ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA SPECIES ISOLATED FROM CANCER PATIENTS

Aws Akram Jabber[1] and Fadhil Sami Zghair[2]

[1]College of Health and Medical Technology, [2]Kufa Technical Institute, Al-Furat Al-Awsat Technical University, Kufa, IRAQ

Corresponding author:
Aws Akram Jabber
Mobile: +9647811020990

E-mail:
awsfm9595@gmail.com

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ABSTRACT:

The purpose of the study was to identify Candida infections in cancer patients and examine treatment response. and evaluating the effectiveness of specific antifungal drugs in treating the illness as well as the resistance of the isolated antifungal strains. The number of samples (serum) were 125, collected from cancer patients, whose ages ranged between (20 year - 60 years) with a date of infection with candidiasis during the period from (October to December 2023). All specimens were collected from Al-Najaf Governorate.

The results showed that 59% of cancer cases are associated with fungal infections (candidiasis), with 49 samples being Candida albicans, 4 samples being C.tropicalis, 5 samples being C.glabrata, and one sample being C.parapslosis, while the results of the sensitivity test showed 45 samples are sensitive to two types of antifungals (Nystatin and Fluconazole), and 14 samples are resistant to these antifungals.

Samples	Antifungal
2 samples	resistant to fluconazole
6 samples	resistant to nystatin
6 samples	resistant to fluconazole and nystatin

Keywords*: Candida albicans, antifungal compound, cancer*

1. INTRODUCTION:

The global the prevalence of severe fungal infections may be related to antifungal overuse the use of tainted equipment, immunosuppressive medication [(1)].

In most cases, it is difficult to control fungal infections because they need specialized antifungals, in addition to the lack of vaccines against fungi, and the careless application of antifungals has resulted in the emergence of pathogenic strains [(2)]. *Candida* spp are the most prevalent fungi that cause candidiasis in the mouth, vagina, and gastrointestinal tract in humans., causing severe morbidity worldwide.

Candidiasis may have an association with a variety of cancer types [(3)]. Candidiasis is an opportunistic fungal infection on or within any body part, such as fungi, caused by any species of *Candida*, the most frequent causal species being *Candida albicans*. These infections are wide-ranging; they can range from minor conditions like vaginitis and oral thrush to serious, frequently fatal systemic infections.

The cancer-microbiota relationship has been found both in several types of tumors. Nowadays, it is clear that certain infectious pathogens, are strong causes of cancer [(4)].

A complex illness, cancer is the second largest cause of death globally, taking the lives of around 10 million people in 2020 [(5)].

When it comes to antifungal medications, azoles are the most commonly used and favored medication for treating *Candida* infections. Depending on the type of

infection, the anatomical location where it appears, and the sensitivity profile of the species, additional antifungals may also be use. Polyenes, echinocandins, nucleoside analogs, and allylamines are a few of them [6]. Concerns have been raised regarding the possibility for antifungal drugs to select and disseminate resistant fungus strains or species due to their present use [7]. Research has shown that the frequency of infections brought on by yeasts that are either innately resistant to the medicine being used or have developed resistance to it is rising [8&9].

2.MATERIALS AND METHODS:

2.1 Samples Collection:

The number of samples (serum) were 125, collected from cancer patients, whose ages ranged between (20 year - 60 years) with a date of infection with candidiasis during the period from (October to December 2023),

2.2 Preparation of Culture Media:

The manufacturer's instructions, which were attached to the container, were followed in the preparation of all culture medium. They were autoclaved at 121°C for 15 minutes. and 15 pressure to sterilize them. However, chromo agar media was boiled for 1 minutes without overheating ,15 mL of media was poured into disposable Petri dishes and stored at 4 C° until use.

2. 3 Identifying the isolates of fungal:

The microscopic characteristics, the culture, and the identification of the fungal isolates were used to the form, size, color, edge, and appearance of the yeast isolates were assessed on SDA media during a 24- to 48-hour incubation period. From the yeast growth on SDA, a single cell was taken out and cultivated using the loop method, which involved incubation at 37°C for 24 to 48 hours. *Candida* species could be identified based on color with the help of the chromagar test [10&11].

A popular phenotypic technique for identifying Candida species is **Chromagar** Candida [12].

2.4 Germ Tube Formation test:

Took a part of the colony with 5.0 ml of human blood serum and incubate it for two to four hours at 37°C in a sterile test tube.

