CANDIDA PARAPSILOSIS COMPLEX SPECIES AND ANTIFUNGAL SUSCEPTIBILITY PROFILE IN PATIENTS OF INTENSIVE CARE UNITS OF MANSOURA UNIVERSITY HOSPITALS.

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ABSTRACT

Candida parapsilosis is an important non-albican species responsible for invasive fungal infections and hospital acquired infections especially in critical care patients. C*. parapsilosis* complex has been renamed according to genetic bases into 3 different species *C. parapsilosis sensu stricto, C. metapsilosis and C. orthopsilosis.* This study was designed to describe the distribution and antifungal susceptibility profile of the three members of *Candida parapsilosis* complex among patients of intensive care units (ICUs) in Mansoura University Hospitals. *Candida parapsilosis* was identified by Analytic Profile Index (API) 20 C. *C. parapsilosis sensu stricto, C. orthopsilosis* and *C. metapsilosis* were recognized according to the secondary alcohol dehydrogenase (*SADH)* restriction pattern using *BanI* restriction enzyme. Antifungal susceptibility testing was performed by E test. A total of 68 *C. parapsilosis* isolates were included in this study. Sixty-two isolates (91.2%) were identified as *C. parapsilosis sensu stricto,* 4 (5.9%) were identified as *C. orthopsilosis*, and 2 isolates were identified as *C. metapsilosis* (2.9%). All isolates of the *C. parapsilosis* complex species were sensitive to amphotricin B. Fifty isolates (80.6%) of *C. parapsilosis sensu stricto* were susceptible to fluconazole; 7 isolates (11.3%) were susceptible-dose dependent (SDD) to fluconazole, and 5 isolates (8.1%) were resistant to fluconazole. Most of *C. parapsilosis*

sensu stricto isolates were sensitive to itraconazole 59 (95.2%). No itraconazole or fluconazole resistance were found among the *C. metapsilosis* and *C. orthopsilosis* isolates; there was single *C. orthopsilosis* isolate SDD to both itraconazole and fluconazole.

Keywords: Candida, C. parapsilosis, Fluconazole, Resistance, Antifungal.

INTRODUCTION

Candidiasis is a serious infection in hospitals worldwide, especially in intensive care units (ICUs) patients [1-2]. Candidiasis can result from an endogenous colonization; however, hospital transmission and emergence of resistance to antifungal agents represent new and remarkable problems [3].

Although the main candidal species causing infections worldwide is still *Candida albicans,* there is an alarm from the increase of invasive infections caused by non-albicans species. *Candida parapsilosis* has emerged as the second most common causative agents of candidemia in Latin America, Asia [4-5] and in many European surveys [6-8]. *C. parapsilosis* is considered one of the main causes of invasive fungal infections in USA especially in transplant patients [9].

Isolates of *C. parapsilosis* cannot be distinguished phenotypically. However, genetic analysis by randomly amplified polymorphic DNA revealed that *C. parapsilosis* complex is composed of three different species, originally they were designed group (I, II, and III). This designation is replaced later by C*. parapsilosis sensu stricto, C. orthopsilosis and C. metapsilosis,* for group (I, II, and II) respectively [10]. This three genetically different species can be identified by restriction analysis of secondary alcohol dehydrogenase *(SADH)* gene which is present in all groups [11].

Candida infections are mostly treated with amphotericin B (AMB) and its lipid formulations [12-14]. However, *C. parapsilosis* resistance to amphotericin B has been reported [15].

Fluconazole (FLU) is an effective and safe alternative option for treat-

ment of patients with candidemia [16-17] and in particular for candidemia caused by *Candida parapsilosis complex* (18). Several studies reported resistance in *C. parapsilosis* to *fluconazole* [19-20].

This study aimed at giving insight into the prevalence of the different *C. parapsilosis* complex species; C*. parapsilosis sensu stricto, C. metapsilosis,* and *C. orthopsilosis a*nd their distribution among patients of ICUs of Mansoura University Hospitals. Moreover, this study describes the susceptibility profile of these species to antifungal agents commonly used for treatment of candidal infections namely, AMB, FLU and Itraconazole (ITC).

METHODS

This cross sectional study was carried out including all patients aged ≥18 years with candidal infection during their hospital stay in ICUs during period extending from February 2013 to December, 2013 (11 months period). Our local ethical committee approved the protocol. Urine, respiratory samples, blood, and oral swabs were collected from cases with suspected candidal infections clinically.

