Multilaboratory Study of Epidemiological Cutoff Values for Detection of Resistance in Eight Candida Species to Fluconazole, Posaconazole, and Voriconazole


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Although epidemiological cutoff values (ECVs) have been established for Candida spp. and the triazoles, they are based on MIC data from a single laboratory. We have established ECVs for eight Candida species and fluconazole, posaconazole, and voriconazole based on wild-type (WT) MIC distributions for isolates of C. albicans (n = 11,241 isolates), C. glabrata (7,538), C. parapsilosis (6,023), C. tropicalis (3,748), C. krusei (1,073), C. lusitaniae (574), C. guilliermondii (373), and C. dubliniensis (162). The 24-h CLSI broth microdilution MICs were collated from multiple laboratories (in Canada, Brazil, Europe, Mexico, Peru, and the United States). The ECVs for distributions originating from ≥6 laboratories, which included ≥95% of the modeled WT population, for fluconazole, posaconazole, and voriconazole were, respectively, 0.5, 0.06 and 0.03 μg/ml for C. albicans, 0.5, 0.25, and 0.03 μg/ml for C. dubliniensis, 8, 1, and 0.25 μg/ml for C. glabrata, 8, 0.5, and 0.12 μg/ml for C. guilliermondii, 32, 0.5, and 0.25 μg/ml for C. krusei, 1, 0.06, and 0.06 μg/ml for C. lusitaniae, 1, 0.25, and 0.03 μg/ml for C. parapsilosis, and 1, 0.12, and 0.06 μg/ml for C. tropicalis. The low number of MICs (<100) for other less prevalent species (C. famata, C. kefyr, C. orthopsilosis, C. rugosa) precluded ECV definition, but their MIC distributions are documented. Evaluation of our ECVs for some species/agent combinations using published individual MICs for 136 isolates (harboring mutations in or upregulation of ERG11, MDR1, CDR1, or CDR2) and 64 WT isolates indicated that our ECVs may be useful in distinguishing WT from non-WT isolates.

Severe candidal infections are seen worldwide among immuno-compromised hosts and nonimmunocompromised patients. Irrespective of the species, these infections are associated with high mortality and morbidity rates (1, 2). In addition to the different amphotericin B formulations, the triazoles are recommended as primary (fluconazole and voriconazole) and prophylactic (fluconazole and posaconazole) treatments for invasive infections caused by Candida spp. (3, 4). The azoles block the pathway of ergosterol biosynthesis by inhibiting the 14-α-lanosterol demethylase enzyme. The wide use of fluconazole and other triazoles has led to in vitro resistance among Candida and other fungal isolates to fluconazole and, to a lesser extent, the newer triazoles, voriconazole and posaconazole (5). Various molecular mechanisms are associated with in vitro resistance to triazoles among Candida spp., such as (i) modifications in the quality or quantity of the target enzyme, reduced access of the drug to the target, mutations in the ERG genes participating in ergosterol biosynthesis, or a combination of these mechanisms, and (ii) active efflux of azole out of the cell through the activation of multidrug efflux transporters encoded by the MDR and CDR genes (6–12). The Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Tests has adjusted the breakpoints (BPs) for fluconazole and voriconazole to be species specific (13). A recent study defined triazole epidemiological cutoff values (ECVs) (12) based on data from a single laboratory for the triazoles and several species of Candida; however, BPs are not available for posaconazole and any fungal species or for the less prevalent species and fluconazole and voriconazole. The ECV, defined as the highest susceptibility endpoint of the wild-type (WT) population MIC, has been shown to detect the emergence of in vitro resistance or to separate WT isolates (without known mechanisms of resistance) from non-WT isolates (with mechanisms of resistance and reduced susceptibilities to the agent being evaluated) (12, 14–16). The data from multiple laboratories used to define ECVs in the present study should be more representative of the susceptibilities of these species to the triazoles evaluated.

The purpose of this study was (i) to define the wild-type susceptibility endpoint distributions of fluconazole, posaconazole, and voriconazole for 5 common and 3 less common Candida spp.
originating from ≥6 laboratories and (ii) to propose ECVs for these 3 triazoles using the 24-h CLSI broth microdilution method (17). We aggregated MICs obtained in 15 independent laboratories (29 to 11,241, species and agent dependent). MIC distributions for other less prevalent Candida species (C. famata, C. kefyr, C. orthopsilosis, C. rugosa) also are documented. In addition, since our isolates have not been assessed for mechanisms of resistance, we evaluated our ECVs using available studies where MICs for individual isolates, determined using broth microdilution methods, and the presence or absence of mechanisms of resistance were reported for some of the species included in the present study (6–8, 10, 18–26).