2.5 Sensitivity test:

Note: The sensitivity tests were conducted in accordance with the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) and the equipment manufacturer

1. Isolated colonies from an 18- to 24-hour agar plate should be made into a simple broth or saline solution in order to prepare the inoculum.
2. Add a cotton swab that has been sterilized to the suspension. Press down hard on the tube's interior wall above the fluid level while rotating the swab multiple times. This clears the swab of extra fluid.
3. Used the swab to inoculate the agar plate's whole dry surface. agar's sterile surface. Make sure the inoculum is evenly dispersed by streaking two more times, each time rotating the plate by around 60 degrees. Finally, use a wipe to clean the agar's rim.
4. Let the lid remain open for a maximum of 15 minutes, but for three to five minutes.

Setting up the disks:

1. Placed the antifungal using sterile forceps or a disk dispenser, place a disk on the surface of the dried and inoculated plate.
2. To guarantee that the disk and the agar surface are completely in contact, lightly press take it out with the tool immediately. Once a disk comes into touch with the agar surface, it should not be moved since some drug diffusion happens instantly.

3. Arrange the disks so that they are at least 24 mm from the petri dish's edge and no closer than 10 to 15 mm. Twelve disks may be arranged on a 150 mm plate and a maximum of six disks in a 9-cm petri dish. If overlapping zones are present, decrease the number of disks put per plate.

Ethical consideration:

These samples were collected after the approval of the Ethics Committee in accordance with the order issued by the Najaf Health Department No. 966 dated 10/20/2023, After being moved to the lab, every sample was examined and identified.

3. RESULT:

3.1 Examining Plates and Determining Outcomes:

To detect isolates resistant to antifungals, the plates were inspected, the zones of inhibition were measured, and the results were compared with international tables**.**

The present study includes a collection of 125 serum samples from cancer patients, these samples were obtained from Al-Najaf Governorate, from (October to December 2023). Out of 125 only 59 were considered positive growth while 66 samples were negative growth, as in Table (1).

Table 1: The number of fungal infections appears in several types of cancers.

Type of cancer	Number of samples	Positive	negative
Lung Cancer	12	7	4
Breast Cancer	27	12	14
Rectum Cancer	19	5	14
Uterus Cancer	23	9	16
Pancreas Cancer	16	9	7
H.L Cancer	8	2	6
Colon Cancer	20	15	5
Result	125	59	66

The following results data are recorded in the present study: *C.albicans* which exhibits with the highest frequency in comparison with the other species of *Candida* which is 49 isolates of *candida*. *spp* while other samples were represented in Table (2) [12].

Table 2: show candida spp. on chromagar

Candida spp	Number of samples
C.tropicalis	4
C.glabrata	5
C.parapsilosis	1
C.albicans	49

3.2 Diagnosis of Isolates:

From cancer patients, 59 *Candida* samples were identified. Initially, colonies emerged and took shape. Their outward appearance and microscopic features were used to determine their identity. surface that contrasts in color between cream and white, has edges, and is glossy Figure (1). This result is consistent with [13] and [14] in terms of the phenotypic characteristics of the colonies.

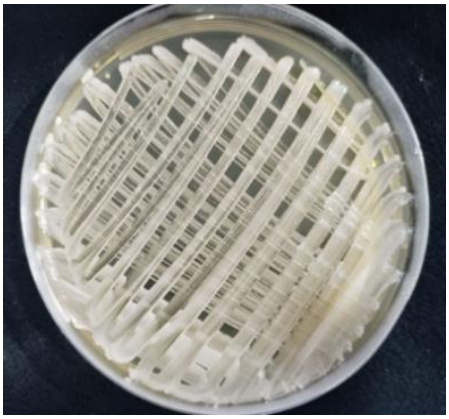

Figure 1: The external appearance of single colonies of *Candida spp*

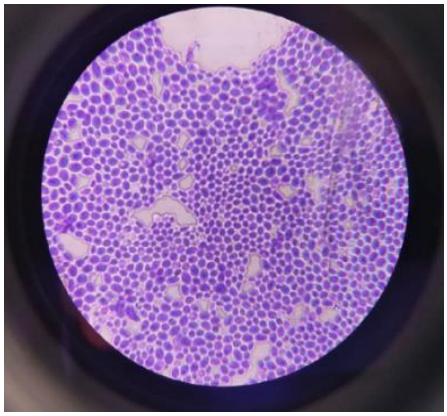

Figure 2: Cells of *Candida spp* isolates are stained with simple stain at 100X magnification.

