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Isolation, Characterization, Distribution and Antifungal Sensitivity *of Candida albicans* from Oral Cancer Patients

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Abstract
The aim of this study was to investigate the prevalence of oral candidiasis in cancer patients and their antifungal susceptibility. A total of 50 cancer patients were included in this study, oral examination was done and oral swabs were taken from all the participants for yeast culture, identification and susceptibility testing to Fluconazole and two volatile oils by agar test. Oral candidiasis was prevalent in 23 (46%) of all cancer patients. The highest rate of total candidiasis was seen in male 12 (52.2%) than female 11 (47.8%). The highest prevalence of *Candida albicans* was in the age group 41-60 years which was 13 (56.52%) while the lowest was in children 2(8.96%). The overall sensitivity of *C. albicans* to Fluconazole and Clove oil was 100% and 99% respectively, and no effect of coriander oil was found. The study revealed that the oral candidiasis was prevalent in patients who use chronic antibiotics, chewing qat, and tobacco smoking.
Keywords: Antifungal Sensitivity, Candidiasis, *Candida albicans*, Prevalence.

INTRODUCTION

The term *Candida* originates from the Latin word candid, meaning white. The spores of *Candida* are a commensally, harmless form of a dimorphic fungus that becomes invasive and pathogenic pseudohyphae when there is a disturbance in the balance of flora or in debilitation of the host (Raju and Rajappa 2011). Candidiasis is one of the most common diseases of human caused by several species of *Candida* (Jasim *et al.,* 2016). Oral Candidiasis is a common opportunistic infection of the oral cavity caused by an over growth of *C. albicans* (Al-Hakami et al., 2016).

Oropharyngeal candidiasis is a common fungal infection in immunocompromised individuals. Conditions like malignancies, chemotherapy, and radiotherapy compromise the cell mediated immunity predisposing the person to fungal infections (Jayachandran *et al*., 2016). According to the literature, *C. albicans, C. tropicalis, C. parapsilosis, C. krusei, C. guillermondii, C. glabrata* and *C. dubliniensi*s have been isolated in the oral cavity. *C. albicans* possess pathogenic site factor that allows it to develop disease more frequently than other species of *Candida* (Castellot and Soriano 2013).

Currently, an increase in the number of yeasts that are resistant to antifungal drugs is recognized worldwide; therefore, the use of in vitro laboratory tests may aid in choosing an appropriate therapy (Jasim *et al.,* 2016). The pathogenesis of candidiasis combines three factors: host, fungus and oral microenvironment-modifying factors (Castellot and Soriano 2013). Cancer is one of the major causes of morbidity and mortality in the modern era (Jain *et al*., 2016). A routine oral examination of cancer patients has revealed greater incidence of candida infections than that in most other type of patients in the United States, Europe, and other countries and little is known about this problem in developing countries (Al-Abeid *et al.,* 2004). Cancer progression can be stopped by depriving the cancer cells from vital nutrients (Irfan *et al.,* 2016). Essential oils are used to inhibit the growth of *C. albicans* (Carvalho *et al.,* 2018).

The aim of this study was to determine the prevalence of *Candida albicans* in oral cavity in Yemeni Hospital cancer patients and evaluate the antifungal susceptibility pattern of the isolates to choose the appropriate antifungal drug, and to predict the outcome of the therapy.

MATERIALS AND METHODS

Patients

This study was conducted in the National Cancer Center - Sana'a & Al-Gomhori hospital lab- Sana‘a by using Cohort Descriptive methodology. It took a complete one year (January to December 2017) from preparing phase to data analysis & typing phase and was approved by ethical committee of Sanaa's university. The 50 patients' samples attending the national cancer center - Sana'a, were chosen in order to come up with reliable and adequate results. Thus, the personal data, risk factors, clinical symptoms and oral cavity swabs of the chosen patients were taken. The questionnaire was used to collect patient`s data. Data was analyzed using Excel program.