MATERIALS AND METHODS

Isolates. Each isolate originated from a unique clinical specimen from 1 of 18 independent laboratories. In the present study, the MICs of the three triazoles used for ECV definition were obtained at the following medical centers: VCU Medical Center, Richmond, VA; Instituto de Medicina Tropical Alexander Von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru; Unidad de Microbiologia Experimental, Hospital Universitario La Fe, Valencia, Spain; University of Texas Health Science Center, San Antonio, TX; The University of Alberta, Edmonton, Alberta, Canada; Universidad Nacional Autónoma de México, Mexico; The Innsbruck Medical University, Innsbruck, Austria; Centers for Disease Control and Prevention, Atlanta, GA; Hospital Universitario de Valme, Seville, Spain; Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, Netherlands; The Adolfo Lutz Institute, São Paulo City, Brazil; University of Texas Health Science Center, Houston, TX; Hospital General Universitario Gregorio Marañón, Faculty of Medicine, Universidad Complutense, Madrid, Spain; University of Iowa, Iowa City, IA; Mycology Department, Adolfo Lutz Institute, São Paulo City, Brazil; Institut National de Santé Publique du Québec, Laboratoire de Santé Publique du Québec, Quebec, Canada; Adolfo Lutz Institute, Araçatuba City, Brazil; Facultat de Medicina, IISPV, URV, Reus, Spain. These laboratories were coded 1 to 20 (for several studies), but because some laboratories were excluded from the study or did not provide triazole MIC data for some species, we used data from the remaining 15 laboratories. Species were identified and stored at each medical center using standard and molecular methodologies (27), and isolates were not evaluated for azole resistance mechanisms.

We aggregated the available 24-h CLSI MIC data of each agent for 11,241 C. albicans, 162 C. dublinensis, 7,538 C. glabrata, 373 C. guilliermondii, 1,073 C. krusei, 574 C. lusitaniae, 6,023 C. parapsilosis, and 3,748 C. tropicalis isolating from 6 to 15 different laboratories and for four other less prevalent species (49 C. famata, 36 C. kefyr, 68 C. orthopsilosis, and 76 C. rugosa isolates) from 3 to 9 different laboratories (Tables 1 to 3). One or both quality control (QC) isolates (C. parapsilosis ATCC 22019 and C. krusei ATCC 6258) were used by the participating laboratories (13, 17).

In addition, we included triazole MIC distributions from previously published studies (5 species, 73 to 200 isolates, 20 to 64 WT MICs and 53 to 136 non-WT MICs [agent dependent]), all tested for the presence (non-WT) or absence (WT) of either intrinsic or acquired azole resistance mechanisms (e.g., substitutions and missense mutations in or upregulation of ERG11, MDR1, CDRI, and CDR2), in order to assess the ability of the various fluconazole, posaconazole, and voriconazole ECVs to discriminate non-WT from WT strains of Candida spp. at the molecular level (6–8, 10, 18–26).

Antifungal susceptibility testing. The MICs were obtained at each center by following the CLSI M27-A3 broth microdilution method (standard RPMI 1640 broth [0.2% dextrose], final inoculum concentrations that ranged from 0.5 × 10^3 to 5 × 10^4 CFU/ml, and 24 h of incubation); MICs were the lowest drug concentrations that produced ≥50% growth inhibition compared to the growth control (17). MIC data for the two QC reference strains, utilized during the years of testing in each center, were obtained each time that a set of isolates was tested following the CLSI M27-A3 broth microdilution method (13, 17). The majority of MIC ranges (98 to 100%) for the two QC strains were within the CLSI established reference range in each laboratory that had data included in the analyses; a certain degree of interlaboratory modal variability (mostly ±2-fold dilution) was observed.