3.3 Germ Tube Formation test:

The results of Figure (3) showed that most of the isolates were unable to form germ tubes after being placed in a tube containing Blood serum for 2-4 hours, which is considered diagnostic for the species C.*albicans* This result was identical to the results of[15] who reported that *C.albicans*. has the ability to form germ tubes. With the exception of some isolates (which do not have the ability to form germ tubes).

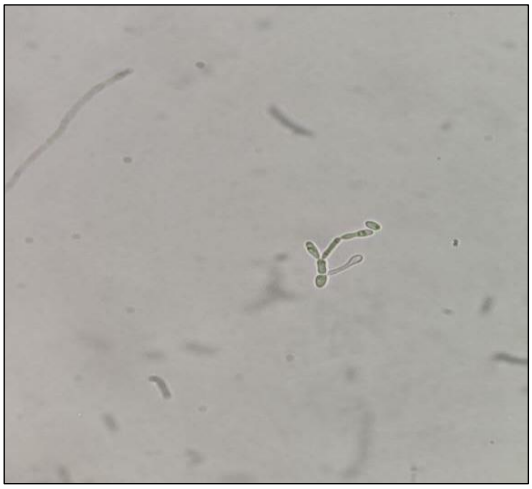

Figure 3: Germ tube formation of *Candida albicans*

3.4 Growth Test on Chrom agar:

The *Candida* spp isolates were diagnosed on Chromagar medium, as they appeared in different colors, each colour. It is considered a diagnostic characteristic for a specific type of *Candida* spp. The isolates were (Blue, purple, green, Creamy white color). The light green color indicates *C.albicans*., the blue color indicates *C.tropicalis* and the cream color indicates to *C.Parapsilosihs*. and the violet color to *C. glabrata*. according to the instructions of the company that prepared the medium Figure (4). These results are consistent with the findings [16].

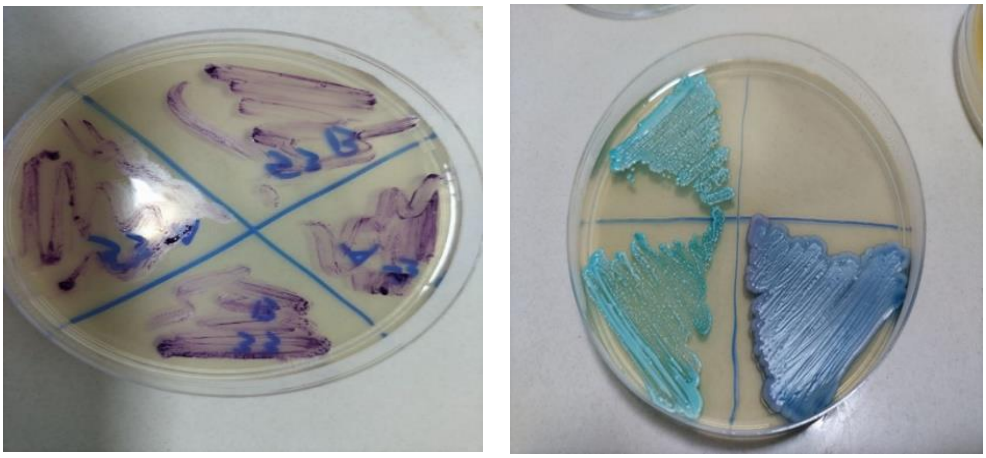

Figure 4: Candida spp. on chromagar
C. albicans C.tropicalis C. glabrata C .Parapsilosis

3.5 Susceptibility testing:

The results showed that isolates of yeast varied in the extent of their resistance to antifungal, the isolates showed Sensitive to both nystatin and fluconazole by 65 % and by 35%that isolates are resistance to antifungal when concentration 100mcg in each disk for nystatin, and in concentration 10mcg in each disk for fluconazole. The resistance of isolates to the above antifungal may be attributed to: The mechanism of overexpression of fks genes and thus an increase in transport proteins that will pump the antifungal out of the cell. The result is consistent with [17] the susceptibility test. Figures (5 & 6)

