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بالرسالة صفحات

لم ترد بالأصل

HOSSAM MAGHRABY

COMPARATIVE EVALUATION OF 3 SUSCEPHBILITY TEST METHODS OF CANDIDA ALBICANS FOR KETOCONAZOLE AND FLUCONAZOLE

Thesis

submitted for Partial fulfillment of M.Sc Degree in Medical Microbiology

- Plasma, Serum

BY

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Opportunistic fungal infections are increasingly important cause of morbidity and mortality particularly due to ***Candida*** species. There is also an increase of Candidosis especially ascribed to acquired or induced immunodeficiency syndromes or in the event of long-term antibiotic, immuno-suppressor or cytotoxic therapies (***Mallie and Bastide 1996).***

Parallel to the higher incidence of mycoses has been the development and more frequent use of antifungal agents. As a result, clinical laboratories are expected to assume a greater role in the selection and monitoring of antifungal chemotherapy ***(Espinel and Ingroff, 1994)***.

The widespread use of antifungal therapy, particularly the azoles, for extended periods has raised concerns regarding the development of resistance among isolates of ***Candida*** species (Korting, et al., 1988).

Both ketoconazole and fluconazole are broadspectrum agents, active against a variety of fungi, including yeasts, dimorphic organisms, dermatophytes, and opportunistic pathogens. Reports indicate that some patients with oropharngeal and eosophageal candidiasis are

failing Fluconazole therapy, despite an initial favorable response to the agent *(Willocks, et al., 1991)*.

Susceptibility tests with antifungal agents are performed for the same reasons as with antibacterial agents, to provide reliable data which will permit selection of the most active for use in treatment of human infections. Such data may be either quantitative i.e. determination of minimal inhibitory concentrations (MICs),or qualitative, i.e. prediction of probable clinical responses *(Shadomy,1980)*.

Routine antifungal susceptibility testing of azoles and other antifungal agents are still not definitely standardized, and is not widely used because of the remarkable interlaboratory differences obtained with the same isolates as well as with various media. Several methods have been published so far, but the correlation between in vivo and in vitro findings are still limited.

The National committee for clinical laboratory standard (NCCLs) subcommittee on antifungal susceptibility tests, considered the broth macrodilution test as the reference method for antifungal susceptibility *(NCCLs, 1992)*.

Although standardized broth dilution methods for antifungal susceptibility testing are available, the search for an easier testing procedures are desirable. The E test is one of these alternatives, for testing antifungal agents. E test is a predefined antimicrobial gradient that was developed for susceptibility testing of bacteria and now is being adopted for antifungal-testing *(Espinel-Ingroff, 1994)*.

First studies with ***Candida*** strains comparing the NCCLS method and the ready to use Agar based E test produced comparable results *(Shawar, et al., 1992)*. However, studies that compared different susceptibility test methods using clinical isolates are still limited *(Ruhnke, et al., 1996)*.

The aim of this work is to compare the three antifungal susceptibility methods (broth macrodilution and microdilution methods with the E test) of ***Candida albicans*** for ketoconazole and fluconazole.

CANDIDA

Lagenbeck (1839) was the first to demonstrate a yeast like fungus. The fungus was named ***Odium albicans*** by ***Robin (1853)***, and ***Manila albicans*** by ***Hansen (1888)***. ***Bekhout (1923)*** proposed the generic name ***candida*** to include these fungi which develop a pseudomycelium and reproduced by budding.

Candida species were classified on morphological and biochemical basis. ***Skinner and Zsolt (1963)*** defined over 100 species in the literature. Of these species only ***Candida albicans*** is considered to be pathogenic. Other ***Candida*** species that occasionally cause disease include **candida parapsilosis, Candida tropicalis** and ***Torulopis glabrata***. Other ***Candida*** species that live in soil occur in normal human flora and are rarely implicated in human disease includes, ***Candida pseudotropicalis, Candida krusei, Candida stellatoidea*** and ***Candida gilliermondii***.

CANDIDA ALBICANS

Candida albicans is normally isolated from alimentary tract of most mammals and birds and mucocutaneous areas as well. *Candida* species colonize the mucosal surfaces of all humans during or soon after birth ***(Rotrosen et al., 1986)***.

Morphology and staining:

According to ***Szaniszlo et. al., (1972) Candida albicans*** occurs principally as an oval, budding yeast cells (3-6um in size). ***Candida*** exists basically in two morphological forms i.e. it is a dimorphic. These forms are yeasty form and mycelia form.

Using Gram's stain they are Gram positive, round or oval cells. They divide asexually by budding. They form yeast colonies on artificial media at 37C°. Pseudohyphae are formed when the buds continue to grow but fail to detach, producing chains of elongated cells that are pinched or constricted at the separations between cells ***(Midgley and Hay, 1997)***

True hyphae can be formed by ***Candida*** species. They are composed of long thread like branching structure or septate branched filaments. It occurs in yeasts which reproduce by fission ***(Moore and Jaciow, 1979)***. On nutritionally deficient media ***C. albicans*** produces large, spherical chlamydospores. Chlamydospores represent resting resistant spores and consequently are formed in old culture or on relatively poor nutrient media deficient in protein ***(Ladder et. al., 1958)*** and reducing sugars. They occur also in cases of low oxygen tension at a temperature between 21-30 °C and pH 5-6 or 7.4-9. ***(Reid et. al., 1953)***.

SPECIES OF MEDICAL IMPORTANCE

Of the more than 80 species of yeasts that have been classified in the genus ***Candida***, only about 10 have been identified as capable of producing or contributing to disease of humans and animals ***(Martin, 1993)***. The most common species of interest are, ***Candida albicans, Candida tropicalis, Candida guilliermondii, Candida parapsilosis, Candida stellatoidea, Candida pseudotropicalis, Candida kruse,***. The importance of this species lies in its resistance to amphotericin B. *C. **glabrata,*** formerly called ***Torulopsis glabrata***, is capable of infecting the urinary tract and disseminating to major organs in immunocompromised patients, with manifestations similar to those associated with the other species ***(Martin, 1993)***.

Of the various species in the genus ***Candida,*** ***Candida albicans*** is the most common cause of superficial and systemic candidiasis. Several of the more than 80 other species classified in this genus can also be responsible for clinical disease under certain circumstances (e.g., host immunosuppression, indwelling catheters, intravenous drug delivery). Most of these infections are systemic, but can be localized. Together, ***Candida albicans and Candida tropicalis*** account for about 80 percent of the species isolated from medical specimens ***(Martin, 1993)***.