

# Oral *Candida* Carriage in Waterpipe and Cigarette Smokers with Various Dietary Habits

ORIGINAL

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## Abstract

**Background:** Tobacco smoking and diet have been both linked to increased oral *Candida* carriage. The aim of this study is to investigate the distribution of *Candida* species in the oral cavity of young individuals smoking cigarettes and waterpipe who have various dietary habits.

**Methods:** Participants were recruited from university students during December 2013, and were divided into three groups; a control non-smoking, a cigarette smoking, and a waterpipe smoking groups. The study sample completed a pre-structured questionnaire on smoking, and dietary habits. Oral swab samples were collected from all participants to determine oral *Candida* carriage and the colonizing *Candida* species. Data were statistically analyzed to determine the significance of association between *Candida* carriage on one hand and smoking status, and dietary habits on the other hand.

**Findings:** A total of 238 students participated in the study and among those only 30 (12.8%) students had oral *Candida* carriage. *Candida albicans* was the most common species to be recovered (56.7%), followed by *Candida dubliniensis* (23.3%). The association between smoking, and perceived dietary habits on one hand and oral *Candida* carriage on the other was not statistically significant.

**Conclusions :** Smoking, and perceived dietary habits seem to have no association with oral *Candida* carriage among a young cohort of university smokers. It is recommended that similar studies are conducted on older age groups to investigate if age has any influence.

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## Keywords

Oral *Candida* Carriage; Colonization; Diet; Narghile; Waterpipe; Cigarettes.

## Background

*Candida* species are fungi that colonize the gastrointestinal tract, and the oral cavity of healthy humans [1, 2]. The reported incidence of *Candida* carriage in the oral cavity demonstrated a wide variation in healthy persons ranging 40-60% [1].

*Candida* species becomes an opportunistic pathogen in immunocompromised hosts, causing various systemic, superficial and invasive infections, where *C. albicans* is considered the most common *Candida* species causing such infections [3, 4].

Whereas immunocompromised individuals are the most susceptible category of patients to *Candida* infections, a number of local oral factors can predispose to such infections in healthy individuals. These factors include persistent dry mouth, and oral appliances like dentures and fixed and removable orthodontic appliances [5]. Certain habits like high-sugar diet, and tobacco smoking [3, 6] have also been implicated in the development of oral *Candida* infections.

Waterpipe, which is also called hookah, shisha/sheesha, hubble-bubble, argihleh and narghile, is a special instrument used to smoke tobacco. Waterpipe smoking has been a traditional type of smoking in wide areas of Asia and Africa. A new trend has been witnessed lately in waterpipe smoking showing a high popularity among adolescents and youth, increased popularity among youth in Europe and North America particularly university students, and inclination of females of all age groups to smoke waterpipe in societies that discourage women smoking cigarettes [7].

In waterpipe smoking the smoker inhales through a plastic or leather hose, connected to a bowl of water, in order to draw air over the burning charcoal which is placed on top of perforated aluminium foil, which in turn contains tobacco or maassel (a mixture of tobacco, fermented chopped fruits, glycerin and flavorings).

Health risks of waterpipe smoking have been studied extensively in recent years, but the micro-

biological and infectious aspects have received little attention. On the other hand, there has been some controversy about the relationship between smoking and oral *Candida* carriage, so it is important to investigate this relationship among waterpipe smokers as well as cigarette smokers. The importance of identifying *Candida* species is becoming essential for studying the epidemiology, pathogenicity and treatment of oral candidiasis [8]. This becomes more important when smoking habits are combined with the reported high sugar consumption among adolescents in some parts of the world [9, 10].

The aim of this study is to investigate the distribution of *Candida* species in the oral cavity of young individuals smoking cigarettes and waterpipe with a particular reference to their self-reported dietary habits.

## Methods

This was a double blind cross-sectional study where sample collection and laboratory investigations were performed by two different teams. The study was ethically approved by the Faculty of Postgraduate Studies at The University of Jordan.

Participants were recruited from university students who were invited to an oral health awareness campaign on campus during December 2013. Inclusion criteria were: both genders, either an exclusive cigarette smoker on a daily basis, or an exclusive waterpipe smoker on a daily\ weekly basis, or a non-smoker, and is not known to be medically compromised with no past history of intake of anti-fungal medications. After signing a consent form, participants completed a pre-structured questionnaire. It was written in Arabic and it included questions on:

- Age, gender, daily/weekly frequency of waterpipe smoking for waterpipe smokers, daily quantity of cigarettes for cigarette smokers and age of onset for each type of smoking.

- Dietary habits consisting of three options; mainly soft, mainly fresh fruits and vegetables, and mainly sweets.

Questionnaires were completed by participants who were guided throughout the questionnaires by three senior dental students who were trained and calibrated for this purpose to ensure that correct and accurate answers were given. Reliability testing was performed for them to ensure that they consistently produced the same outcome.

Oral swab samples of the buccal mucosa were collected using sterile cotton swabs pre-moistened with 0.9% saline and transported using 1 ml normal saline as a transport medium. All oral samples were transferred immediately to the Microbiology research laboratory of the Faculty of Medicine at the University of Jordan.

