Iraqi Journal of Biotechnology, 2017, Vol. 16, No. 3, 216-222

Isolation and identification of opportunistic fungi from patients with different types of leukemia in Baghdad province

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Received: 13 August 2017 / **Accepted**: 10 October 2017

Abstract: The study is concern on determine the type of opportunistic fungal *spp*.in leukemia patients that were infected with many types of opportunistic fungal infections including oral candidiasis as a result to their immune suppression due to their submission to radiation and chemotherapy treatment. From 125 patients who diagnosed with different types of leukemia, 140 samples were collected, 110 samples were positive, and 30 samples were negative. The number of dermatophytes isolates was 21 which represent (19.09%) of the total cases. The number of *Candida* isolates was 60 which represent (73.17 %) of the total cases. The number of opportunistic fungal isolates was 29 which represent (26.12%) of the total cases. The result showed that the most common isolates were *C. guillermondii* 19 isolates which represent (31.66 %) of cases, then followed by *C. itermedia* 11 isolates which represent (18.3 %), *C. kyfer* 8 isolates which represent (13.3 %), *A. niger* 17 isolates which represent (58.62 %), *A. fumigatus* and *A. flavus* 3 isolates which represent (10.34 %), *Penicillium* 5 isolates which represent (17.24 %), and *Alternaria* 1 isolate which represent (3.44 %).

Keywords: leukemia, immunocompromized patients, opportunistic fungal infections, candidiasis.

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Introduction:

Candida is a genus of yeasts. Many species are harmless (commensals or endosymbionts) of hosts including humans, but other species when present in wrong location, could cause disease. Many species are found in gut flora, including *C. albicans* in mammalian hosts, whereas others live as endosymbionts in insect host (1, 2). *C. albicans* can cause infection like candidiasis or thrush in humans especially in immunocompromised patients (3). *Candida* infections are one of the most commonly occurring fungal infections in humans, (4) affecting mucosa, skin, nails and internal organs of the body, it also a common opportunistic infections in immunocompromised patients (5).

The incidence of candidiasis that caused by *Candida spp*. Continues to increase in proportion to the growing number of immunocompromised, cancer, and postoperative patients. Antibiotics promote yeast infections, including gastrointestinal *candida*

overgrowth, and penetration of the gastrointestinal mucosa (6). Certain factors, like prolonged antibiotic use, individuals with diabetes or impaired immune systems, such as those with Human Immunosuppression Virus (HIV), are more susceptible to yeast infections. Immunodeficiency (or immune-deficiency) is a state in which the immune system's ability to fight infectious disease is compromised or entirely absent. Immunodeficiency may also decrease cancer immunosurveillance. Opportunistic Mycoses are fungal infections that do not normally cause disease in healthy people, but do cause disease in people with weakened immune defenses (immunocompromized people) (7). Species that caused opportunistic infections includes: *Candida*, *Cryptococcus*, *Aspergillus*, *Mucor* and *Rhizopus*, in addition to *Pneumocystis*(7).

A person who has an immunodeficiency of any kind is said to be immunocompromised. An immunocompromised person may be particularly vulnerable to opportunistic infections, in addition to normal infections that could affect everyone (8). There for, immunodeficiency is defined as the failure of the immune system to protect against disease or malignancy, it includes two types, primary immunodeficiency which caused by genetic or developmental defects in the immune system. These defects are present at birth but may show up later on in life, and secondary or acquired immunodeficiency which means the loss of immune function as a result of exposure to disease agents, environmental factors, immunosuppression, or aging (8).

One of the most common types of immunosuppressive diseases is leukemia; Leukemia is a type of cancer of the blood or bone marrow characterized by an abnormal increase of immature white blood cells called "blasts". In turn, it is part of the even broader group of diseases affecting the blood, bone marrow, and lymphoid system. Patients with hematologic malignancies such as leukemia are susceptible to infectious complications, because the host is immunocompromised by both malignancy and cytotoxic treatment (9).

Materials and Methods:

Sample collection: During the period that confined between "October 2013 to May 2014", 140 samples (oral swabs, skin fragment and nail clippings) were collected from 125 patients with different types of leukemia who diagnosed clinically by specialist doctors. All patients were admitted at Baghdad Educational Hospital, 7th floor, Baghdad province. Oral swab samples were cultured on plates with Sabouraud's Dextrose Agar Medium (SDA), for 2-3 days, at temperature 37 °C, while nail clippings and skin fragments were examined directly by microscopic examination and then they were cultured on plates with SDA medium, for 3-7 days, at temperature 37°C.

In this study a swab of a tensional site as a relatively simple method for detection the growth of *candida spp.* was taken from leukemia patients whom admitted at Baghdad Educational Hospital, in Baghdad Province. The collection of samples involves gently rubbing over the tensional tissue by using a sterile transport media cotton

swab. Then all samples were subsequently inoculating in a primary isolation medium such SDA.

Note: in original theses samples were collected from 125 healthy individuals (control group) to compare the frequency of fungal isolates.