Samples were collected and processed at the Medical Mycology unit and Microbiology Diagnostic and Infection Control unit in Medical Microbiology and Immunology department, Faculty of medicine, Mansoura University.

All media were prepared according to the manufacturer's instructions. Processing of specimens was performed according to *Koneman et al.* [21].

Candida was identified according to colonial morphology on Sabouraud Dextrose Agar (SDA), Gram stained film, and non *albicans Candida* were differentiated from *Candida albicans* by germ tube test.

Candida parapsilosis was identified using API 20 C AUX (bioMérieux), according to the the manufacturer's instructions.

DNA extraction.

DNA Extraction Kit QIAamp was used to extract genomic DNA from Candida parapsilosis strains according the manufacturer's instructions. The DNA obtained was finally suspended in 100 μ1 TE buffer and stored at -20° C until use.

PCR amplification and SADH gene restriction analysis.

SADH gene was amplified by PCR for confirmation of *C. parapsilosis*, the reaction was done as described previously by Tavanti et al. [11] using the following primers Fwd, 5'- GTTGATGCTGTTGGATTGT-3' and Rev, 5'-CAATGCCAAATCTCCCAA-3'. PCR reaction was done in a PTC-100 ^{TM} instrument.

Isolates displaying *SADH* fragment sized of 716 bp were confirmed to be *Candida parapsiliopsis* complex and used in the study.

The products of PCR reaction were treated with the *BanI enzyme* (Thermo Fisher Scientific) in a tube containing 10 μl of the amplification products and 2 μL of *BanI*. The products of restriction reaction were detected by agarose gel electrophoresis. *Candida parapsiliopsis* species were distinguished as *C. parapsilosis sensu stricto, C. metapsilosis and C. orthopsilosis* according to the SADH restriction pattern. DNA bands were visualized using a UV transilluminator.

Antifungal susceptibility testing: was performed by E test (Liofillchem, Italy), and MIC results were interpreted according to the CLSI [22] guidelines.

RESULTS

This study enrolled 68 isolates of *Candida parapsiliopsis* complex as identified by API 20 C AUX (bioMérieux) and confirmed by PCR amplification of *SADH* gene.

For the *C. parapsilosis* complex, the amplified *SADH* fragment (716 bp) was cut by BanI restriction enzyme. According to the *BanI* restriction profile described before [11], isolates with single *BanI* restriction site (at position 196) were identified as *C. parapsilosis sensu stricto*, isolated with no restriction site were classified as *C. orthopsilosis* and isolates with three *BanI* restriction sites (at positions 96, 469, and 529) were identified as *C. metapsilosis.*

Distribution of *C. parapsilosis* complex species: The sex distribution and age groups of patients are presented in table (1). Prevalence of the *C. parapsilosis* complex species and their distribution in different clinical samples are described in table (2). About ninety percent (91.2%) of the isolates (62 isolates) were identi-

fied as *C. parapsilosis sensu stricto.* Four isolates representing (5.9%) were identified as *C. orthopsilosis.* Only two isolates representing (2.9%) were identified as *C. metapsilosis. C. parapsilosis sensu stricto* were detected in all types of collected clinical samples including blood. However, *C. orthopsilosis and C. metapsilosis* were isolated only from urine and mucosal samples.

Antifungal susceptibility pattern: v Susceptibility profile to azole agents (fluconazole, itraconazole) and AMB are described in table (3). All isolates were sensitive to AMB. Regarding fluconazole sensitivity, fifty isolates (80.6%) of *C. parapsilosis sensu stricto* were sensitive to FLU; 7 isolates (11.3%) were SDD to FLU, and 5 isolates (8.1%) were resistant to FLU. No azoles (fluconazole and itraconazole) resistance were detected among *C. metapsilosis* and *C. orthopsilosis* isolates; there was single *C. orthopsilosis* isolate SDD to both ITC and FLU.

Table (1) Epidemiological features of patients

Sex	**NO (%)**
Male	29 (42.6)
Female	39 (57.4)
Age	
Mean ±SD (min-max)	47.1± 13.7 (18-68 years)
Age groups	**NO (%)**
≥18 - ≤29	8 (11.8)
>29 - ≤39	12 (17.6)
>39-≤49	10 (14.7)
>49-≤60	17 (25)
>60	21 (30.9)

Table (2) Distribution of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* in different clinical samples

Clinical Sample	No. (%) of isolates			
	C. parapsilosis sensu stricto	*C. orthopsilosis*	*C. metapsilosis*	Total
Urine	29	3	1	33
Respiratory tract	17	0	0	17
Blood	6	0	0	6
Mucosal surface	10	1	1	12
Total	62 (91.4)	4 (2.3)	2 (2.9)	68 (100)

Table (3): Susceptibility profile of the three *Candida parapsilosis* spp. and their antifungal MIC range.