Oral samples were inoculated directly onto Sabouraud dextrose agar plates (SDA, Oxoid, UK) and incubated at 37°C for 48 hours. The presence of white to creamy white growth of yeast-like colonies on the culture plates was indicative of *Candida* species.

These were identified initially by preparing germ tube test in human serum followed by sub-culturing the isolated colonies on CHROMagar *Candida* plates and incubating them at 37°C for 48 hours. *Candida* isolates that were observed on CHROMagar *Candida* medium were identified primarily by pigmentation and colony morphology according to the manufacturer's instructions and as described by Odds and Bernaerts [11]. Optimum color intensity of some *Candida* isolates was recorded after 48 hours of incubation. Throughout the study reference standard strains of *Candida albicans* (ATCC 10231), *Candida krusei* (ATCC 6258) and *Candida glabrata* (ATCC 90876) were included on plates of CHROMagar *Candida* medium as control strains.

Confirmation of the identified of *C. albicans* and *C. dubliniensis* was further done by PCR. Extraction of DNA from fresh *Candida* growth in Yeast Pep-

tone Dextrose broth was performed using Wizard Genomic Purification Kit (Promega, USA) according to manufacturer's instructions. Identity of *C. albicans* and *C. dubliniensis* was confirmed using primers derived from unique rDNA sequences specifically ITS-1 and ITS-2 regions of rDNA and duplex PCR. Other *Candida* species were detected using three 10-mers (OPA-18, OPE-04, OPE-18), and RAPD PCR was carried out with some modifications [12]. The amplification of these sequences was performed in a total volume of 25 µL. Qiagen PCR buffer 10x (2.5 µl) and Qiagen MgCl<sub>2</sub> 25 mM (0.375 µl) and Dntps mix 10 mM (2 µl) were added to the mixture. The primer quantities used in the PCR reaction were; (0.5 µl) of *C. albicans* (CAL) primers and (0.5 µl) of *C. dubliniensis* (CDU) primers. 0.25 µl KAPA Taq polymerase and 4 µl of the extracted DNA were added to the reaction mixture. A final volume of 25 µL of the PCR reaction mixture was achieved by adding nuclease-free water. PCR conditions were set in a PCR thermocycler with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for one minute, then annealing at 55°C for 30 seconds, and extension at 72°C for one minute, and a final extension at 72°C for 10 minutes. The PCR product with a volume of 8 µl was loaded into wells of 2% agarose gel with 1X TBE buffer and was run for one hour at 110 volts. A 100- bp DNA ladder was used as the molecular size marker. The agarose gel was visualized by UV Transilluminator (UVP) system.

### Statistical analysis

Data generated from the study were tabulated as Microsoft Excel sheets and uploaded to Statistical Package for Social Sciences (IBM SPSS version 20). It was used to calculate; frequency and percentage for the categorical data, values of significant differences between the groups and to carry out regression analysis. Regression analysis was used to calculate the values of significance for the variable risk factors

associated with waterpipe smoking and cigarette smoking. In all statistical tests, the differences were considered to be statistically significant if P-value was < 0.05.

## Results

### Description of sample

A total of 238 students participated in the study. Their ages range was (16-28) years. Students were categorized into three groups. The first group were non-smokers or control (n=80) age range of 16-28 years (mean=21.02, SD=2.225) with 27 males and 53 females. The second group were cigarette smokers (n=77) age range of 18-26 years (mean=21, SD=1.923) with 75 males and two females. Finally, the third group constituted waterpipe smokers (n=81) age range of 17-26 years (mean=21, SD=1.42) with 42 males and 39 females.

**Table 1** shows the age of onset of smoking and the quantity for waterpipe and cigarette smokers

### Oral *Candida* carriage rate and the influence of dietary habits

A total of 30 (12.8%) *Candida* species were recovered from oral swab samples on SDA that belonged to smokers and control groups. Isolated *Candida* spp were mostly *C. albicans* (17, 56.7%), *C. dubliniensis* (7, 23.3%), and other *Candida* spp (6, 20%). There were 9 (11.25%) out of 80 non-smokers, 9 (11.7%) out of 77 cigarette smokers, and 12 (14.8%) of waterpipe smokers who had oral *Candida* carriage. Details of the isolated *Candida* spp from the three groups are shown in **Table 2**.

According to frequency of smoking waterpipe, 8/81 (9.9%) of waterpipe smokers, had *Candida* species colonizing their oral cavity, smoke waterpipe daily, and 4/81 (4.9%) waterpipe smokers, had *Candida* species colonizing their oral cavity, smoke waterpipe weekly. **Table 1** shows that there was no statistically significant difference in *Candida* carriage among the three study groups. There was

also a non-significant statistical difference between dietary habits and oral *Candida* carriage (P=0.983)

**Table 1.** Frequency of waterpipe smoking, quantity of cigarette smoking and age of onset for each type of smoking. P value indicates the significance of association between the shown variables and oral *Candida* carriage.