Collection of isolates

The isolates were collected from 50 cancer patients suffering from oral candidiasis from the National Cancer Center - Sana'a. The sampling approach involves gently rubbing a sterile cotton swab over the lesion tissue of oral cavity and then subsequently inoculating to the medium Sabouraud Dextrose Agar (SDA).

All samples were cultured on (SDA), and then were incubated aerobically at 37ºC for 24-48 hrs. *Candida* isolated was identified depending on the morphological features on culture medium and germ tube formation (Jasim *et al*., 2016).

A single colony was transferred from a sterilized loop to a clean slide containing a drop of KOH to examine under light microscope. *Candida* is a yeast so it can be identified by its oval shape and budding cells. After observing the budding cells, a small amount of the same colony was sub cultured and streaked on a Petri dish containing SDA, and then the media was incubated aerobically at 37°C for 24 hrs in order to obtain a pure culture for the second step.

Germ tube test

This test was used for the identification and differentiation of *C.albicans* from other yeasts (Giri, 2015) in plate 1.

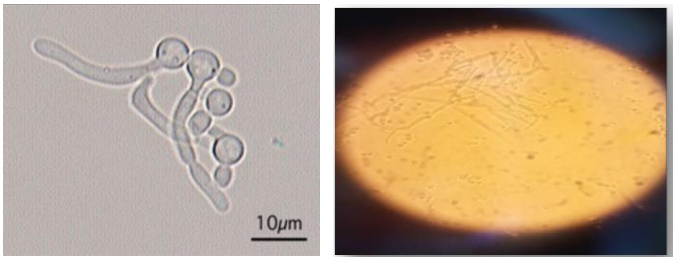

Plate 1. Germ tube formation in *C. albicans* when inoculated at 37 °C in plasma after 2-3hrs

Antifungal activity assay

The antifungal activities of the volatile oils were evaluated by disc diffusion method (Prabuseenivasan *et al.,* 2006; Chedia *et al.,* 2013). The cultures were adjusted to approximately 10^6 Colony Forming Unit/ml with sterile saline solution. One hundred microliters of the suspensions were spread over the plates containing Sabouraud Dextrose Agar (SDA) using a sterile cotton swab in order to

get a uniform microbial growth on both control and test plates.

Fluconazole (10 µg/ ml) was used as positive control. The plates were then incubated at 28 ^{o}C for 24 h for yeast and 3 days for molds after which zones of inhibition were measured and recorded. The zone of inhibition was taken to be the diameter of the zone visibly showing the absence of growth including the 5 mm disk. If there was no inhibition the value of 0 mm was assigned to the test sample. The percentage of inhibition was calculated as followed:

$$\text{Inhibition (\%)} = \frac{\text{Growth diameter of the sample} - \text{Control growth diameter}}{\text{Control growth diameter}} \times 100$$

The zone of inhibition of the tested fungi by the volatile oils were measured using HI Antibiotic Zone Scale - ^{TM}C reader model PW297 (India).

RESULTS AND DISCUSSION

The isolation and identification of *Candida albicans*

In this study the yeast like colonies on SDA agar were identified by classical methods; study of micromorphology and formation of germinative tubes.

The 50 oral cancer patients were from different cities in Yemen, different age and different sex. *C.albicans* was found in 23 (46 %) patients according to KOH test and germinative tube (Plates 2,3, 4 and figure 1). Our results agree with (Samaranayake, 1991) who found 56% were C*. albicans,* also (Jasim *et al.,* 2016) isolated *C. albicans* with 49.09 %. All of our isolates gave positive germ tube test which is against a previous study by Jayachandran et al. (2016) who showed that 95% of the *C. albicans* gave positive germ tube test. There are many species of *Candida* but the most prevalent one which is recovered from the oral cavity, in both commensal state and in cases of oral candidiasis is *C. albicans.* It is estimated that this species accounts for over 80% of all oral yeast isolates (Smith and Shashanka, 2011).

