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Review

The role of epidemiological cutoff values (ECVs/ECOFFs) in antifungal susceptibility testing and interpretation for uncommon yeasts and moulds

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ABSTRACT

The role of antimicrobial susceptibility testing is to aid in selecting the best agent for the treatment of bacterial and fungal diseases. This has been best achieved by the setting of breakpoints by Clinical Laboratory Standards Institute (CLSI) for prevalent *Candida* spp. versus anidulafungin, caspofungin, micafungin, fluconazole, and voriconazole. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) also has set breakpoints for prevalent and common *Candida* and *Aspergillus* species versus amphotericin B, itraconazole, and posaconazole. Recently, another interpretive category, the epidemiological cut off value, could aid in the early identification of strains with acquired resistance mechanisms. CLSI has postulated that epidemiological cut off values may, with due caution, aid physicians in managing mycosis by species where breakpoints are not available. This review provides (1) the criteria and statistical approach to establishing and estimating epidemiological cut off values (ECVs), (2) the role of the epidemiological cut off value in establishing breakpoints, (3) the potential role of epidemiological cut off values in clinical practice, (4) and the wide range of CLSI-based epidemiological cut off values reported in the literature as well as EUCAST and Sensititre Yeast One-ECVs. Additionally, we provide MIC/MEC (minimal inhibitory concentrations/minimum effective concentrations) ranges/modes of each pooled distribution used for epidemiological cut off value calculation. We focus on the epidemiological cut off value, the new interpretive endpoint that will identify the non-wild type strains (defined as potentially harboring resistance mechanisms). However, we emphasize that epidemiological cut off values will not categorize a fungal isolate as susceptible or resistant as breakpoints do, because the former do not account for the pharmacology of the antifungal agent or the findings from clinical outcome studies.

Utilidad clínica de los puntos de corte epidemiológicos (ECVs/ECOFFs) para interpretar los datos de la sensibilidad antifúngica de los mohos y levaduras de poca prevalencia

Palabras clave:
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RESUMEN

Las pruebas de sensibilidad a los antimicrobianos tienen como finalidad ayudar en la selección del mejor fármaco para el tratamiento de las infecciones fúngicas y bacterianas. El establecimiento de puntos de corte para la anidulafungina, la caspofungina, la micafungina, el fluconazol y el voriconazol en las especies de *Candida* más prevalentes por parte del Clinical and Laboratory Standards Institute (CLSI) permite alcanzar este objetivo. El European Committee on Antimicrobial Susceptibility Testing (EUCAST) también ha establecido puntos de corte para la anfotericina B, el itraconazol y el posaconazol en las especies más comunes de *Candida* y *Aspergillus*. En los últimos tiempos se ha propuesto una nueva categoría, la de los puntos de corte epidemiológicos, que puede ayudar a identificar de manera temprana los aislamientos que han adquirido mecanismos de resistencia. Según el CLSI

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los puntos de corte epidemiológicos podrían, con la debida cautela, ayudar a los médicos en la selección del tratamiento en aquellas micosis causadas por especies para las que no se han establecido puntos de corte. Esta revisión repasa: 1) los criterios y la aproximación estadística seguida para establecer y estimar los puntos de corte epidemiológicos, 2) el papel de los puntos de corte epidemiológicos para establecer los puntos de corte, 3) el papel de los puntos de corte epidemiológicos en la práctica clínica, y 4) el amplio rango de puntos de corte epidemiológicos que aparecen en la literatura establecidos mediante los métodos del CLSI, EUCAST o Sensititre® YeastOne®. Se muestran también los rangos de las concentraciones mínimas inhibitorias (CMI) y concentraciones mínimas efectivas (CME) utilizados para el cálculo de los puntos de corte epidemiológicos. Incidimos de manera especial sobre estos últimos por tratarse de una nueva interpretación de los CMI y los CME que permite identificar aquellos aislamientos que no son salvajes y potencialmente resistentes. No obstante, insistimos en que los puntos de corte epidemiológicos no pueden utilizarse para calificar como resistente o sensible a un determinado aislamiento, como lo hacen los puntos de corte, puesto que los puntos de corte epidemiológicos no se rigen por las características farmacológicas de los agentes antifúngicos ni por la evolución clínica de los pacientes.