Definitions. The ECV (also known as the wild-type cutoff, or CO_WT) definition and the definitions of the two populations (WT and non-WT MIC populations or isolates) that will be discussed have been provided above (12, 14–16, 28). Briefly, a non-WT organism shows reduced susceptibility to the agent being evaluated compared to the WT (without resistant mechanisms) population, but it may or may not respond to treatment with the drug being evaluated. ECVs are calculated by taking into account the MIC distribution, the modal MIC of each distribution, and the inherent variability of the test (usually within one doubling dilution) and should encompass ≥95% of isolates (28).

Data analysis. As previously described (28–32), the MIC distribution of each species obtained in each coded laboratory (numbered 1 to 20) was listed in an Excel spreadsheet and screened for (i) grossly skewed distributions that precluded statistical fitting (distributions that had a modal MIC [most frequent value] at the lowest or highest concentration tested and/or which were bimodal in the presumptive wild-type distribution),
(ii) distribution size (data from ≥3 laboratories and the total pooled distribution had ≥100 isolates), and (iii) unusual modal variation (modes that were ≥2-fold dilutions from the others). Skewed distributions were removed from each pooled distribution of each species/agent used for the analysis (28, 31). The resulting screened and pooled MIC distributions were used to calculate the ECVs by the statistical method where the modeled population is based on fitting a normal distribution at the lower end of the MIC range, calculating the mean and standard deviation of that normal distribution, and using those parameters to calculate the MIC that captures at least 95%, 97.5%, and 99% of the modeled WT population (28).

RESULTS AND DISCUSSION

The ultimate goal of susceptibility testing is to predict with some reliability the clinical outcome when an infected patient is treated with the specific agent evaluated. The endpoint that categorizes an MIC as susceptible or resistant is the BP (14–16). However, in the fungal world, there are many species and agent combinations for which BPs have not been proposed. The reason for that is the lack of sufficient data correlating clinical outcomes and which BPs have not been proposed. The reason for that is the lack of sufficient data correlating clinical outcomes and in vitro results used to establish BPs. That is the case for posaconazole and Candida spp., the other triazoles for some of the less prevalent Candida species, and for C. glabrata versus voriconazole (13). Although the ECV is not a BP, ECVs serve as an early indication of emerging changes in the patterns of susceptibility of organisms to the agent being evaluated. Species-specific ECVs have been previously defined based on MIC data from a single laboratory (12), which may not completely represent the WT MIC population for each species of Candida and each of the three triazoles evaluated in the present study. Because of this, we used data from multiple laboratories to define ECVs for fluconazole, posaconazole, and voriconazole, for the five most prevalent Candida spp., and for C. dubliniensis, C. guilliermondii, and C. lusitaniae (Tables 1 to 4). In addition, MIC distributions for another four less prevalent species are provided (Tables 1 to 3).

Eighteen laboratories submitted MIC data for the present study. MICs from between 1 and 7 laboratories, depending on the species and antifungal agent, were not included in the final analysis due to truncated distributions (modal MIC at the lowest concentration tested). All of the MIC distributions were typical for WT organisms and covered 3 to 5 2-fold dilution steps surrounding the modal MIC. The remaining aggregated MIC distributions for the three triazoles that originated in 3 to 15 laboratories are

### TABLE 2 Pooled MIC distributions of posaconazole for 12 Candida species

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of labs</th>
<th>No. of isolates</th>
<th>No. of isolates for which the MIC (μg/ml) was:&lt;sup&gt;b&lt;/sup&gt;</th>
<th>0.008</th>
<th>0.016</th>
<th>0.03</th>
<th>0.06</th>
<th>0.12</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>≥8</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>9</td>
<td>3,210</td>
<td>105, 1,768, 670, 291, 140, 114, 84, 21, 11, 2, 4</td>
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<tr>
<td>C. dubliniensis</td>
<td>7</td>
<td>152</td>
<td>52, 86, 7, 3, 2, 1</td>
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<tr>
<td>C. glabrata</td>
<td>11</td>
<td>4,176</td>
<td>13, 208, 556, 1,476, 983, 304, 166, 162, 172, 112, 24</td>
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<tr>
<td>C. guilliermondii</td>
<td>12</td>
<td>369</td>
<td>3, 42, 126, 119, 33, 20, 12, 8, 1, 1</td>
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<td>C. krusei</td>
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<td>930</td>
<td>1, 8, 36, 136, 476, 207, 53, 12</td>
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<tr>
<td>C. lusitaniae</td>
<td>8</td>
<td>142</td>
<td>23, 61, 51, 6</td>
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<tr>
<td>C. parapsilosis</td>
<td>8</td>
<td>2,337</td>
<td>188, 986, 547, 334, 119, 80, 46, 26, 11</td>
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<tr>
<td>C. tropicalis</td>
<td>8</td>
<td>3,127</td>
<td>547, 912, 893, 441, 155, 69, 43, 31, 21, 8, 7</td>
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<tr>
<td>C. famata</td>
<td>9</td>
<td>53</td>
<td>6, 10, 23, 10, 3, 1</td>
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<tr>
<td>C. kefyr</td>
<td>7</td>
<td>34</td>
<td>4, 17, 11, 1, 1</td>
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<tr>
<td>C. orthopsilosis</td>
<td>4</td>
<td>66</td>
<td>9, 30, 17, 9, 1</td>
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<tr>
<td>C. rugosa</td>
<td>9</td>
<td>39</td>
<td>1, 6, 34, 12, 3, 3</td>
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</table>