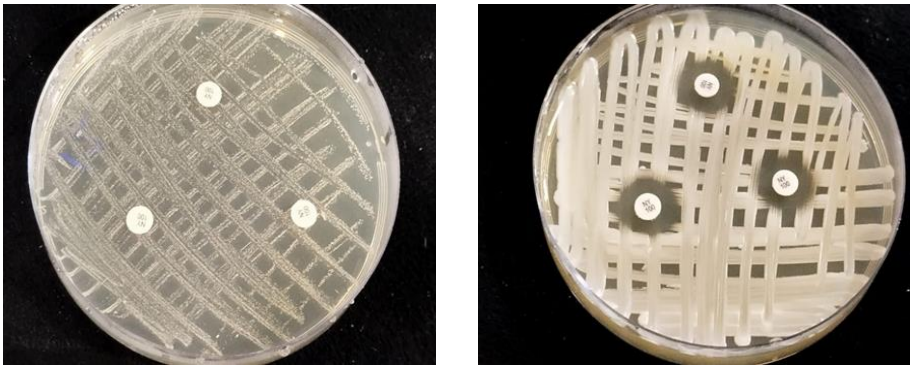

Figure 5: Sensitive test for nystatin

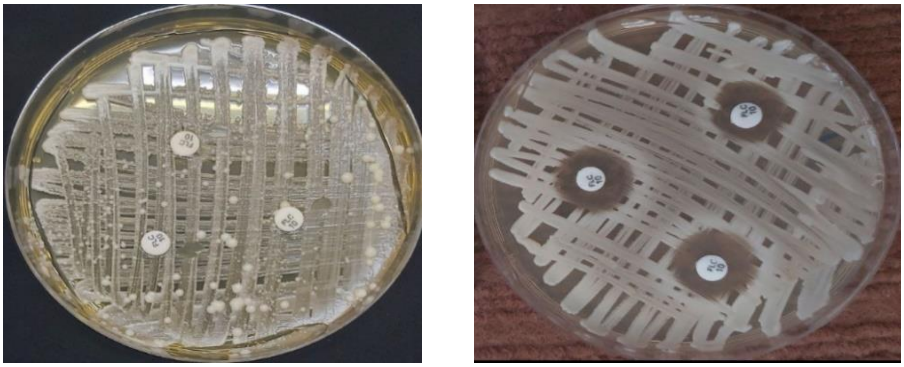

Figure 6: Sensitive test for Fluconazole

4. DISCUSSION:

Weakness of the immune system as a result of chemotherapy and radiotherapy leads to an increased chance of opportunistic infections, including fungal infections, specifically Candida, according to the results shown. We also note differences in the ability of the antifungals Nystatin and Fluconazole to inhibit the growth of Candida species. We found 45 samples sensitive to the antifungals and 14 samples resistant to them. The reason for this resistance is due to many reasons, including the presence of mutations in resistance genes. Through the results and statistics, it is indicated that colon cancer leads to an increased probability of developing it. As in Table (3).

Table 3: Susceptibility testing to antifungal for a number of Candida Spp isolates.

Candida Isolate	Fluconazole	Nystatin	Cancer type	gender
C.a 1	Resistant	Resistant	pancreas	m
C.t 1	sensitive	Resistant	colon	m
C.t 2	Resistant	Resistant	colon	m
C.t 3	sensitive	sensitive	lung	m
C.t 4	sensitive	sensitive	lung	m
C.g 1	sensitive	sensitive	lung	m
C.g 2	Resistant	sensitive	colon	f
C.g 3	sensitive	sensitive	rectum	m
C.g 4	sensitive	Resistant	uterus	f
C.g 5	sensitive	sensitive	colon	m
C.P 1	sensitive	sensitive	colon	m
C.a *2*	Resistant	sensitive	lung	m
C.a *3*	sensitive	sensitive	H.L	m
C.a 4	sensitive	sensitive	breast	f
C.a 5	sensitive	Resistant	breast	f
C.a 6	sensitive	sensitive	colon	f
C.a 7	sensitive	sensitive	breast	f
C.a 8	sensitive	sensitive	breast	f
C.a 9	Resistant	Resistant	pancreas	m
C.a 10	sensitive	sensitive	rectum	f
C.a 11	sensitive	sensitive	uterus	f
C.a 12	Resistant	Resistant	uterus	f
C.a 13	sensitive	Resistant	colon	f
C.a 14	Resistant	Resistant	breast	f
C.a 15	sensitive	sensitive	breast	f
C.a 16	sensitive	sensitive	rectum	m
C.a 17	sensitive	sensitive	colon	m
C.a 18	sensitive	sensitive	breast	f
C.a 19	sensitive	sensitive	breast	f
C.a 20	sensitive	Resistant	colon	m
C.a 21	sensitive	Resistant	colon	m
C.a 22	sensitive	sensitive	uterus	f
C.a 23	sensitive	sensitive	pancreas	f
C.a 24	sensitive	sensitive	pancreas	f
C.a 25	sensitive	sensitive	uterus	f
C.a 26	Resistant	Resistant	breast	f
C.a 27	sensitive	sensitive	lung	m
C.a 28	sensitive	sensitive	rectum	m
C.a 29	sensitive	sensitive	lung	m
C.a 30	sensitive	sensitive	breast	f
C.a 31	sensitive	sensitive	breast	f

