25	NR	0.25	0 (0)
				MFG	CLSI	0.25–2	1	1	NR	0.25	14 (87.5)
				AmB	CLSI	0.5–8	2	4	NR	1	12 (75)
Rudramurthy et al. [8]	74	India (74)	Blood (74)	FLU	CLSI	0.06–8	1	2	0.52	64	30 (40.5)
				ITR	CLSI	0.03–2	0.06	0.25	0.08	0.25	7 (9.5)
				PSC	CLSI	0.03–1	0.12	0.25	0.1	0.12	18 (24.3)
				VRC	CLSI	0.03–4	0.5	2	0.36	1	9 (12.2)
				AFG	CLSI	0.03–2	0.12	0.25	0.16	0.25	22 (29.7)
				MFG	CLSI	0.03–2	0.12	1	0.12	0.25	14 (18.9)
				AmB	CLSI	0.06–8	1	2	0.52	1	10 (13.5)
Morales-López et al. [19]	17	Colombia (17)	Blood (13)	FLU	VITEK®2	16 to >64	32	>64	NR	NA	NA
			OS (4)	VRC	VITEK®2	<0.12–2	0.25	2	NR	NA	NA
				MFG	VITEK®2	<0.06–0.25	0.12	0.12	NR	NA	NA
				AmB	VITEK®2	8 to >16	8	>16	NR	NA	NA
				AmB	Etest	0.5–2	1	2	NR	NA	NA

(*continued on next page*)

Table 4 (*continued*)

Reference	Total no. of isolates	Country (no.)	Source (no.)	Drug	Method	MIC (mg/L)				dECV	No. (% R or non-WT)
						Range	MIC_{50}	MIC_{90}	GM		
Arendrup et al. [5][c]	123	India (123)	Blood (100)	FLU	CLSI	4 to ≥64	≥64	≥64	43.38	64	91 (74)
				FLU	EUCAST	0.5 to ≥64	≥64	≥64	53.74	64	108 (87.8)
				ISA	CLSI	0.016–4	0.12	0.5	0.095	0.5	5 (4.1)
				ISA	EUCAST	≤0.008–2	0.12	0.5	0.09	1	5 (4.1)
				ITR	CLSI	0.03–2	0.12	0.25	0.11	0.25	9 (7.3)
				ITR	EUCAST	0.008–1	0.12	0.5	0.13	1	0 (0)
				PSC	CLSI	0.016–8	0.016	0.12	0.035	0.12	11 (8.9)
				PSC	EUCAST	≤0.008–0.5	0.032	0.12	0.033	0.12	4 (3.3)
				VRC	CLSI	0.032–16	0.5	4	0.66	1	41 (33.3)
				VRC	EUCAST	≤0.008–4	0.5	2	0.54	2	5 (4.1)
				AFG	CLSI	0.016–8	0.12	0.5	0.22	0.25	29 (23.6)
				AFG	EUCAST	0.002–2	0.12	1	0.17	0.25	33 (26.8)
				MFG	CLSI	0.016–8	0.12	0.25	0.12	0.25	10 (8.1)
				MFG	EUCAST	0.002–4	0.12	0.25	0.13	0.25	8 (6.5)
				AmB	CLSI	0.12–8	0.5	2	0.66	1	12 (9.8)
				AmB	EUCAST	0.25–1	1	1	0.91	1	0 (0)
Khan et al. [20]	56	Kuwait (56)	Various sources	FLU	Etest	128–256	256	256	252.8	≥32[a]	56 (100)
				VRC	Etest	0.06–6	1.5	3	1.2	≥1[a]	41 (73.2)
				MFG	Etest	0.006–4	0.094	0.12	0.093	≥4[a]	1 (1.8)
				AmB	Etest	0.047–3	1.5	2	1.05	≥2[a]	1 (1.8)
Berkow and Lockhart [22]	100	Various countries		AFG	CLSI	0.12 to >16	NR	NR	NR	NR	NR
				MFG	CLSI	0.03 to >8	NR	NR	NR	NR	NR
				AmB	CLSI	0.12–4	NR	NR	NR	NR	NR
Escandón et al. [23]	85	Colombia	Various sources	FLU	CLSI	2–64	8	16	NR	64	0
				ISA	CLSI	0.016–1	0.12	0.5	NR	0.5	1 (1.18)
				PSC	CLSI	0.016 – 0.5	0.06	0.25	NR	0.12	10 (11.8)
				VRC	CLSI	0.016–4	0.12	0.5	NR	1	1 (1.18)
				AFG	CLSI	0.016–2	0.12	2	NR	0.25	33 (38.8)
				MFG	CLSI	0.03–2	0.25	1	NR	0.25	29 (34.1)
				AmB	Etest	0.25–4	0.5	2	NR	1	NA
Current study	73	Spain (73)	Blood (56)	FLU	EUCAST	>64	>64	>64	>64	64	56 (100)
				FLU	SYO	>256	>256	>256	>256	NA	NA
				FLU	Etest	256 to >256	>256	>256	256	NA	NA
				ISA	EUCAST	0.03–2	0.06	0.12	0.066	1	1 (1.8)
				ISA	Etest	0.016–1	0.12	0.5	0.117	NA	NA
				ITR	EUCAST	0.06–0.5	0.12	0.25	0.157	1	0 (0)
				ITR	SYO	0.06–0.5	0.12	0.5	0.15	NA	NA
				PSC	EUCAST	0.03–0.12	0.06	0.12	0.053	0.12	0 (0)
				PSC	SYO	0.015–0.25	0.06	0.125	0.057	NA	NA
				PSC	Etest	0.016–0.5	0.25	0.5	0.201	NA	NA
				VRC	EUCAST	0.5 to >8	2	4	2	2	10 (17.9)
				VRC	SYO	0.5 to >8	2	4	1.7	NA	NA
				VRC	Etest	8–64	64	64	52.43	NA	NA
				AFG	EUCAST	0.016–0.5	0.03	0.06	0.035	0.25	2 (3.6)
				AFG	SYO	0.06–0.5	0.125	0.25	0.121	NA	NA
				AFG	Etest	0.008–1	0.06	0.12	0.051	NA	NA
				MFG	EUCAST	0.03–0.12	0.06	0.06	0.052	0.25	0 (0)
				MFG	SYO	0.03–0.5	0.06	0.06	0.057	NA	NA
				MFG	Etest	0.008–1	0.03	0.06	0.031	NA	NA
				AmB	EUCAST	0.06–2	0.12	0.25	0.136	1	0 (0)
				AmB	SYO	0.25–1	0.5	0.5	0.38	NA	NA
				AmB	Etest	0.016–2	1	2	0.407	NA	NA

