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Fenticonazole Activity Measured by the Methods of the European Committee on Antimicrobial Susceptibility Testing and CLSI against 260 *Candida* Vulvovaginitis Isolates from Two European Regions and Annotations on the Prevalent Genotypes▽

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The activity of fenticonazole was studied against 260 West and Southeast European vulvovaginal candidiasis isolates, and low MICs were displayed. Fenticonazole was assessed by European Committee on Antimicrobial Susceptibility Testing and CLSI microdilution methods for the first time, and the results showed excellent agreement (97%) and significant interclass correlation coefficient ($P < 0.0001$). Also, the levels of agreement for the results for itraconazole, fluconazole, and ketoconazole were 84%, 90%, and 98% ($P < 0.0001$), respectively. Multilocus typing by PCR fingerprinting and subsequent cluster analysis delineated geographically associated alignments for *Candida albicans* and fluconazole resistance-related clusters for *Candida glabrata*.

Uncomplicated vulvovaginal candidiasis (VVC) affects approximately 75% of women at reproductive age (13, 17, 22); *Candida albicans* is a major cause and *Candida glabrata* accounts for approximately 5% of cases worldwide (30). The recommended first-line therapy for uncomplicated VVC is topical azoles (4, 7, 25, 27, 28), unless resistance of the isolate is substantiated or azole hypersensitivity is diagnosed (4, 8). Identifying antifungal resistance in vitro is clinically important, but variable host responses to treatment and unpredictable fungal load in the vulvovaginal mucosa (*in loco*) invariably weaken in vitro with in vivo correlations. However, standardized susceptibility testing of isolates to local antifungals could provide data on the in vitro activity of newer topical antifungals.

Recording agreement of the results of the CLSI (24) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (10) reference methods in determining the susceptibility of VVC isolates from Belgian and Greek patients to fenticonazole, a topical imidazole (8, 12), forms the basis of this report. Subsequently, PCR fingerprinting was used to investigate whether distinct geographical and azole-resistant clinical isolate subpopulations can be recognized.

A total of 260 baseline *C. albicans* and *C. glabrata* isolates from pregnant, nonpregnant, and diabetic women were tested (Table 1). Isolates were identified in Chromagar medium (Chromagar, Paris, France) and identified with the API ID 32 C system (bioMerieux, Marcy l'Etoile, France). All *C. albicans* isolates were screened for *Candida dubliniensis* (3, 18, 31) and *C. glabrata* strains were screened for *Candida nivariensis* and *Candida bracarensis* (1, 19) to ensure that susceptibility testing and PCR fingerprints corresponded only to *C. albicans* and *C. glabrata* isolates.

Stock fluconazole (Pfizer Inc., Sandwich, Kent, United Kingdom) solutions and a range of concentrations of itraconazole and ketoconazole (Janssen, Beerse, Belgium) were prepared as described for each reference method. Fenticonazole compound (Recordati S.A, Milan, Italy, and Galenica, Athens, Greece) was prepared as a 100× stock in dimethyl sulfoxide (Merck, Darmstadt, Germany) at a final concentration range of 0.0312 μg/ml to 32 μg/ml. Test medium, inoculum prepara-

TABLE 1. Origin of isolates from 260 patients with uncomplicated vulvovaginitis

Patient group (no.)	Mean age (yr)	No. of patients of indicated national origin with isolates of indicated species				Total no. of isolates
		Belgium		Greece		
		C. albicans	*C. glabrata*	*C. albicans*	*C. glabrata*	
Pregnant (65)	30	61	4	NI[a]	NI	65
Nonpregnant (152)	40	132	2	17	1	152
Diabetic (43)	42	NI	NI	39	4	43
All (total no. of patients)		193	6	56	5	260

[a] NI, patient group not included.

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TABLE 2. Susceptibilities of 260 VVC isolates and quality control strains determined by the CLSI M27-A2 and EUCAST broth microdilution methods

