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Susceptibility of Candida albicans isolates to Terbinafine and Ketoconazole

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Abstract: The prevalence of drug resistance has become an important issue in various yeast infections, which have a significant effects on both human animal health. In this study, an attempt has been made to determine susceptibility pattern of two antifungal agents Terbinafine and Ketoconazole against 45 oral and non oral Candida albicans isolates using broth microdilution method. Under in vitro conditions, results showed that (42/45) 93% of the C. albicans isolates had MIC values indicating susceptibility to Ketoconazole (≤0.125 µg/ml) and MICs ranged from ≤0.03125-8.0 µg/ml. According to Terbinafine, (40/45) 88.9% of isolates had MICs less than 4 µg/ml and MICs ranged from 0.25-8.0 µg/ml. This is the first report of in vitro antifungal susceptibility data to be published from Palestine against clinical isolates of Candida albicans. Availability of sensitive and highly accurate antifungal susceptibility testing methods, can permit analysis of data in vitro and with outcome in vivo, important to assist physician for making appropriate drug choices and patient management decision. These data indicated that Terbinafine and Ketoconazole are still active against C. albicans and may therefore have clinical applications against some of these organisms.

Key words: C. albicans, Antifungal agents, Terbinafine, Ketoconazole, MIC.

الملخص: إن انتشار مقاومة الدواء بين الكائنات الحية المهجرية تعتبر مسألة في غاية الأهمية بالنسبة للالتهابات الفطرية، والتي أصبح لها تأثيرات سلبية كبيرة على صحة الإنسان والحيوان .في هذه الدراسة، فقد تم تحديد نمط الحساسية لاثنين من العلاجات الفطرية هما تربينافين و كيتوكونازول ضد 45 عزلة من المبيضة البيضاء (Candida albicans) الفموية وغير الفموية و ذلك باستخدام طريقة التخفيف الدقيق المتسلسل (Broth Microdilution Method). أظهرت تجارب المختبر أن 93% (42/45) من عزلات المبيضة البيضاء كانت قيم تركيز الحد الأدنى المثبط لها (Minimum Inhibitory Concentration (MIC)) تشير إلى حساسية المبيضة البيضاء

لكيتوكونازول (≥0.125ميكروغرام/مل) ، وتراوحت قيم تركيز الحد الأدنى المثبط بين ≥ 0.03125 -8.0 ميكروغرام / مل. أمابالنسبة لتربينافين فإن 88.9% (40/45) من عزلات المبيضة البيضاء كانت قيم تركيز الحد الأدنى المثبط لها أقل من 4 ميكروغرام / مل وتراوحت قيم تركيز الحد الأدنى المثبط بين 0.25 - 8.0 ميكروغرام / مل. يعتبر هذا هو أول تقرير يتم نشره من فلسطين عن حساسية عزلات سريرية من المبيضة البيضاء. إن توافر طرق حساسة ودقيقة للغاية لفحص اختبار الحساسية، يساعد الطبيب على اختيار العلاج المناسب و مراقبة حالة المريض بشكل صحيح. وأشارت النتائج إلى أن المضادان تربينافين و كيتوكونازول لا يزالان علاجان فعالان ضد المبيضة البيضاء.

الكلمات الدالة: المبيضة البيضاء،المضادات الفطرية، تربينافين، كيتوكونازول، تركيز الحد الأدنى المثبط.

Introduction:

Recently, several new antifungal agents have become important and available for both the topical and systemic treatment of fungal infections. Most antifungal drugs have adverse side effects, broad prophylactic usages and long-term treatments with those agents become ineffective against some fungi, and lead to development of resistance. The prevalence of drug resistance has become a serious issue in various yeast infections, which has a significant effects on human health [1,2].

Candida albicans is considered one of the most frequently implicated pathogen, causing localized, invasive or disseminated disease in normal or immunocompromised hosts, promoted by the use of broad spectrum antibiotic, steroids or other immunosuppressive drugs, diabetes mellitus, AIDS, cancer chemotherapy and organ transplantation etc. Candida infection is involving every part of the body and is considered the fourth most prevalent organism found in blood stream infections. C. albicans represents more than 80% of isolates recovered from clinical infection [3,4]. In addition to that, rarely other Candida species can cause infection. The importance of Candida species is not only due to the severity of their infections but also due to their ability to develop resistance against antifungal drugs. In vitro antifungal susceptibility testing plays an increasingly important role in guiding the selection of antifungal therapy, as an aid in drug development studies, and to detect shifts toward resistance as early as possible in epidemiologic studies [5-7]. Although Candida species have various degrees of susceptibility to frequently used antifungal agents, antifungal resistance is rare [8]. Antifungal susceptibility

testing is not routinely done in Palestine as well as in other countries, mainly due to economic resources are very limited and low demand. By performing antifungal susceptibility testing, this will avoid unnecessary usage of antifungal agents and more importantly, the patients do not have to bear the unnecessary side effects or toxicity of these antifungal agents. The changing epidemiology of Candida infections and the increase in serious fungal infections, the emergence of resistant Candida strains and the availability of new antifungal agents can assist in their clinical use, may influence the choice of antifungal agents for the patients [9]. In this study, an attempt has been made to determine susceptibility pattern of two antifungal agents (Terbinafine and Ketoconazole) against 45 oral and non oral Candida albicans isolates using broth microdilution method.

