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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Regression analysis and categorical agreement of fluconazole disk zone diameters and minimum inhibitory concentration by broth microdilution of clinical isolates of *Candida*



Analyse de régression et accord catégorique des diamètres de la zone des disques de fluconazole et de la concentration minimale inhibitrice par microdilution au bouillon d'isolats cliniques de Candida

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KEYWORDS

Candida;
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Disk diffusion;
Broth microdilution;
CLSI

Summary

Aim and objectives. – Rampant use of fluconazole in *Candida* infections has led to predominance of less susceptible non-*albicans* *Candida* over *Candida albicans*. The aim of the study was to determine if zone diameters around fluconazole disk can be used to estimate the minimum inhibitory concentration (MIC) for clinical isolates of *Candida* species and vice versa. Categorical agreement between the Clinical & Laboratory Standards Institute (CLSI) recommended disk diffusion and CLSI broth microdilution method was sought for.

Material and methods. – Antifungal susceptibility testing by disk diffusion and Broth microdilution was done as per CLSI document M44-S3 and CLSI document M27-S4 for *Candida* isolates respectively. Regression analysis correlating zone diameters to MIC value was done. Pearson's

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MOTS CLÉS

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La méthode de
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CLSI

correlation coefficient was calculated to determine correlation between disk zone diameters and MICs.

Results. – *Candida albicans* (33.3%) was clearly outnumbered by other non-*albicans* species predominantly *Candida tropicalis* (42.5%) and *Candida glabrata* (18.4%). Ten percent of the strains were resistant to fluconazole by disk diffusion and 13% by broth microdilution. MIC range for *Candida albicans* and *Candida tropicalis* ranged from ≤ 0.25 –64 $\mu\text{g/ml}$ while that of *Candida glabrata* ranged from ≤ 0.25 –128 $\mu\text{g/ml}$. Categorical agreement between disk diffusion and broth microdilution was 86.8%. Pearson's coefficient of correlation was -0.5975 indicating moderate negative correlation between the two variables.

Conclusion. – Zone sizes can be used to estimate the MIC values, although with limited accuracy. There should be a constant effort to upgrade the guidelines in view of new clinical data, and laboratories should make an active effort to incorporate them.

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Résumé

Le but et les objectifs. – L'utilisation éffrénée du fluconazole pour les infections à *Candida* a entraîné à la prédominance du non-*albicans* *Candida* moins sensibles sur *Candida albicans*. L'étude visait à déterminer si les diamètres de la zone autour de disque de fluconazole peuvent être utilisés à estimer la *minimum inhibitory concentration* (MIC) pour les isolats cliniques des espèces de *Candida* et vice versa. Un accord catégorique entre la méthode de diffusion de disque et de microdilution du bouillon recommandé par le Clinical & Laboratory Standards Institute (CLSI) était recherché.

Le matériel et les méthodes. – Test de sensibilité antifongique par la diffusion de disque et la microdilution du bouillon a été fait selon le document CLSI M44-S3 et le document CLSI M27-S4 pour *Candida* isolé, respectivement. L'analyse de régression corrélant les diamètres de zone à la valeur de MIC a été effectuée. Le coefficient de corrélation de Pearson a été calculé pour déterminer la corrélation entre des MIC et des diamètres de zone de disque.

Résultats. – *Candida albicans* (33,3 %) était clairement moins nombreux que les autres espèces non-*albicans*, principalement *Candida tropicalis* (42,5 %) et *Candida glabrata* (18,4 %). Dix pour cent des souches étaient résistantes au fluconazole par la diffusion de disque et 13 % par la microdilution du bouillon. La plage de MIC pour *Candida albicans* et *Candida tropicalis* varie de 0,25 à 64 g/mL tandis que celle de *Candida glabrata* varie de 0,25 à 128 g/mL. Un accord catégorique entre la diffusion de disque et la microdilution du bouillon était 86,8 %. Le coefficient de corrélation de Pearson était $-0,5975$ indiquant une corrélation négative modérée entre les deux variables.

Conclusion. – Les tailles de zone peuvent être utilisées à estimer les valeurs de MIC, malgré avec une précision limitée. Il devrait y avoir un effort constant pour améliorer les lignes directrices en vue de nouvelles données cliniques et des laboratoires devraient faire un effort actif de les incorporer.

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Introduction

Candida has established itself as an important human pathogen, particularly in the hospitalized and immunocompromised patients [1,2]. While *Candida albicans* (*C. albicans*) was the predominant species worldwide, it is now being replaced by several of the non-*albicans* *Candida*, particularly *Candida tropicalis* (*C. tropicalis*) and *Candida glabrata* (*C. glabrata*) [3]. The reason often cited is rampant use of fluconazole to treat infections caused by *Candida*. Several species such as *C. glabrata*, *Candida krusei* (*C. krusei*) as well as *C. tropicalis* are known to be inherently less susceptible to this antifungal agent; thus leading to inadvertent selection of these *Candida* species [4]. In light of this fact and the rising resistance to antifungal agents, it becomes essential to perform antifungal susceptibility testing (AFST) routinely.

The reference method suggested by the Clinical & Laboratory Standards Institute (CLSI) is the recently updated broth microdilution (BMD) method as per the CLSI M27-S4 guidelines [5]. However, this is not easily incorporated into the laboratory's workflow because of its labor-intensive and cost-ineffective nature. Thus, more than a decade back, CLSI released guidelines for AFST by disk diffusion method similar to antimicrobial susceptibility testing [6]. The last update was provided in 2009 (CLSI M44-S3) [7]. While being easy, reproducible and inexpensive on one hand, disk diffusion method does not provide the minimum inhibitory concentrations (MICs), which can assist in treatment of critical patients as well as demonstrate creeping MICs (gradually increasing MICs indicating emerging trend in resistance).

Thus, the present study was conducted with the primary aim of determining whether the zone diameters around fluconazole disk (determined by CLSI recommended disk

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