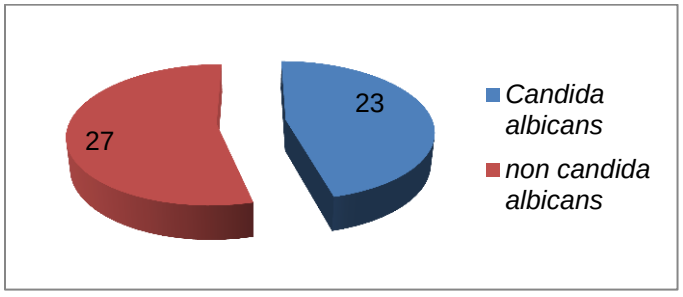

Fig.1. Percentage of *C. albicans* and non-*Candida* isolated from oral cancer patients.

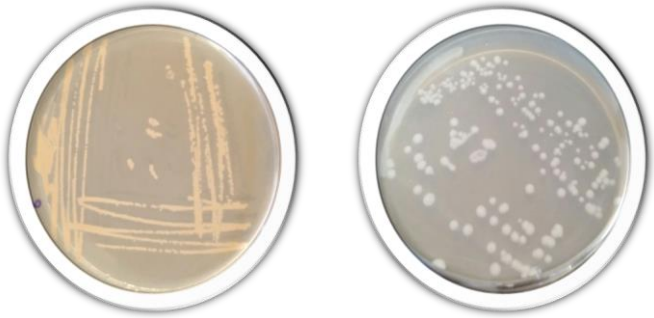

Plate 2. *C. albicans* on SDA medium.

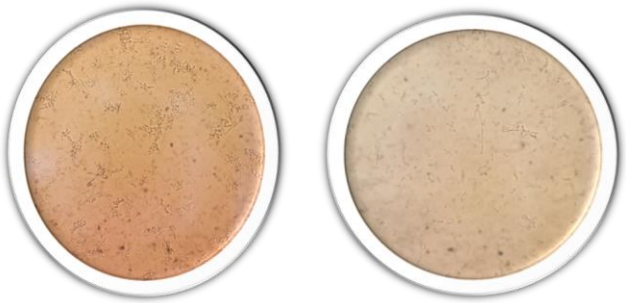

Plate 3. Germ tube test for *C. albicans*

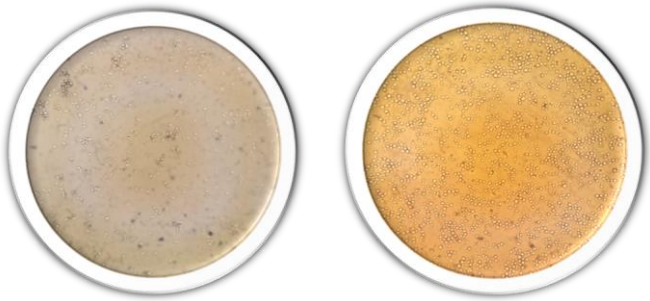

Plate 4. KOH test for *C. albicans*

Isolation of *Candida albicans* from oral cancer patients according to their sex

In our study among 50 oral cancer patients 33 were male and 17 female. We obtained 12 (36.4 %) *C. albicans* isolates from male while *C. albicans* isolated from female were 11 (64.7 %). 21 (77.8 %) non- *C. albicans* were isolated from male and 6 (22.2 %) from female. Our results did not agree with (Grimoud *el al.,* 2003), they isolated *C. albicans* from women with 77.3 % higher than man with 22.7 % (Table 1). This type of candidiasis particularly affects people with immune system disorders and people with dental prostheses. It can also be found in patients receiving chemotherapy for cancer treatment, or taking

immunosuppressive drugs to protect transplanted organs or in patients infected by HIV. In children and young people, it may be involved with oral ("thrush") and lingual disease (Epstein and Polsky, 1998).

Table 1. Distribution of *C. albicans* from oral cancer patients according to gender.