Candida isolate (no.) or QC strain[a]	Method	Itraconazole		Fenticonazole		Fluconazole		Ketoconazole	
		MIC_{50} range (μg/ml)	GM[b] (μg/ml)	MIC_{50} range (μg/ml)	GM (μg/ml)	MIC_{50} range (μg/ml)	GM (μg/ml)	MIC_{50} range (μg/ml)	GM (μg/ml)
C. albicans (249)	CLSI	0.03–0.5	0.07	0.03–0.5	0.14	0.12–16	1.86	0.12–4.0	0.78
	EUCAST	0.03–0.5	0.06	0.03–0.25	0.10	0.12–32	1.84	0.12–4.0	0.54
C. glabrata (11)	CLSI	0.03–0.5	0.10	0.03–1	0.34	2.0–≥64	7.51	0.5–4.0	1.86
	EUCAST	0.03–0.5	0.10	0.03–0.5	0.28	2.0–≥64	7.51	2.0–8.0	2.13
C. parapsilosis ATCC 22019	CLSI	0.06–0.25	0.14	0.03–1[c]	0.16	2–8	3.73	0.12–0.25	0.17
	EUCAST	0.06–0.12	0.09	0.03–0.25[d]	0.13	0.5–4	2.14	0.12–0.5	0.21
C. krusei ATCC 6258	CLSI	0.12–0.5	0.10	0.06–2[e]	0.23	16–64	45.25	0.25–0.5	0.33
	EUCAST	0.03–0.12	0.08	0.06–1[f]	0.20	32–64	45.25	0.12–1	0.26

[a] Results were obtained for 20 independent tests. QC, quality control.
[b] GM, geometric mean.
[c] One of 20 test results (MIC, 1 μg/ml) was out of the observed range of 0.03 to 0.5 μg/ml.
[d] No test result was out of range.
[e] One of 20 test results (MIC, 2 μg/ml) was out of the observed range of 0.06 to 0.5 μg/ml.
[f] One of 20 test results (MIC, 1 μg/ml) was out of the observed range of 0.06 to 0.25 μg/ml.

tions, and reading of results were as described in the respective guidelines (10, 24). *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as control strains for both methods (Table 2). No CLSI or EUCAST out-of-range MICs were observed for itraconazole, fluconazole, or ketoconazole.

No differences in susceptibilities among isolates from the three patient groups were observed, but in contrast to previous reports (21), no geographical associations in susceptibility were recorded for isolates from the two European regions. Fluconazole resistance (Table 2) in *C. albicans* was rare (6.9%), whereas 45% of the *C. glabrata* isolates were resistant (6, 11). Fluconazole and ketoconazole cross-resistance was inferred for 20/249 (8.03%) *C. albicans* isolates and for 3/11 (27.2%) *C. glabrata* isolates. Generally, lower MICs were recorded for fenticonazole than for the other drugs (Table 2), but their clinical relevance cannot be assessed without correlating the in vitro responses and *in loco* fenticonazole pharmacokinetics and pharmacodynamics with the in vivo response. Topical ketoconazole efficacy and drug levels have thus far been assessed ex vivo in human skin specimens and have successfully supported standardized susceptibility testing and clinical investigations (2). However, bioassay systems to complement in vitro studies have not been assessed with topical VVC agents.

Agreement between the CLSI and EUCAST results (29) within ±1 dilution was 84 to 98% (Table 3), and interclass correlation coefficients were statistically very significant ($P < 0.0001$), suggesting that fenticonazole testing with both reference methods gives concordant MICs.

A possible susceptibility-associated relatedness of strains and the population structures of the *C. albicans* and *C. glabrata* isolates from the two geographic regions was studied by PCR fingerprinting using the minisatellite specific oligonucleotide [5′-GAGGGTGGCGGTTCT-3′] M13 (23, 35) as described before (15, 34). All Greek VVC isolates originated exclusively from Greek Caucasians, whereas Belgian strains were isolated from patients of mixed ethnic origin, including African immigrants residing in Belgium.

TABLE 3. Agreement and intraclass correlation coefficients for log_2-transformed data[a] obtained by CLSI and EUCAST reference methods for azole drugs against vulvovaginitis isolates

Antifungal drug	Agreement (%)[b]	ICC[b]	*P*
Itraconazole	84	0.64	$<10^{-4}$
Fenticonazole	97	0.88	$<10^{-4}$
Fluconazole	90	0.90	$<10^{-4}$
Ketoconazole	98	0.88	$<10^{-4}$

[a] SPSS version 10.0 (SPSS Inc., Chicago, IL, 1999) was used to determine these data.
[b] A value of 85% was selected to validate the results. Agreement values and intraclass correlation coefficients (ICCs) were calculated from the results obtained for all *C. albicans* and *C. glabrata* isolates.