<sup>a</sup> Number of laboratories contributing data to each MIC distribution. 
<sup>b</sup> MICs determined at 24 h as described in the CLSI M27-A3 reference method (17). The modal MIC (most frequent value) for each distribution is underlined. 
<sup>c</sup> Data from the other 2 to 4 labs were not used due to abnormal MIC distributions (the mode and lowest concentration tested were the same).
shown in Tables 1 to 3. Overall, the distributions were quite normal; similar-sized “bars” were observed with some of the agent and species combinations (e.g., at fluconazole MICs of 0.12 and 0.25 µg/ml for C. albicans and 0.12 and 0.25 µg/ml for C. dubliniensis), which indicated that the mode lies between those two concentrations. The fluconazole modal MICs ranged from 0.12 to 16 µg/ml; the lower value was for C. albicans, and the highest was for C. krusei (Tables 1 and 4). The lowest posaconazole modes were for C. albicans and C. lusitaniae (0.008 µg/ml), and the highest were for C. glabrata and C. krusei (0.25 µg/ml). Overall, the voriconazole modes were lower (0.016 to 0.03 µg/ml) than those of the other two azoles for most of the species, with the exception of C. glabrata and C. krusei (voriconazole modes, 0.06 and 0.12 µg/ml, respectively). Our MIC distributions are similar to those observed by other authors (33–37). Previously reported azole MICs for C. dubliniensis (fluconazole MIC₉₀ range, 8 to 32 µg/ml) and C. guilliermondii (fluconazole MIC₉₀ range, 16 to 32 µg/ml; voriconazole MIC₉₀ range, 4 to 8 µg/ml) were higher than those observed in the present study (Tables 1 to 3).

The triazole ECVs based on the aggregated MIC distributions of 8 of the 12 Candida spp. evaluated are shown in Table 4. Although the MIC distributions are provided for four less prevalent species (C. famata, C. kefyr, C. orthopsilosis, and C. rugosa), their ECVs were not calculated because the current criterion for ECV definition requires that the total pooled distribution have ≥100 isolates from ≥3 laboratories. The ECVs were defined using ≥95%, ≥97.5%, and ≥99% of the modeled MIC populations; we focused on the more conservative values (lower ECVs encompassing ≥95% of the modeled population). This decision was corroborated by the genetic information discussed below for the four more prevalent species and C. dubliniensis, although the ECVs encompassing ≥97% of the population were similar to those at 95%. ECVs defined using CLSI MIC data from a single laboratory are the same or 1 to 2 dilutions higher than those in the present study (12); in contrast, ECVs using YeastOne MIC data are mostly higher (38). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has cutoff values for Candida and the azoles, and it has established ECVs (ECOFFs) for fluconazole, posaconazole, and voriconazole, respectively, of 1, 0.06, and 0.125 µg/ml for C. albicans, of 32, 1, and 1 µg/ml for C. glabrata, of 128, 0.5, and 1 µg/ml for C. krusei, and of 2, 0.06, and 0.12 µg/ml for C. parapsilosis and C. tropicalis (see http://www.EUCAST.org). For the most part, with the exception of C. glabrata and fluconazole, these values are comparable to those reported herein.