Groups	*C. albicans*		Non-*C. albicans*		Total isolates	
	No.	%age	No.	%age	No.	%age
Male	12	36.4	21	77.8	33	66
Female	11	64.7	6	22.2	17	34
Total	23	100	27	100	40	100

Isolation of *Candida albicans* from oral cancer patients according to their places:

Our study found that out of 20 patients from Sana'a, 11 (47.8%) isolates were *C. albicans*, while 9 (33.4%) isolates were non-*C. albican*s. From 9 patients belonging to AL hodaidah, we isolated 3 (13%) *C. albicans* isolates and 6 (22.2 %) non- *C. albican*s isolates. From Thamar, we collected 6 isolates, 3 (13 %) were *C. albicans*, while 3 (11.1 %) were non- *C. albican*s. From Hajah, we collected 7 isolates, 1 (4.4%) was *C. albicans*, while 6 (22.2%) isolates were non- *C. albican*s. Our study found that 2 patients from Sada contained *C.albicans* (8.6 %). In Taiz, Almahwet, and Ibb we collected 2 isolates each, we found 1 (4.4%) isolate of *C.albicans*, while 1 (3.7 %) isolate of non- *C. albican*s (Table 2).

Table 2. Distribution of *C. albicans* from oral cancer patients according to places.

Isolates	*C. albicans*		*Non- C. albicans*		Total samples	
	No.	%	No.	%	No.	%
Sana'a	11	47.8	9	33.4	20	40
AL Hodaidah	3	13	6	22.2	9	18
Thamar	3	13	3	11.1	6	12
Sada	2	8.6	0	0	2	4
Hajah	1	4.4	6	22.2	7	14
Taiz	1	4.4	1	3.7	2	4
AL Mahwet	1	4.4	1	3.7	2	4
Ibb	1	4.4	1	3.7	2	4
Total	23	100	27	100	50	100

Isolation *Candida albicans* from oral cancer patients according to their age:

We divided the patients into 4 groups according to their age, group 1 (0-20) years, group 2 (21-40) years, group 3 (41-60) years, group 4 (over 60) years. In group 1, we obtained 2 *C.albicans* isolates out of total 3 samples with 8.8% prevalence, while 1(3.7 %) specimen was non *C.albicans*. In group, 2 we obtained 5(21.7%) *C.albicans* isolates from total 9 samples, while 4(14.8 %) samples were non *C.albicans*. In group 3, we isolated 13 (56.5 %) *C. albicans* isolates from total 29 samples while 16 (59.3 %) samples were non *C. albicans*. In group 4, we isolated 3(13%) *C. albicans* isolates from total 9 samples while 6 samples were non *C.albicans (*22.2%) (Table 3).

Pathology and risk factors of oral cancer

We collected some information about 50 oral cancer patients and wrote it as a questionnaire. This questionnaire included the relationship of the presence of *C.albicans* in the oral cavity for oral cancer patients with habits and their history. In our research we studied the site of infection or oral lesions and time of infection for the 50 oral cancer patients that were suspect infection with *C.albicans*, these lesions were different in places from patients to other. The tongue lesions were the highest in 33 (66 %) patients among 50 samples; we isolated *C. albicans* from 13 (39.4 %) patients suffering from tongue lesions, cheek lining lesions were in 10(20 %) patients; from these lesions we isolated 7(70 %) *C. albicans* isolates. Jaw lesions were in 5(10%) patients; all these patients had C*. albicans*.

Table 3. Distribution of *C. albicans* from oral cancer patients according to age.

Age Groups (years)	*C. albicans*		*Non C. albicans*		Total samples	
	No.	%	No.	%	No.	%
0-20	2	8.8	1	3.7	3	6
21-40	5	21.7	4	14.8	9	18
41-60	13	56.5	16	59.3	29	58
>61	3	13	6	22.2	9	18
Total	23	100	27	100	50	100

Gum lesions were in 4(8 %) patients, two (50 %) of them have *C. albicans* and two others patients had non *C. albicans* isolates. Lips lesions were in 3(6 %) patients, two (66.7 %) of them had *C. albicans* isolates, under teeth lesions were in 1 patient with 2 % prevalence, this patient was not infected with *C. albicans* (Table 4, plates 5, 6, 7 and figure 2).