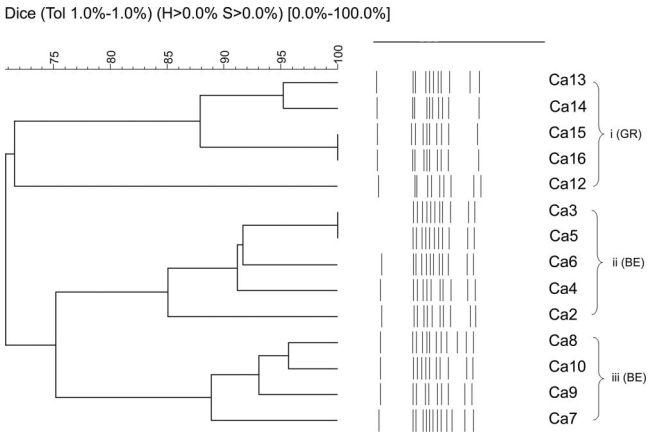

FIG. 1. Summary of results for 249 *Candida albicans* VVC isolates. A dendrogram representing the three *C. albicans* clusters of VVC isolates is shown. Groups were defined by 75% similarity. Of the 194 Belgian isolates, 137 (70.6%) grouped in cluster ii and 57 (29.40%) grouped in cluster iii. All 55 Greek isolates grouped in a single cluster, cluster i.

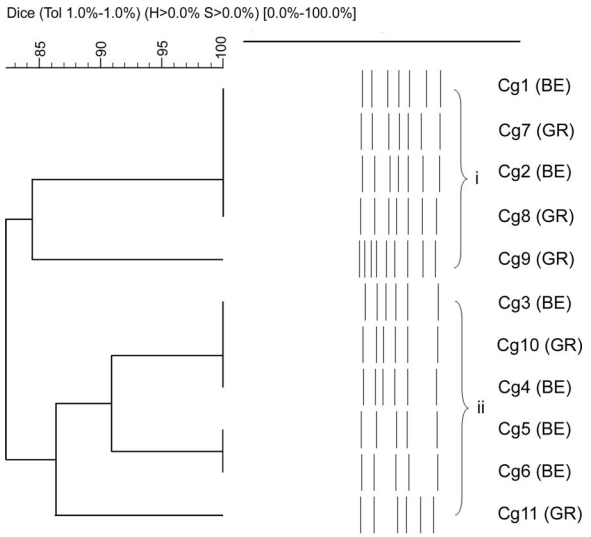

FIG. 2. *Candida glabrata* clusters. Groups were defined by 86% similarity. *C. glabrata* (Cg) isolates 3, 4, 5, 6, 10, and 11, with fluconazole resistance, clustered in group ii.

Each strain was tested on five independent occasions to ensure the reproducibility of the results. Cluster analysis was performed using Bionumerics version 4 (Bio-Maths, Kortrijk, Belgium; analysis done at the National Centre for Meningococcal Disease, Athens School of Public Health, Athens, Greece) and the Dice coefficient of similarity and cluster analysis with the unweighted-pair group method with arithmetic averages, with 1.00% position tolerance and no optimization, to obtain the greatest variation in similarity.

Discrete non-nosocomial and epidemiologically unrelated *C. albicans* subpopulations in the two European regions were identified (Fig. 1). Despite a microsatellite fingerprinting inference to the contrary (5, 20), our minisatellite typing did not associate fluconazole-resistant *C. albicans* isolates with any particular cluster. Similarly, multilocus sequence typing (MLST) did not significantly connect isolates with specific azole susceptibility profiles to particular clades (26). At a global level, MLST analysis of *C. albicans* isolates with different geographical and anatomical origins has shown clades with geographical enrichment (32, 33). Also, microsatellite analysis has even separated German from Austrian *C. albicans* clades in Central Europe (14), though with no reference to the ethnic origin of the population studied. Our minisatellite assay assembled all strains from Greek Caucasians in a single group (Fig. 1), but irrespective of the geographic origin of the patients, fluconazole-resistant *C. glabrata* isolates grouped in a single cluster (Fig. 2). An association of fluconazole-resistant strains with specific clades has been also shown by MLST analysis (9). M13 typing is not equivalent to MLST, as each method assays different elements of the genome. However, the acute discrimination of the fluconazole-resistant *C. glabrata* subpopulation among only 11 isolates adds confidence that M13 typing may be dependably used in discriminating *C. glabrata* fluconazole-resistant strains. Notably, *C. albicans* and *C. glabrata* isolates from pregnant, nonpregnant, and diabetic women did not associate with specific clusters.

This study showed excellent agreement between the EUCAST and CLSI methods (97%) in testing fenticonazole against *C. albicans* and *C. glabrata* from patients with uncomplicated VVC and limited *C. albicans* fluconazole resistance. Comparative multilocus typing by PCR fingerprinting has clustered fluconazole-resistant *C. glabrata* isolates in a separate group irrespective of their geographic origins, whereas *C. albicans* isolates clustered in geographically distinct groups with no susceptibility associations. The possibility that marker choice (16) and sample size influence the *C. albicans* geographic distinction patterns cannot be excluded. However, assuming that there are no deviations from the Hardy-Weinberg principle, the observed clustering of VVC strains from Greek Caucasian patients may reflect an ad hoc geographically restricted event that nonetheless requires further investigation.

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