Species (no. of isolates)	Antifungal agent	MIC (mg/ml) Range	Mean	MIC 90	MIC 50	No (%) of isolates		
						S	SDD	R
C. parapsilosis sensu stricto (62)	AMB	0.031-1	0.21	0.5	0.125	62 (100%)	0	0
	FLC	2-64	1.3	32	8	50 (80.6)	7 (11.3%)	5 (8.1%)
	ITC	0.031-0.25	0.15	0.25	0.125	59 (95.2%)	3 (4.8%)	0
C. orthopsilosis (4)	AMB	0.062-0.5	0.19	0.5	0.09	4 (100%)	0	0
	FLC	2-32	1.2	3.2	6	3 (75%)	1 (25%)	0
	ITC	0.125-0.5	0.34	0.5	0.375	3 (75%)	1 (25%)	0
C. metapsilosis (2)	AMB	0.25	0.25	-	-	2 (100%)	0	-
	FLC	0.5-8	4.3	-	-	2 (100%)	0	0
	ITC	0.25-1	0.63	-	-	2 (100%)	0	0

S: susceptible
SDD: susceptible dose dependant
R: resistant

DISCUSSION

Three different species of *C. parapsilosis complex* have previously been recognized according to genetic background namely; *C. parapsilosis sensu stricto, C. metapsilosis* and *C. orthopsilosis* [11, 23].

In this study, *C. parapsilosis sensu stricto* represents (91.2%) of all isolated *C. parapsilosis* strains. *C. orthopsilosis* and *C. metapsilosis* represent (5.9% and 1.5 %) respectively. *C. parapsilosis sensu stricto* was the only member of the complex that was isolated from blood samples of the ICUs patients.

This result agrees with results of other studies like Silva et al. and GE et al. [24-25]. This augments the assumption that main member of C*. parapsilosis complex* responsible for hematogenous infections is *C. parapsilosis* sensu stricto. The other two members *(C. orthopsilosis* and *C. metapsilosis)* are responsible for other infections like urinary tract infections and mucosal infections.

The higher prevalence of *C. parapsilosis sensu stricto* may be due to its higher capacity for persistence in the hospital environment which may help its transmission to patients. [26-27] And/or may be explained the its capacity to express virulence determents more than the other two species [28-29] (e.g) adherence to host cells, the ability to form biofilm, and several hydrolytic enzymes production, such as phospholipases, lipases, and proteases [30].

C. parapsilosis sensu stricto was the only species of the complex can that can form biofilms [31,32]. Tavanti et al. [33] found that most of *C. parapsilosis sensu stricto* strains are proteinase producers, the higher producers being recovered from blood and mucosal specimens.

The isolation frequency of the three species of *C. parapsilosis* complex is variable throughout the world. In almost all studies, C*.parapsilosis sensu stricto* is the most common isolated species. However, the prevalence and distribution of the three species is variable. This distribution may vary according to socioeconomic conditions of the affected patients population in different countries and cities throughout the world. For example, *C.parapsilosis sensu stricto*

incidence varies from (95.6%) in Kuwait [34] to (64.5%) in China [35]. Also elevated incidence of *C. metapsilosis* (10–35.5%) was found in studies performed in hospitals from China [35-36] and in Hungary [37] compared to other countries. On the other hand, *C. orthopsilosis* has higher incidence about (9%) in other studies like Bonfietti et al. ([38]) in Brazil.

Antifungal susceptibility tests were performed by E test to itraconazole, fluconazole and amphotericin B. All *C. parapsilosis* sensu stricto, *C. metapsilosis* and *C. orthopsilosis* isolates were susceptible to amphotericin B. The *C. parapsilosis* sensu stricto MIC50 and MIC90 of AMB was 0.125 μg/ml and 0.5 μg/ml respectively. This result agrees with most of studies before that found the *C. parapsilosis* MIC50 and MIC90 average values range from 0.13 to 1 μg/ml and from 0.5 to 1 μg/ml, respectively [39-42].