Tobacco smoking type	No	%	P value
Waterpipe smokers	76.19		
Frequency			
Daily	30	37.0	0.096
Weekly (, %)	51	63.0	
Age of onset (years)			
<18	14	17.3	0.61
≥18	14	17.3	
Can't remember	53	65.4	
Cigarette smokers	77		
Cigarettes per day			
5	9	11.7	0.502
15	33	42.9	
25	35	45.4	
Age of onset (years)			
<18	49	63.6	0.976
≥18	25	32.5	
Can't remember	3	3.9	

**Table 2.** *Candida* species isolated from the oral cavity of the study subjects.

<i>Candida</i> species	Non-smokers (n=80)	Cigarette smokers (n=77)	Waterpipe smokers (n=81)	Total	
				no	%
<i>C. albicans</i>	5	4	8	17	56.7
<i>C. dubliniensis</i>	2	3	2	7	23.3
<i>C. glabrata</i>	1	1	0	2	6.7
<i>C. krusei</i>	1	0	0	1	3.3
<i>C. parapsiiosis</i>	0	1	2	3	10
Total	9	9	12	30	100

## Discussion

The aim of this study was to investigate the prevalence of oral *Candida* carriage in a young cohort of smokers and non-smokers who have various dietary habits known to be popular in this geographic area (soft diet, sweets, fruits and vegetables).

We not only included cigarette smokers but also waterpipe smokers because of the increasing popularity of this type of smoking among youth in wide areas of the world including Europe and North America.

Prevalence of oral *Candida* carriage in our sample was as low as 12.8%; 14.8% among waterpipe smokers, and approximately 12% among cigarette smokers and non-smokers; however, there was no statistically significant difference in the carriage rate among the three groups.

This percentage is relatively low in comparison with the findings of other studies that found a prevalence of up to 60% among healthy humans. This may be explained by the young age of participants as their age mean was around 20 years; it is reported that the prevalence of *Candida* carriage is proportional to age [13], particularly increasing in those who are above 70 years [14]. Similar studies have reported a low prevalence for oral *Candida* carriage in young people. MAJIMA *et al* (2014) reported a carriage rate of around 18% in a cohort of dental students [15]. Another study done by NEGRONI *et al* (2002), also among dental students, showed a low rate of around 21% [16]. There was no statistically significant difference in oral carriage of *Candida* among non-smokers and smokers whether it was cigarettes or waterpipe. Comparable results were obtained in an earlier study conducted among cigarette smokers of a similar age group [16]. Several studies have, on the other hand reported that tobacco smoking either alone or in association with other factors, is associated with increased oral *Candida* colonization [17-19], and the relationship between frequency of smoking and the *Candida* carriage is proportional [20, 21].

The theory that supports a role of smoking in increasing oral *Candida* carriage is based upon the fact that nicotine thickens the epithelial keratinized layer in the oral cavity and causes structural and functional changes in keratinocytes facilitating fungal colonization and increasing the possibility of candidiasis [4]. Furthermore, *C. albicans* uses tobacco constituents as nutritional factors, and its virulence factors change accordingly leading to the formation of some chemical compounds [3, 4]. Studies investigating the influence of waterpipe smoking on oral mucosa particularly on epithelium are unfortunately scarce. Studies that were conducted so far aimed at investigating the carcinogenic potential of waterpipe. SEIFI *et al* [22] found that waterpipe smoking affects oral epithelium by increasing the nucleus/cytoplasm ratio but to a lesser degree than cigarettes. Another study conducted by AL-AMRAH *et al* [23] found that waterpipe smoking causes DNA damage of the buccal cells. Albeit the fact that no thickening of the keratinized layer of oral epithelium due to waterpipe smoking was reported before, SEIFI *et al* [22] found that oral *Candida* carriage is higher in waterpipe smokers than non-smokers. Considering the higher mean age of their study sample (30 years), this may explain the higher oral carriage rate. Other factors that may play a role in the different outcome could possibly be attributed to the frequency and duration of waterpipe smoking.

Although there was no significant association between smoking and oral *Candida* carriage in our study, most subjects with positive *Candida* carriage, can be considered heavy smokers either by using waterpipe daily or smoking more than 20 cigarettes per day.

Local oral factors that were investigated in this study included self-reported dietary habits. Although there was no statistically significant difference between the investigated types of soft, sweet and fruit/vegetable diet, it was noticed that a larger number of *Candida* species colonized the oral cavity of subjects consuming the soft diet. Carbohydrate-rich

diet in general, increases adherence of *Candida* to epithelial cells and hence increasing the risk of infection [24]. It is also known that waterpipe smoking utilizes materials that may be rich in sugar like the tobacco mix and the cooling liquid [7]. This, combined with the fact that sugar consumption encourages oral *Candida* carriage [25], suggest that more research needs to be conducted on that area.

## Conclusions and Recommendations

This study showed that there is no association between waterpipe smoking and oral *Candida* carriage in a sample of young university students. There was also no association between the reported dietary habits with oral *Candida* carriage.

It is recommended that more studies are conducted to study the association between waterpipe smoking and oral *Candida* carriage in an older age group to include the middle aged and elderly

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## Competing and conflicting interests

Authors declare that they don't have conflict of interest

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