Plate 5. *C. albicans* infection in the tongue.

Plate 6. *C. albicans* infection in cheek lining

Plate 7. *C. albicans* infection in jaw and gum lining

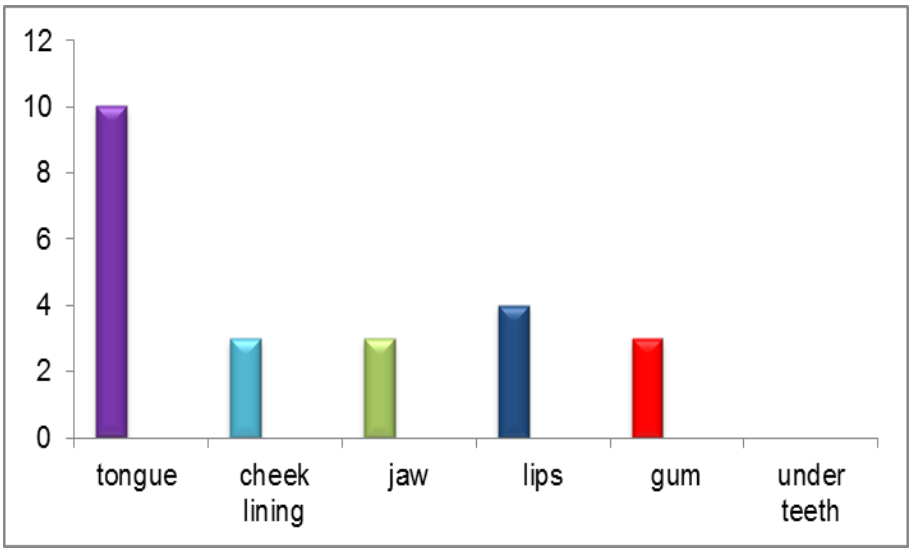

Fig. 2. The prevalence of *C. albicans* according to oral lesions.

Table 4. The presence of oral cancer patients' lesions for *C. albicans* infection.

Site of lesions	*C. albicans* isolates		Non- *C. albicans* isolates		Total samples	
	No.	%	No.	%	No.	%
Tongue	10	43.6	20	74.1	30	60
Cheek lining	3	13	3	11.1	6	12
Jaw	3	13	0	0	3	6
Lips	4	17.4	1	3.7	5	10
Gum	3	13	2	7.4	5	10
Under teeth	0	0	1	3.7	1	2
Total	23	100	27	100	50	100

Among the 50 oral cancer patients infected with *C. albicans* according to their symptoms, we studied 7 symptoms which accompanied to infection with *C. albicans;* pain, oral lesion and itching were in all patients with 100 %, 23 of them were infected with *C. albicans* (46 %) and the rest 27 were non- *C. albicans* isolates.

Abscess was in 48 patients with 96 %, 20 patients were with *C. albicans* infection (41.7 %) and Non- *C. albicans* isolates were in 28(58.3 %), difficulty in swelling was in 23 patients (46 %), 9 (39.1 %) of them had *C. albicans* and 14 had Non- *C. albicans* isolates.

Least symptoms were; redness in 8 patients (16 %), four of them were with *C. albicans* infection and 4 were non- *C. albicans* isolates. Necrosis was in 5 patients, 2 of them were *C. albicans* isolates (Table 5 and figure 3). Jasim et al. (2016) found that dryness of mouth and pain in the oral cavity are the most frequently encountered symptoms for isolation the *C. albicans*.