Materials and Methods:

Candida albicans isolates:

A total of forty-five clinical isolates of C. albicans were recovered from various clinical specimens during the 2008-2009. The specimens used in this study included 27 oral isolates while the rest of the non-oral isolates. These isolates were identified by phenotypic characteristics. C. albicans isolates were differentiated from other Candida and Cryptococcus species by their ability to grow on the Levine formula of EMB agar and to produce germ tubes within 3 hours, and pseudohyphae and budding cells at 18-24 hours when incubated at 35^{o}C in 5-10% CO2. The addition of tetracycline to the Levine formulation aids in the selection of C. albicans from clinical sources that are contaminated with bacteria. A reference strain (C. albicans ATCC 10231) was also included.

Antifungal Susceptibility Testing

Antifungal susceptibility of C. albicans isolates was tested by the broth microdilution technique with endpoints read at 48 hours as standardized by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards [10]. Antifungal agents including Terbinafine and Ketoconazole were used -as commercially supplied- to prepare solutions of a concentration 128 µg/ml. Minimum inhibitory concentration (MIC) was determined in RPMI 1640 medium (Sigma) buffered to pH 7.0 with 0.165 M morpholinopropansulfonic acid (MOPS) buffer (Sigma). Determination of MIC was performed in 96-wells microtiter plates, which were inoculated with 1 x 10^4/ml of C. albicans isolates and incubated at 35ºC for 48 h. The final concentrations of the antifungal agents were 64 to 0.03125 µg/ml

for all antifungal agents. Control wells drug-free were also included in the study. All the testing was done in duplicates.

Results:

A total of 45 isolates were analyzed for their susceptibilities to Ketoconazole and Terbinafine. In-vitro susceptibility data for all isolates tested against these two antifungal agents are summarized in Table 1. Ketoconazole was highly active, our results showed that (42/45) 93% of the C. albicans isolates had MIC values indicating susceptibility to Ketoconazole (≤0.125 µg/ml) and MICs ranged from ≤0.03125-8.0 µg/ml. According to Terbinafine no specific breakpoints proposed, but (40/45) 88.9% of isolates had MICs less than 4 µg/ml and MICs ranged from 0.25-8.0 µg/ml. The geometric mean MIC values were ≤0.054 µg/ml and 2.52 µg/ml for Ketoconazole and Terbinafine, respectively. C. albicans ATCC 10231 had MICs ≤0.03125 µg/ml and 16 µg/ml against Ketoconazole and Tterbinafine, respectively.

Table 1. Minimum Inhibitory Concentrations (MICs) and Geometric Mean Values of 45 C. albicans isolates to Terbinafine and Ketoconazole.

Antifungal agent	MIC (µg/ml)						Geometric mean MIC values (µg/ml)	Total isolate
Ketoconazole	≤0.03125 N= 31	0.0625 N=6	0.125 N=5	1.0 N=1	4.0 N=1	8.0 N=1	≤0.054	45
Terbinafine	0.25 N=2	0.5 N=3	1.0 N=2	2.0 N=14	4.0 N=19	8.0 N=5	2.52	45

Discussion:

Prolonged or repeated exposure to antifungal drugs may be associated with the emergence of antifungal resistance among strains of C. albicans. The determination of the in vitro susceptibility may prove helpful to predict the ability of a given antifungal agent to eradicate Candidal isolates. In our work, the evaluation of in vitro susceptibility showed that the antifungal drugs tested (Ketoconazole and Terbinafine) displayed a high activity against the C. albicans isolates. It is worth mentioning that both Ketoconazole and Terbinafin had low MIC and geometric mean MIC values. These low MICs found for the these two drugs can help to explain the promising results obtained for the treatment of C. albicans with these antifungal agents.