Isolation of fungi: Techniques available for the isolation of *Candida* within the oral cavity include the use of a smear, a plain swab (10), an imprint culture (11), collection of whole saliva (12), and mucosal biopsy. Each method has particular advantages and disadvantages and the choice of sampling technique is primarily governed by the nature of the lesion to be investigated .Where an accessible and defined lesion is evident, a direct sampling approach such as the use of a swab or an imprint is often preferred as this will provide information of the organisms present at the lesion itself.

Detection of *Candida spp*.

The following identification tests were used to identify the *Candida spp.*:

1. Germ Tube Formation (GT)

It includes the production of germ tube by the yeast isolates. It was tested by inoculation a small portion of an isolated colony in 0.5 ml of human serum. The suspension was inoculated at 37°C for 3hrs. Then a drop of this suspension was put on clean glass slide and examined under light microscope. The germ tube appeared as short lateral hyphen filament. The serum used in this test was prepared by aspirating blood from healthy human being into test tube without anticoagulant agent and then incubated in an upright position at room temperature for 30 minutes, then the samples were centrifuged at 3000 rpm for 15 min, then the serum was aspirated carefully by sterilized micropipette and placed in sterilized tube and stored at deep freeze at -8 °C until use (13).

2. Chlamydospores Production

It is a diagnostic characteristic (pathognomonic) of *C. albicans*. An inoculum was taken from an isolated colony and cultured in plates with Corn Meal Agar (CMA) and incubated at 28 °C for 3 days, and then a loopful of inoculation was examined under light microscope.

3. Urease Test

An inoculation was taken from an isolated colony and added into test tubes with urea base agar and incubated at 37 °C for 4 hrs. Then the changing in the color of indicator (phenol red) from yellow "negative" to pink "positive" result was observed.

4. Sugar Fermentation Test

An inoculation was taken from an isolated colony and added into test tubes with Fermentation based media which contain one of the following sugars (Glucose, Maltose, Lactose and Sucrose) and dirham tube, and then these tubes were incubated at 37 °C for 3- 4 days with the observation of color change from red "negative" to yellow "positive" result and with the formation or not of gas in dirham tube (14). (table, 3).

For the identification of opportunistic fungal species colored atlas was used (15).

Results and discussion:

All *Candida* species that were isolated from leukemia patients were illustrated

according to their percentage, from 125 patients who diagnosed with different types of leukemia 82 oral swab samples were collected, 60 samples were positive, and 22 samples were negative. The number of *Candida* isolates was 60 which represent (73.17 %) of the total cases. The most common isolates were *C. guillermondii* 19 isolates which represent (31.66 %) of cases, then followed by *C. itermedia* 11 isolates which represent (18.3 %). (Table, 1, Figure1). The number of opportunistic fungal isolates was 29 which represent (26.12%) from total cases. All opportunistic fungal species that were isolated from both study and control groups were illustrated according to their percentage in each group. The most common isolated species was *A. niger* 17 which represent (58.6%), (Table, 2, Figure 2). One of the important tests for yeast identification is sugar fermentation test (Table, 3).

Table (1): The frequency of *Candida* isolates according to their species

***Candida* spp.**	**Number of isolates**	**Percentage (%)**
C. guillermondii	19	**31.67%**
C. itermedia	11	**18.33%**
C. kyfer	8	**13.33%**
C. albicans	6	**10%**
C. glabrata	5	**8.33%**
C. parasilopsis	4	**6.67%**
C. tropicalis	4	**6.67%**
C. zeylamodes	3	**5%**
Total	**60**	**(100%)**

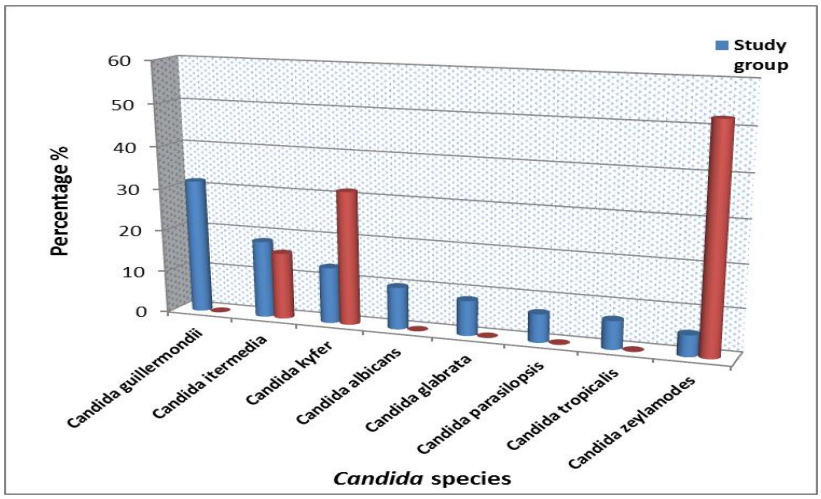

Figure (1): percentage of Candida isolates blue color leukemia patients, red color control

Table (2): The frequency of opportunistic fungal isolates according to their species.