C.a 32	sensitive	sensitive	colon	m
C.a 33	sensitive	sensitive	colon	m
C.a 34	sensitive	sensitive	breast	f
C.a 35	sensitive	sensitive	rectum	m
C.a 36	sensitive	sensitive	uterus	f
C.a 37	sensitive	sensitive	pancreas	m
C.a 38	sensitive	sensitive	lung	m
C.a 39	sensitive	sensitive	colon	m
C.a 40	sensitive	sensitive	colon	f
C.a 41	sensitive	sensitive	pancreas	f
C.a 42	sensitive	sensitive	H.L	f
C.a 43	sensitive	sensitive	colon	f
C.a 44	sensitive	sensitive	pancreas	f
C.a 45	sensitive	sensitive	uterus	f
C.a 46	sensitive	sensitive	uterus	f
C.a 47	sensitive	sensitive	pancreas	f
C.a 48	sensitive	sensitive	pancreas	m
C.a 49	sensitive	sensitive	uterus	f

(C.a---candida.albicans, C.t----candida.tropicalis, C.g----candida.glabrata, C.p---candida.parapsilosis)

Competing interests:

It is worth noting that there is no conflict of interest.

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اختبار الحساسية المضادة للفطريات لأنواع المبيضات المعزولة من مرضى السرطان

أوس أكرم جبار[1] و فاضل سامي زغير[2]

كلية التقنيات الصحية والطبية - جامعة الفرات الأوسط التقنية - الكوفة - العراق 1

معهد الكوفة التقني - جامعة الفرات الأوسط التقنية - العراق2

كان الغرض من الدراسة هو التعرف على عدوى المبيضات لدى مرضى السرطان وفحص الاستجابة للعلاج. وتقييم فعالية أدوية محددة مضادة للفطريات في علاج المرض وكذلك مقاومة السلالات المضادة للفطريات المعزولة. بلغ عدد العينات (المصل) 125 عينة تم جمعها من مرضى السرطان الذين تراوحت أعمارهم بين (20 سنة - 60 سنة) مع تاريخ الإصابة بداء المبيضات خلال الفترة من (أكتوبر إلى ديسمبر 2023). تم جمع جميع العينات من محافظة النجف وأظهرت النتائج أن 59% من حالات السرطان مرتبطة بالعدوى الفطرية (داء المبيضات)، حيث كانت:

49 عينة (Candida albicans) - 4 عينات (C.glabrata) - 5 عينات (C.tropicalis) – 1 عينه (C.parapslosis)

في حين أظهرت نتائج اختبار الحساسية أن 45 عينة حساسة لنوعين من مضادات الفطريات (النيستاتين والفلوكونازول)، و14 عينة مقاومة لهذه المضادات الفطريه,عندما يتعلق الأمر بالأدوية المضادة للفطريات، فإن الآزولات هي الأدوية الأكثر استخدامًا والمفضلة لعلاج عدوى المبيضات. اعتمادًا على نوع العدوى، والموقع التشريحي الذي تظهر فيه، وحساسية النوع، يمكن أيضًا استخدام مضادات الفطريات الإضافية . قمنا بعد ذلك باختبار تكوين الأنبوب الجرثومي بواسطة

اخذ جزءًا من المستعمرة مع 5.0 مل من مصل الدم البشري وقم باحتضانها لمدة ساعتين إلى أربع ساعات عند 37 درجة مئوية في أنبوب اختبار معقم. اما بخصوص أنواع السرطانات لوحظ ان سرطان القولون هو اكثر الأنواع عرضه للاصابه بالمبيضات مقارنة بالانواع الأخرى حسب الاحصائيات .