Susceptibility of Candida albicans isolates

Terbinafine is the only systemic allylamine antifungal agent currently available, which presents a potent in vitro activity against a number of filamentous and dimorphic fungi. Some researchers have proposed the application of this agent against Candida yeasts [11]. Although, its capacity to inhibit yeasts is still controversial. Clinically relevant interpretive breakpoints are not currently available for Terbinafine, but in our study no isolates with higher MICs (>8 μg/ml) was observed. Our results showed that geometric mean MIC value for Terbinafine against C. albicans isolates is consistent with previous report [12], which showed that geometric mean MIC value for Terbinafine against C. albicans was 2.83 μg/ml. These results were in agreement to reports published previously [11,13], who obtained good results for Tterbinafine against Candida spp. isolates. However, these results are in contrast to a previous report [12], who showed that Terbinafine had high MIC values for all Candida spp. evaluated (MIC90 ≥ 64.0 μg/ml). Patients in previous study might have a history of antifungal use or ever unknown the name or function of a medicine prescribed.

Earlier susceptibility testing of Terbinafine against yeasts showed poor activity [15,16], while more recent studies have shown that this drug is clearly effective in inhibiting yeasts [12,13,17]. This is not a surprise due to wide differences existed in the methodologies used to evaluate in vitro activity of Terbinafine by earlier researchers [11,12]. In contrast to earlier studies, the NCCLS macrodilution assay shows reproducible in vitro data for Terbinafine against Candida and other yeasts [10]. Interestingly, in the present study the MICs of Terbinafine against C. albicans were much lower than those obtained by earlier workers [18-21]. They reported high MICs around 25 μg/ml for C. albicans, while in the present study, MIC has ranged from 0.03125–8.0 μg/ml for C. albicans. Our results were consistent with that reported previously [12], which showed that MIC has ranged from 0.03125–4 μg/ml for C. albicans. This may be likely due to using medium is buffered at neutral pH, which provide good results for Terbinafine. While earlier assays used unbuffered media which are rapidly acidified by Candida spp., led to the recording of high MIC values which means that the Terbinafine is much less active at low pH [22,23]. So it was suggested that buffer MOPS should be added to the culture medium since Terbinafine is less effective under low pH conditions as an essential prerequisite for testing antifungal activity against yeasts. The in vitro activity of Terbinafine against C. albicans is thus of interest with regard to potential

clinical efficacy. Clinical studies have shown that topical and oral Terbinafine formulations are active against cutaneous candidiasis and Candida nail infections [13,24].

According to Ketoconazole, our results were inconsistent with studies carried out previously [25-27]. It was shown that 80.3% (57/71) of C. albicans isolates were sensitive to Ketoconazole (MIC ≤ 0.03-4 μg/ml) and 19.7% (14/71) were intermediate susceptibility (MIC 16-32 μg/ml) to Ketoconazole [25]. C. albicans isolated from blood culture of cancer patients showed 28/56 (50%) of isolates were sensitive (MIC ≤0.125-4), 22/58 (39.3%) were intermediate (MIC 16-32) and 6/58 (10.7%) were resistant (MIC≥64) against Ketoconazole [26]. In other study it was found that MIC_{90} of Ketoconazole for all C. albicans isolated from the oral cavities of AIDS patients were >32 μg/mL with a range from 0.064->32 μg/mL [27], this high resistance may be explained by the phenomenon of cross-resistance observed among the family of azole compound. Besides, the selection of resistant isolates to azoles would be explained by the long term of azoles in the prophylaxis therapy for patients with AIDS or other immunosuppressive diseases. Our results were in accordance with the previous results which showed that 93% of C. albicans were sensitive to Ketoconazole [28]. In recent study, all isolates were susceptible in both groups; isolates from HIV-infected patients showed MIC values between 0.03 - 4.0 μg/ml while the MIC values ranged from 0.03-0.25 μg/ml for the isolates from control patients [29]. Also it was found that 88% of C. albicans isolates were sensitive to ketoconazole [9]. Our result was also in agreement with results reported previously [30], these results showed that 90% of C. albicans were susceptible to Ketoconazole and had MIC <0.125 μg/ml).

The in vitro susceptibility of C. albicans to Tterbinafine and Ketoconazole has already been described by different investigators [12,17]. However, there is no information available from Palestine, in this respect as ecology of Candida species and the epidemiology of candidiasis are different in many parts of the world, it is important to know whether these differences also reflect variations in susceptibilities to antifungal agents such as Terbinafine an Ketoconazole [12,17]. This is the first report of in vitro antifungal susceptibility data to be published from Palestine against clinical isolates of C. albicans. Therefore investigations concerning its antifungal activities in vivo against such organism should be pursued. So availability of sensitive and highly accurate antifungal susceptibility testing methods, can permit analysis of data in vitro and with

outcome in vivo, important to assist physician for making appropriate drug choices and patient management decision. These data indicated that Terbinafine and Ketoconazole are still active against C. albicans and may therefore have clinical applications against some of these organisms.

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