The ability of the ECVs encompassing 95% of the statistically modeled population to differentiate strains of Candida spp. with intrinsic or acquiredazole resistance mechanisms (e.g., substitutions and missense mutations in or upregulation of ERG11, MDR1, CDRI, or CDR2) may be seen in the data presented in Table 5. The isolates in the collection depicted in Table 5 were compiled from 13 previously published studies to represent WT and non-WT MIC results for fluconazole, posaconazole, and vori-
TABLE 5 Application of ECVs to MIC distributions of fluconazole, posaconazole, and voriconazole versus Candida species strains tested for the presence of azole resistance mutations by broth microdilution methods*

<table>
<thead>
<tr>
<th>Species</th>
<th>Antifungal agent (no. of isolates tested)</th>
<th>ECV (µg/ml)</th>
<th>No. of isolates by ECV category (no. with acquired resistance mutation[s])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤ECV</td>
<td>&gt;ECV</td>
</tr>
<tr>
<td></td>
<td>Fluconazole (65)</td>
<td>0.5</td>
<td>18 47 (47)</td>
</tr>
<tr>
<td></td>
<td>Posaconazole (65)</td>
<td>0.06</td>
<td>18 (5) 42 (42)</td>
</tr>
<tr>
<td></td>
<td>Voriconazole (65)</td>
<td>0.03</td>
<td>18 (3) 44 (44)</td>
</tr>
<tr>
<td></td>
<td>Fluconazole (38)</td>
<td>0.5</td>
<td>12 26 (26)</td>
</tr>
<tr>
<td></td>
<td>Posaconazole (4)</td>
<td>0.25</td>
<td>1 (2) 1 (1)</td>
</tr>
<tr>
<td></td>
<td>Voriconazole (4)</td>
<td>0.03</td>
<td>4 (3)</td>
</tr>
<tr>
<td></td>
<td>Fluconazole (89)</td>
<td>8</td>
<td>29 60 (57)</td>
</tr>
<tr>
<td></td>
<td>Voriconazole (49)</td>
<td>0.25</td>
<td>12 (7) 30 (30)</td>
</tr>
<tr>
<td></td>
<td>Fluconazole (4)</td>
<td>1</td>
<td>1 3 (3)</td>
</tr>
<tr>
<td></td>
<td>Posaconazole (4)</td>
<td>0.25</td>
<td>1 3 (3)</td>
</tr>
<tr>
<td></td>
<td>Voriconazole (4)</td>
<td>0.03</td>
<td>1 3 (3)</td>
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<tr>
<td></td>
<td>Fluconazole (4)</td>
<td>1</td>
<td>1 3 (3)</td>
</tr>
<tr>
<td></td>
<td>Voriconazole (4)</td>
<td>0.06</td>
<td>1 3 (3)</td>
</tr>
</tbody>
</table>

* Data were compiled from references 6–8, 10, and 18–26. Azole resistance mechanisms included mutations in and overexpression of ERG11 and/or overexpression of MDR or CDR efflux pumps.

conazole, and all the isolates were characterized regarding the presence (non-WT) or absence (WT) of azole resistance mechanisms (6–8, 10, 18–26). A total of 136 isolates harbored molecularly defined azole resistance mechanisms: 47 C. albicans, 26 C. dubliniensis, and 57 C. glabrata isolates and 3 isolates each of C. parapsilosis and C. tropicalis (Table 5). The ECVs for fluconazole and C. albicans, C. dubliniensis, C. glabrata, C. parapsilosis, and C. tropicalis were 0.5 µg/ml, 0.5 µg/ml, 8 µg/ml, 1 µg/ml, and 1 µg/ml, respectively (Table 4). Using these fluconazole ECVs, the CLSI method correctly classified all (100%) of the 136 strains with resistance mutations/mechanisms as non-WT (MIC > ECV) and 61 (95.3%) of the 64 strains with non-demonstrated resistance mutations/mechanisms as WT strains. The ECVs for posaconazole and C. albicans, C. dubliniensis, and C. parapsilosis were 0.06 µg/ml, 0.25 µg/ml, and 0.25 µg/ml, respectively (Table 4). Using these ECVs, the CLSI method with posaconazole correctly classified 46 (86.8%) of 53 strains with resistance mutations/mechanisms as non-WT and all 20 (100.0%) WT strains (MIC < ECV). Although a total of 7 isolates (5 of C. albicans and 2 of C. dubliniensis) with molecularly defined resistance mechanisms were classified as WT for posaconazole, this may be explained by the fact that certain azole resistance mechanisms (e.g., substitutions and missense mutations in or upregulation of ERG11 or MDR1) may affect fluconazole to a greater extent than other azoles (6, 18, 19); all 7 of these isolates were non-WT for fluconazole. The ECVs for voriconazole and C. albicans, C. dubliniensis, and C. parapsilosis were all 0.03 µg/ml, and those for C. glabrata and C. tropicalis were 0.25 µg/ml and 0.06 µg/ml, respectively (Table 4). Using these ECVs, the CLSI method when used for voriconazole correctly classified 83 (89.2%) of 93 strains with resistance mutations/mechanisms as non-WT and 32 (97%) of the 33 WT strains. As with posaconazole, all 10 of the strains with molecularly defined resistance mutations/mechanisms that were classified as WT for voriconazole were non-WT for fluconazole, reflecting more fluconazole-specific resistance mechanisms. These results support the ability of the triazole ECVs to differentiate WT strains of Candida spp. from those harboring clinically important resistance mechanisms; additional data for C. parapsilosis and C. tropicalis are needed.