Although most fungi associated with disease are considered opportunistic pathogens (in particular the yeasts), irrespective of the species, fungi cause a great deal of morbidity and mortality worldwide, especially among the increasing number of immunocompromised patients.[27,34] Such patients are at high risk for life-threatening mycoses. Despite the available antifungal agents (amphotericin B and its lipid formulations, the triazoles and the echinocandins), the mortality rates and emergence of resistance have increased, especially among patients suffering invasive infections due to *Aspergillus* and other filamentous fungi (moulds) infections; e.g., the survival rates could be below 30% depending on the infecting isolate and the immunological status of the host in invasive fusariosis.[1,33] There is therefore a strong motivation to ensure that antifungal therapy is optimized, and consequently that susceptibility testing maximizes its capacity to predict outcome.

Advances have been made in understanding the molecular mechanisms of resistance in some *Candida* spp., *Cryptococcus* spp. and *Aspergillus* spp. and the triazoles as well as in *Candida* spp. with the echinocandins. For example, the emergence of triazole resistance among patients with aspergillosis has been reported where isolates with *CYP51A* gene mutations have reduced azole affinity or increased target quantity that confers azole resistance; this problem has increased significantly in some European countries.[47] However, the genetics of other important species (e.g., *Fusarium* spp. and the Mucorales) are yet to be investigated. Therefore, there is a need to identify these resistant isolates with reduced antifungal susceptibility.

Currently the best predictors of patient outcome with antifungal therapy are the formal breakpoints (BPs) established by the standards setting organizations, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). However, CLSI BPs are only available for some *Candida* spp. versus two triazoles and three echinocandins,[6] while EUCAST had established BPs for the prevalent *Candida* spp. and some common *Aspergillus* spp. for three other antifungal agents (amphotericin B, itraconazole, posaconazole). BPs by definition are used to indicate those isolates that are likely to respond to (susceptible) or fail (resistant) treatment with a given antimicrobial agent administered at the approved dosing regimen for that agent (http://www.eucast.org/clinical_breakpoints/).[5] They are established using a combination of in vitro, in vivo and clinical data, including the distribution of the minimal inhibitory concentrations (MICs) and/or minimal effective concentrations (MECs), in vitro, animal model pharmacokinetics/pharmacodynamics (PK/PD), and clinical/microbiological outcome data (EUCAST SOP 1.1 Setting breakpoints for new antimicrobial agents at http://www.eucast.org/documents/sops/).[5]

One of the first steps in establishing BPs is the determination of what constitutes wild-type (WT) strains, defined as strains without any phenotypically-expressed resistance mechanism(s). Rather than being a single value, MICs/MECs of WT strains follow a log-normally distribution. The upper end of that distribution is defined as the epidemiological cutoff value (ECV or ECOFF). Estimation of the ECV has the added benefit of acting as a sensitive indicator of the emergence of strains with reduced susceptibility to a given agent. Over the last few years, ECVs have been proposed for a variety of moulds and *Cryptococcus* spp. as well as for some of those species of *Candida* for which BPs have not been established. Recently, method-dependent ECVs for the commercial colorimetric Sensititre® YeastOne® assay (SYO) for *Candida* spp. have been estimated; these values have been integrated in the tables, given that the high degree of caspofungin MIC variability for *Candida* spp. has so far prevented reproducible testing of this agent by reference methodology.[14] The CLSI has described guidelines and listed CLSI ECVs as per CLSI criteria, while the EUCAST publishes visual ECVs on their MIC distribution website when the data are of sufficient quality (http://mic.eucast.org/Eucast2/).

This review presents information regarding both interpretive categories or enpoints that encompass: (1) steps needed for statistical ECV estimation and/or establishment as per CLSI criteria, (2) the role of the ECV in establishing BPs, (3) the role of ECVs on clinical practice versus the BP's fundamental role, (4) and the wide range of CLSI ECVs reported in the literature as well as EUCAST and SYO-ECVs. Other interpretive endpoints that have been proposed as either BPs or ECVs especially for *Aspergillus fumigatus* species complex (SC) in the last few years are briefly discussed.

Epidemiological cutoff value estimation

Basic requirements or criteria

In order to ensure that clean and comparable data are included in the estimation of ECVs, there are a number of conditions/criteria that must be fulfilled. These include conditions that apply to the data generated in the contributing laboratory, as well as methods applied to accepting and rejecting particular distributions before pooling for analysis. The pooling of data for the estimation of the ECV (See Figs. 1 and 2) is built on the assumption that the WT of an individual species does not vary over time or place (e.g., anywhere in the world). The following are widely accepted criteria for generating, reviewing, excluding and pooling the data required for the estimation of ECVs; many of which were recently described by Kahlmeter:[25]