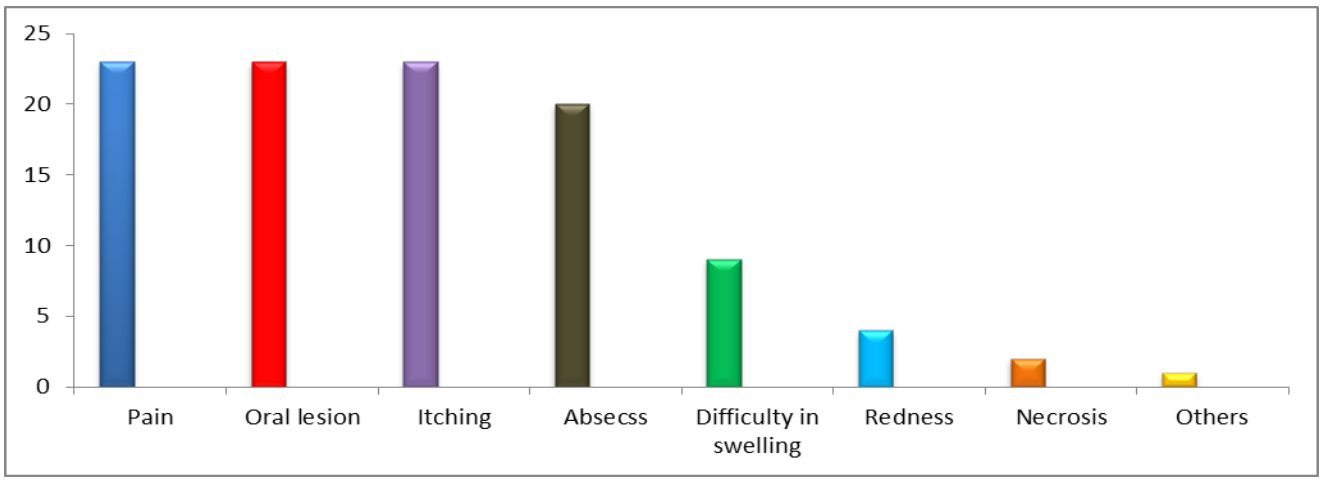

Fig. 3. The distribution of *C. albicans* according to their symptoms

Table 5. The prevalence of oral cancer patients suspected with candida infection according to symptoms.

Patient symptoms	*C. albicans* isolates		Non- *C. albicans* isolates		Total	
	No.	%	No.	%	No.	%
Pain	23	46	27	54	50	10
Oral lesion	23	46	27	54	50	10
Itching	23	46	27	54	50	10
Abscess	20	41.7	28	58.3	48	96
Difficulty in swelling	9	39.1	14	60.9	23	46
Redness	4	50	4	50	8	16
Necrosis	2	40	3	60	5	10
Others	1	25	3	27	4	8

We studied the risk factors for the oral cancer patients with *C.albicans*. Malnutrition was in 40 patients, 20 among them contained *C. albicans*. From 42 qat chewing patients, we isolated 19 *C. albicans*, 34 patients used Shammah, among them we isolated 17 *C. albicans* isolates, 39 patients were using chronic antibiotics, and among them 17 *C. albicans* were isolated with 43.6 % prevalence. 21(42 %) oral cancer patients were suffering from Immuno compromised (diabetes), only 10 patients were infected with *C. albicans*. Smoking factor was found in 9 patients, only 3 of them were infected with *C.albicans*, and just 7 patients were suffering from Rheumatoid disorders, three 3 of them were infected with *C. albicans*. We study risk factors increase the isolation of *C. albicans,* the malnutrition was the highest risk factor for isolation the *C. albicans* with (50 %) (Table 6). A previous study by Curado and Hashibe (2009) showed that tobacco smoking is a major risk factor for oral and oro-pharyngeal cancer.