ECVs for C. krusei and the less prevalent species C. guilliermondii and C. lusitaniae are also depicted in Table 4. Since MIC distributions encompassed less than 100 values, ECVs were not defined for the other four less prevalent species (C. famata, C. kefyr, C. orthopsilosis, and C. rugosa), but their MIC distributions (Tables 1 to 3) are provided so that they may serve as a reference for other studies using the CLSI method. As expected, the fluconazole ECV for C. krusei was higher (32 µg/ml) than those for C. lusitaniae and C. guilliermondii (1 and 8 µg/ml, respectively). ECVs for C. guilliermondii, C. krusei, and C. lusitaniae and the other two triazoles were 0.5, 0.5, and 0.06 µg/ml (for posaconazole) and 0.12, 0.25, and 0.06 µg/ml (for voriconazole), respectively. To our knowledge, information regarding mechanisms of resistance is only available for C. krusei, and resistance to fluconazole has been postulated to be due to either a decreased sensitivity of the target enzyme or target mutations of the efflux pumps (11, 39, 40). More recently, only a 2-fold decrease in the fluconazole MIC (32 to 8 µg/ml) was observed in 1 of the 21 isolates evaluated (fluconazole MICs ≥ 16 µg/ml) by using the efflux pump inhibitor carbonyl cyanide 3-chloro-phenylhydrazone; no changes were observed among the voriconazole MICs (range, 0.06 to 0.25 µg/ml) (37), and hence the results are inconclusive regarding the efflux pumps. Due to the innate resistance of C. krusei to fluconazole, the CLSI does not recommend the interpretation of MICs for this species and agent (13), a recommendation that should be extended to the ECV. The CLSI and EUCAST susceptibility BP for C. krusei and voriconazole is ≤0.5 µg/ml (versus our ECV of 0.25 µg/ml); the EUCAST posaconazole ECV for C. guilliermondii is 0.25 µg/ml (versus our ECV of 0.5 µg/ml) (Table 4) (13, 29, 41).

In conclusion, we have defined ECVs for 8 of the 12 Candida spp. evaluated and the three triazoles (fluconazole ECVs ranged from 0.5 µg/ml for C. albicans and C. dubliniensis to 32 µg/ml for C. krusei, posaconazole ECVs ranged from 0.06 µg/ml for C. albicans and C. lusitaniae to 1 µg/ml for C. glabrata, and voriconazole ECVs ranged from 0.03 µg/ml for C. albicans, C. dubliniensis, and C. parapsilosis to 0.25 µg/ml for C. glabrata and C. krusei). These ECVs encompass 95% of the statistically modeled population and will serve to differentiate WT from non-WT strains of Candida for the three systemically active triazoles. We have demonstrated the ability of the species-specific ECVs for all three triazoles to identify those strains of Candida spp. harboring azole resistance mechanisms in a population of 200 well-characterized Candida species. The ECVs for fluconazole, posaconazole, and voriconazole and the CLSI broth microdilution method will help in monitoring the emergence of azole resistance among target species of Candida.

**ACKNOWLEDGMENTS**

L. Ostrosky-Zeichner has received research grants from and is a consultant and/or speaker for Pfizer, Merck, and Astellas. The findings and conclusions of this article are those of the authors

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