Breakpoints for Susceptibility Testing Should Not Divide Wild-Type Distributions of Important Target Species

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The fluconazole MIC distributions for Candida glabrata from testing 34 different clinical isolates and performing 51 tests on a single isolate mirrored each other. Since what is perceived as biological variation in isolates without resistance mechanisms is mainly methodological variation, breakpoints which divide this distribution not only lack a sound biological basis but also result in poor reproducibility of susceptibility characterization. This makes 2, 4, 8, and possibly 16 μg/ml unsuitable breakpoints for C. glabrata and fluconazole.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) adopted the basic tenet that breakpoints for susceptibility testing should not divide wild-type distributions for target microorganisms. This is to ensure that all individuals of a species that lack mechanisms of resistance to the drug in question are uniformly categorized as either susceptible, intermediate, or resistant and to ensure the highest possible reproducibility of test results (1). This principle was adopted throughout the soon-finalized process of harmonizing European breakpoints for antibacterial drugs.

The EUCAST subcommittee on antifungal susceptibility testing has collated the fluconazole MICs for 26,447 strains of Candida species (3) and proposed a clinical breakpoint of ≤2 μg/ml for C. albicans, C. parapsilosis, and C. tropicalis. The EUCAST subcommittee on antifungal susceptibility testing has refrained from assigning breakpoints for fluconazole to C. krusei and C. glabrata. C. krusei exhibits high MICs, and this species is considered to be inherently resistant, whereas for C. glabrata the median MIC was 8 μg/ml and the range was 1 to 128 μg/ml with the majority of MICs being 4 to 16 μg/ml (3). In routine susceptibility testing MICs of 2 μg/ml are occasionally encountered. In order to investigate whether these isolates were truly more susceptible to fluconazole than were isolates with other MICs belonging to what we perceive as the wild-type distribution, we performed repeat testing (51 tests on separate occasions over 3 months) on a single clinical isolate of C. glabrata (SSI-M68190-2007) with a MIC of 2 μg/ml using the method recommended in the EUCAST definitive document EDef 7.1 (4). MICs were compared with those of 34 individual and unselected consecutive C. glabrata isolates received for routine testing in the same time period. Fluconazole as a pure substance was purchased from Sigma-Aldrich, Brøndby, Denmark (F8929 fluconazole, ≥98% pure [high-pressure liquid chromatography]) and dissolved in dimethyl sulfoxide (15438; Sigma-Aldrich, Brøndby, Denmark) according to the manufacturer’s instructions. A single lot of microtitration plates was prepared and stored at −80°C until use. Following incubation, the wells were read using a spectrophotometer and a wavelength of 490 nm. The MIC was defined as the lowest drug dilution yielding a 50% reduction in growth compared with the control growth well. C. parapsilosis ATCC 22019 was included as a control twice in the initial test of the batch and subsequently once weekly. All results were within the acceptable range (0.5 to 2 μg/ml) (4).

The results are shown in Fig. 1. The MICs for 31 of the 34 (91.2%) consecutive isolates and the 51 MICs obtained from repeated testing of C. glabrata SSI-M68190-2007 all ranged between 2 and 8 μg/ml (geometric mean, 4.1 μg/ml for the consecutive isolates and 3.6 for the repeat tests). Three of the total of 85 results were outside the 2- to 8-μg/ml range—all three with higher MICs (16, 16, and 32 μg/ml). Of these, two could belong to the same population as those with MICs of 2 to 8 μg/ml and could represent technical variation outside 2 standard deviations, or one or both could represent isolates with fluconazole resistance mechanisms.

Both CLSI and EUCAST standards allow a 3-log two-step range of MICs corresponding to the best achievable precision of all hitherto employed MIC testing procedures for a carefully selected reference control strain (2, 4). In agreement with this, repeated testing of a single clinical C. glabrata isolate using a single lot of microtitration plates generated a “Gaussian” MIC distribution spanning three dilution steps. This was exactly mirrored by the distribution of MICs for the clinical C. glabrata isolates. There are a number of conclusions that can be drawn from this. The methodological variation obscures any biological variation in these distributions, and the biological variation among strains lacking resistance mechanisms is too small to be detected with ordinary MIC testing and in all probability too small to be clinically utilizable. What is perceived as the wild-
type distribution of a species against an antimicrobial should not be artificially split by breakpoints; the whole distribution should be uniformly categorized as susceptible, intermediate, or resistant. Splitting the wild-type distribution will result in a random susceptible, intermediate (or susceptible-dose-dependent), and resistant categorization of isolates with identical susceptibilities. This is futile and confusing and will undermine the credibility of the susceptibility testing. For fluconazole and C. glabrata, this rules out 2, 4, and 8 μg/ml as breakpoints, any of which would perhaps otherwise have been acceptable. However, the three-dilution range of the wild-type distribution or of the repeat testing of a single isolate can be achieved only under ideal conditions. When taking into account results from several batches of media, several technicians, and many laboratories and other necessarily allowed variations in testing conditions, the base of the perceived wild-type distribution broadens to four to a maximum of five dilution steps. In the EUCAST MIC distribution displays (http://217.70.33.99/Eucast2/SearchController/regShow.jsp?Id=3282), the C. glabrata fluconazole distribution spans 2 to 32 μg/ml (3).

To achieve robust and clinically meaningful breakpoints, it is sometimes necessary to set species-specific breakpoints. This is accepted by both EUCAST and CLSI with antibacterial breakpoints and should be acceptable for antifungal breakpoints. It requires that laboratories can identify microorganisms to the correct species and genus level. For each antifungal drug, a species-related evaluation of clinical results, of pharmacokinetics/pharmacodynamics, and of MIC distributions will determine which susceptibility category is appropriate for isolates in the wild-type range.

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