The sensitive activity of fluconazole and some volatile oils on *Candida albicans.*

In this research eight (8) *C.albicans* isolates were chosen to study the effect of one antibiotic fluconazole, and two volatile oils of clove and coriander plants with 100 % concentration at three concentrations (15, 10 and 5µl). Our results showed that Fluconazole inhibited the growth of all isolates; the inhibition zone for Fluconazole on *C. albicans* was (1.5-2.4mm), while (Jayachandran *et al.,* 2016) showed a resistance of *C. albicans* for Fluconazole at 5.8% isolates. Results of our study showed that Clove Oil had high activity against the tested *C. albicans* isolates, while coriander oil did not have activity against *C. albicans* isolates. Clove oil gave different inhibition zones in diameter according to different concentrations (Table 7 and figure 4). These results agreed with Radwan et al. (2014), they showed clove oil completely inhibited the growth of *C. albicans*.

Table 6. The prevalence of oral cancer patients suspected with candida infection according to risk factors.

Risk factors	*C. albicans* isolates		Non- *C. albicans* isolates		Total	
	No.	%	No.	%	No.	%
Malnutrition	20	50	20	50	40	80
Qat chewing	19	45.2	23	54.8	42	84
Shammah	17	50	17	50	34	68
Antibiotic chronic uses	17	43.6	22	56.4	39	78
Immunocompromised (diabetes)	10	47.6	11	52.4	21	42
Smoking	3	33.3	6	66.7	9	18
Romatoid disorders	3	42.9	4	57.1	7	14

Table 7. The effect of Fluconazole and Clove oil on *C. albicans* by disk diffusion method.

No. of isolates	fluconazole	Volume of clove volatile oil (concentration 100%)		
		5 µl	10 µl	15 µl
	Inhibition zone (mm)			
1	2.1	2.4	2.6	3
2	2.2	2.7	3.1	3.1
3	2.4	2.6	2.9	3
4	1.5	2.1	2.3	2.5
5	1.6	2.3	2.5	2.8
6	2	2.6	2.7	3
7	2	2.3	2.5	2.7
8	2.3	3	3.1	3.2

Note: µl= microliter; Inhibition Zone = mm.

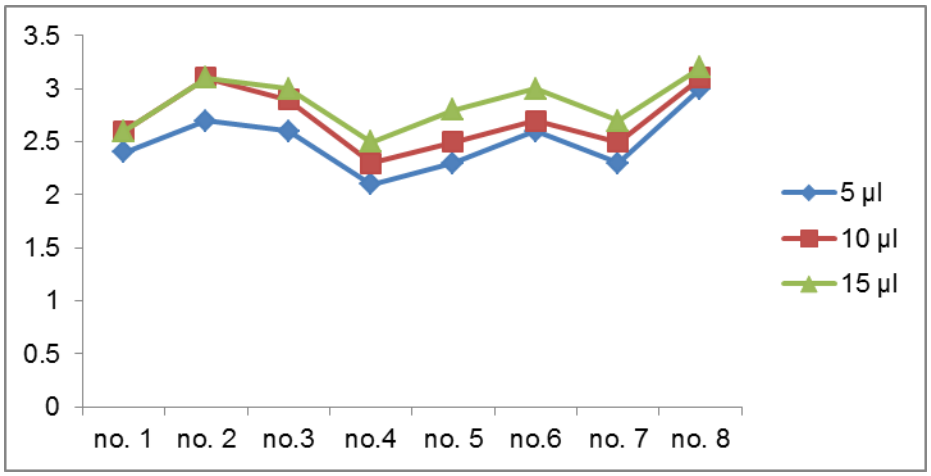

Fig. 4. The sensitivity results of *C. albicans* against Clove oil.

CONCLUSION

The presence of *Candida albicans* in the oral cavity was evaluated in 50 cancer patients, 23(46%) samples from oral candidiasis infection had *C. albicans*. The highest incidence of *C. albicans* was in the age group (41-60) years while children were least infected. Patient coming from Sana'a had more candidiasis infections than those coming from other Governments. Among patients who use chronic antibiotics and chewing Qut, tobacco (smoking or shamae) have the most infection of oral candidiasis. To choose the appropriate antifungal drug, the in vitro antifungal sensitivity results showed Fluconazole and Clove oil were more effective but no effect of cardinor oil was found.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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