Regarding azole antifungal agents, about eighty percent of *C. parapsilosis sensu stricto isolates* were susceptible to fluconazole, (11.3%) were SDD and (8.1%) were resistant. About ninety five percent of the isolates were sensitive to Itraconazole and (4.8%) were SDD. No FLU-resistant or ITC resistance was detected among *C. metapsilosis* and *C. orthopsilosis* isolates. Only one isolate of *C. orthopsilosis* were SDD to Flucoazole and Itrconazole (MIC 32 μg/ml and 0.5 respectively).

Fluconazole-resistance has been reported in clinical isolates of *C.parapsilosis sensu stricto* around the world [43-48].

We observed only one *C. orthopsilosis* isolate was SDD to fluconazole and itraconazole (MIC 32 μg/ml and 0.5 respectively). However, because of the small number of isolates belonging to the new species, this study may not present a complete picture about the antifungal susceptibility pattern of *C. orthopsilosis*, and C. metapsilosis.

This study has some limitations. First, the current study did not investigate possible risk factors for *C. parapsilosis* infections. Furthermore, the study did not search the virulence factors of *C. parapsilosis* complex and the differences of virulence fac-

tors between members of the complex that may increase the prevalence of *C. parapsilosis sensu stricto* infections among these patients. So, further studies are required to discuss these factors.

Conclusion

C. parapsilosis sensu stricto represent majority of C*. parapsilosis comlex* causing infections in ICUs patients. AMB retains its activity against the members of the *C. parapsilosis complex.* There is an alarming of azoles resistance in the members of the complex especially fluconazole.

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الملخـص العربـى

انواع و حساسية ميكروب الكانديدا باراسليوبسز لمضادات الفطريات فى مرضى العناية المركزة بمستشفيات جامعة المنصورة.

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كلية الطب-جامعه المنصورة

ميكروب الكانديدا باراسليوبسز هو واحد من الأنواع الكانديدا الهامة المسؤولة عن العدوي الفطرية الغازية وعدوى المستشفيات المكتسبة وبخاصة في المرضى الرعاية الحرجة.و قد تمت إعادة تسمية مجمع ميكروب الكانديدا بارابسليوبسز وفقا لقواعد وراثية الي ثلاثة انواع سانسيستريكتو ,اورثوبسليوبسز و ميتابسليوبسز.

وقد صممت هذه الدراسة لوصف توزيع وخصائص حساسية اعضاء مجمع الكانديدا بارابسليوبسز لمضادات الفطريات بين المرضى من وحدات العناية المركزة في مستشفيات جامعة المنصورة. وقد تم التعرف علي اعضاء مجمع ميكروب الكانديدا بارابسليوبسز بواسطة مؤشر الملف التحليلى API20 C ووفقا لخصائص قطع جين SADH باستخدام انزيم القطع و تم إجراء اختبار الحساسية لمضادات الفطريات عن طريق اختبار E.

و قد تم عزل ٦٨ عزلة لميكروب الكانديدا بارابسليوبسز تتضمن ٦٢ عزلة من نوع سانسيستريكتو تشكل نسبة (٩١,٢٪) و اربع عزلات من نوع اورثوبسليوبسز بنسبة ((?5.9و عزلتين من النوع ميتابسليوبسز بنسبة (٢,٩٪) جميع العزلات كانت حساسة لعقار الامفوتريسين-ب. و ما يزيد عن ثمانين في المئة (٨٠,٦٪) من العزلات من نوع سانسيستريكتو كانت حساسة لعقار الفلوكونازول و(٨٠,٦٪) العزلات (١١,٣٪) ذو حساسية متغيرة علي حسب الجرعة و خمس عزلات (٨,١٪) مقاومة لعقار الفلوكونازول. و معظم العزلات (٩٧,٩٪) كانت حساسة لعقار الاتراكونازول. لم يتم عزل اي عزلات من الانواع اورثوبسليوبسز و ميتابسليوبسز مقاومة لعقارات الفلوكونازول و الاتراكونازول في هذة الدراسة. و كانت هناك عزلة واحدة من النوع اورثوبسليوبسز ذو حساسية متغيرة علي حسب الجرعة لعقارات الفلوكونازول و الاتراكونازول.

و نستخلص من هذا البحث ان نوع سانسيستريكتو يشكل غالبية مجمع ميكروب الكانديدا بارابسليوبسز المسبب للعدوي في مرضي العناية المركزة و لازال عقار امفوتريسين-ب يحتفظ بفاعليتة ضد جميع انواع مجمع ميكروب الكانديدا بارابسليوبسز و لكن هناك زيادة فى مقاومة الميكروب لعقارات الازولات خاصة عقار فلوكونازول.