Opportunistic fungal spp.	**Number of isolates**	**Percentage (%)**
A. niger	17	**58.62%**
Penicillium	5	**17.24%**
A. fumigatus	3	**10.34%**
A. flavus	3	**10.34%**
Alternaria	1	**3.44%**
Total	**29**	**(99.98%)**

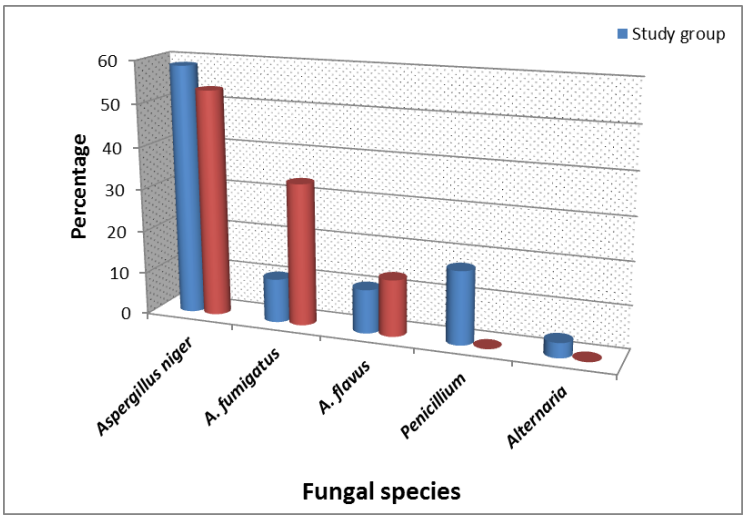

Figure (2): Opportunistic fungal species blue color leukemia patients, red color control

Table (3): Biochemical reactions for yeast identification

Species	GT	UR	Fermentation of			
			G	S	L	M
Candida albicans	+	-	+	-	-	+
C. guilliermondii	-	-	+	+	-	-
C. intermdia	-	-	+	+	-	+
C.kefyr (C. pseudotropicall)	-	-	+	+	+	-
C. krusei	-	+	+	-	-	-
C. parapsilosis	-	-	+	-	-	-
C. stellattodea	+	-	-	-	-	-
C. tropicalis	-	-	+	-	-	+
C. zeylamodes	-	-	+	+	-	+
Cryptococcus difflueas	-	+	-	-	-	+
Cr. Laurentii	-	+	-	-	-	-
Rhodotrule glutinis	-	+	-	-	-	-
R. rubra (R. mucilayinosa)	-	+	-	-	-	-
Saccharomyces cerevisiae	-	+	-	-	-	-
Torulopsis candida (C. famata)	-	-	+	+	-	+
T. slabrata (C. glabrata)	-	-	-	-	-	-
Trichosporon beigeieii	-	-	+	+	-	-
(Tr. cutaneum)	-	+	-	-	-	-
Tr. capitatum	-	-	-	-	-	-

GT: Germ Tube Formation

UR: Urease Test

G: Glucose, **S:** Sucrose, **L:** Lactose, **M:** Maltose

Statistical analysis showed significant difference at level of probability p (<0.05) between *Candida* species in study group.

Candidiasis is the most common opportunistic fungal infection. *Candida* infections are a major problem in the world, especially among cancer

patients, (16- 17). *C. albicans* is the most common cause of candidiasis (18). Superficial candidiasis may involve the epidermal and mucosal surfaces, including those of the oral cavity, pharynx, esophagus, etc…Aspergillosis a disease which caused by *Aspergillus spp*., Zygomycoses due to members of zygomycetes, all these cases are increased in people with immunno-difeciancy syndrome due to courses of broad spectrum antibiotics, cytotoxic chemotherapy, corticosteroids, and vascular catheters (18). Fungal infections remain an important cause of morbidity and mortality in patients with acute or chronic leukemia (19). *Candida* infections are a major problem in the world, especially among cancer patients (20) *Candida spp*. is members of normal microbiota which could invade the living tissues and cause oral candidiasis or life-threatening diseases (21). In this study, oral candidiasis was the most common disease in study group; this result was agreed with previous work of (22) who indicated that the fungal infections are more frequent in hematological malignancy patients than patients with solid tumors. There was agreement with (23) who concluded that oral candidiasis was the only disease (22%) in study group (100 leukemia patients). *Candida* may cause opportunistic infections in immunocompromised hosts, such as patients suffering from AIDS, leukemia and diabetes (24), *C.guillermondii* was the most frequent *Candida* species (19) isolates.

Statistical analysis had shown significant difference at a level of probability P (< 0.05) between opportunistic fungal infections in both groups of this study.

An opportunistic microorganism such as *Aspergillus* sp. which is generally harmless in its normal environment may acts as pathogenic in a compromised patient (24).

An immunocompromised person may be particularly vulnerable to opportunistic infections, in addition to normal infections that could affect everyone. Fungal infections are often classified as opportunistic or primary. Opportunistic infections are those that develop mainly in immunocompromised hosts; primary infections can develop in immunocompetent hosts (25).

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