MIC, minimum inhibitory concentration; $MIC_{50/90}$, MIC that inhibits 50% and 90% of the isolates, respectively; GM, geometric; dECV, derivative epidemiological cut-off value; R, resistant; non-WT, non-wild-type; OS, other source; NR, not reported; FLU, fluconazole; ITR, itraconazole; VRC, voriconazole; MFG, micafungin; AmB, amphotericin B; ISA, isavuconazole; PSC, posaconazole; AFG, anidulafungin; Echino., echinocandin; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; SYO, Sensititre® YeastOne®; NA, not applicable.

[a] Breakpoint applied by the author.

[b] MIC data of 123/350 isolates have been published previously [5].

[c] CLSI MIC data of 90/123 isolates have been published previously [6].

by CLSI and 3.25% by EUCAST, whilst all isolates in the current study would be WT by EUCAST [5,9,21] (Table 4). In the current study, the percentage of VRC non-WT isolates according to the EUCAST ECV (2 mg/L) was 17.8%, in contrast to the reported 3.25% [5] (Table 4). For both echinocandins (CLSI dECV 0.25 mg/L), high variability in non-WT isolates has been observed among authors who assayed more than 50 isolates: from 23.6% to 81.5% for AFG [1,5] and from 5.4% to 38.8% for MFG [1,9] (Table 4). In general, the percentage of non-WT isolates is greater for Indian isolates. Using EUCAST (dECV 0.25 mg/L), 3.6% and 0% of isolates in the current study would be non-WT for AFG and MFG, respectively, whilst Arendrup et al. reported the greatest percentage of non-WT isolates (26.8% and 6.5% for AFG and MFG, respectively) [5].

Finally, for bloodstream isolates, essential agreement between MIC results obtained by EUCAST and SYO was >93% for all antifungal agents, with the best agreement being for MFG and AFG (98%). By MTS, agreement was >83% only for ISA, AFG and MFG. More studies are required to confirm the poor correlation of VRC and AmB with the reference EUCAST method.

There are potential limitations to this study. First, although the isolates were from individual patients, the isolates are clonal in nature and gave rise to an outbreak. Second, isolates with known

resistance mechanisms were not included for comparison of methods. Lastly, another limitation could be that most of the published susceptibility studies with *C. auris* include Indian isolates, therefore the true percentage of resistance is difficult to determine.

In summary, here we provide MIC data for a clade of *C. auris* that is genotypically distinct from those previously described, isolated during a Spanish hospital outbreak. We decided not to use the CDC recommendation about using breakpoints for *Candida* spp. because both breakpoints and ECVs should be method- and species-dependent; the CLSI recommendation is to use the species-specific ECV when a breakpoint is not available. In this study, all isolates were classified as AmB and echinocandin WT and FLU non-WT. The number of isolates above the ECV varied according to the percentage of ECV applied (97.5% recommended by the CLSI versus the lower 95%, and dECV recommended by Arendrup et al.) and the susceptibility testing method. According to 99% ECV by EUCAST, all of the isolates were WT except to ISA (1.8% non-WT). Furthermore, good correlation was observed between EUCAST MIC results and the commercial method SYO, which is perhaps one of the methods most frequently used in clinical laboratories. The poor correlations between EUCAST and MTS for VRC and AmB require additional studies.

Declarations

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Competing interests

None declared.

Ethical approval

Not required.

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