Vol.66: e23230400, 2023
https://doi.org/10.1590/1678-4324-2023230400
ISSN 1678-4324 Online Edition

Article - Human and Animal Health

Oral Yeast Load and Species of Young Individuals Aged 18-25

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Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 02-May-2023; Accepted: 31-May-2023

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HIGHLIGHTS

- Individuals between aged18-25 carry Candida in their mouths without symptoms.
- Specific or antibiotic media should be used to determine the oral yeast load.
- MALDI-TOF MS is a fast, inexpensive, and effective method for yeast identification.

Abstract: Microbiota is considered an organ that affects the health of the human body and includes many microorganisms such as bacteria, yeast, mold, viruses, protozoa, and archaea. Microorganism such as *Candida* sp., a part of the microbiota, is known to cause diseases in the case of opportunistic pathogens under various conditions. Yeast loads and species in the oral mycobiota of young individuals aged 18-25 were determined in our study. Two methods, centrifugation and dilution, were used to determine the oral yeast load. Samples were taken from 31 individuals for the centrifugation method, including 29 for the dilution method and five of these individuals. The samples were inoculated on Sabouraud 2% Dextrose Agar (SDA) and SDA containing chloramphenicol (SCAF). As a result, in young individuals aged 18-25, the oral yeast load was 0.01±0.01 and 1.87±0.01 log CFU in SCAF, and it was 0.01±0.01 and >3.00±0.01 log CFU in SDA. A total of 400 isolates were taken from the counting plates, and 140 were determined to be yeast by gram staining. Germ tube test of the isolates determined to be yeasts determined that 108 were probably *Candida albicans* or *Candida dubliniensis*. 140 isolates were also identified by MALDI-TOF MS. Isolates were identified as *C. albicans* (38.58%), *C. albicans* or *C. africana* (3.57%), *C. dubliniensis* (32.86%), *C. parapsilosis* (20%), *C. inconspicua* (2.14%), *Pichia manshurica* (2.14%), and *Wickerhamomyces subpelliculosus* (0.71%). It has been determined that young individuals between the ages of 18-25 carry *Candida* species in their oral mycobiota.

Keywords: oral mycobiota, oral yeast load, *Candida* spp., MALDI-TOF MS.

INTRODUCTION

Like all other complex multicellular eukaryotes, the human body has many microorganisms inside or on its surface. Bacterial components make up 99% of the total microbial load. They are therefore referred to as the core microbiome, while the remaining less abundant and more diverse microbiota forms a “rare biosphere”. Fungal microbiota (mycobiome) constitutes an essential part of the “rare biosphere”. Although the percentage of mycobiome is low, its impact on human health and disease is far-reaching. For example, over 600 of the 5.1 million fungal species that make up the rare biosphere are estimated to cause human diseases ranging from mild superficial disorders to severe diseases to life-threatening systemic infections [1].

There are many microorganisms in the oral cavity, and this microbial community is influenced by different oral structures and tissues, diet, dental hygiene, xenobiotic, and host genetics. Some microorganisms isolated from the oral cavity play a role in forming oral diseases such as dental caries, periodontitis, and tonsillitis. In short, the main components necessary for the balance between health and disease depend on the microorganisms in the oral cavity and their interrelationships. In addition, examining oral microbiology, the gateway to the gastrointestinal tract and respiratory system, is very important and will help us understand the microbial communities in health and disease [2-4].

Many individuals carry *Candida* species asymptomatically in their mouths, and the prevalence of *Candida* species increases with age. However, *Candida* species can cause various acute and chronic infections. Clinical manifestations of oral candidal infection range from acute pseudomembranous plaques characterized by inflamed and red-white areas on the mucosa to erosive erythroplakia and leukoplakia lesions of chronic atrophic candidal mucositis. Burning and stinging sensations, the classic symptoms of candidal infection, are due to damage to the mucosal surface. Microbial production of carcinogenic acetaldehyde has been associated with oral cancer. The oral cavity can also act as a source of deep or systemic candidiasis in severely immunocompromised patients, such as AIDS, or immunocompromised patients, such as neutropenic patients [4-6].

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used since the late 1990s to identify microorganisms. This method produces unique spectral fingerprints for each microorganism and strain typing, and microorganisms are identified. This identification technique has revolutionized the routine identification of microorganisms in clinical microbiology laboratories by offering an easy, fast, accurate, cost-effective, and highly efficient system. It supports potential applications not only for clinical laboratories but also in many fields, including medical diagnostics, biological defence, environmental monitoring and food quality control. Therefore, it is suitable for high-throughput and rapid microbial identification at low costs and is an alternative to traditional phenotypic characteristics and gene sequence identification techniques [7].

As a result, the microbiota is a very important part of the human body and is considered an organ. The oral microbiota, which contains many microorganisms such as bacteria, yeast, mold, protozoa and archaea, is an important part of the human microbiota. While bacterial microbiota is generally determined in microbiota studies, there are fewer studies on fungal microbiota. Therefore, this study aims to determine the cultured yeast load in the oral mycobiomes of individuals aged 18-25 and to identify the isolated yeast strains with traditional methods and MALDI-TOF MS.

MATERIAL AND METHODS

Research Population and Sample

The research method is a cross-sectional and descriptive study. The research universe consists of 4th-grade students of Çanakkale Onsekiz Mart University, Faculty of Health Sciences, Department of Nursing and Midwifery in the 2018-2019 academic year.

We obtained permission for our study from the school administration and ethics committee approval from Çanakkale Onsekiz Mart University Clinical Research Ethics Committee (No:18920478–050.01.04–E180031135). Before starting the data collection process, researchers informed the participants about the purpose and scope of the research, and we obtained their verbal and written consent that they agreed to participate in the study. The research was carried out by the principles of the Declaration of Helsinki.

Sampling Method

The sampling procedure was chosen to take into account the previous studies. Sampling was done between 11:00 and 14:00. Students were told not to eat or brush their teeth in the last hour before sampling. For the dilution method, samples were taken from 29 participants at 12:00 on 30.04.2019. For the centrifuge

method, samples were taken from 31 participants (5 from the dilution method group) at 12:00 on 08.05.2019. In the sampling, the mouthwash method was applied for 1 minute using 10 mL of sterile PBS [8-10]. Samples were transported to the laboratory in the cold chain immediately after collection and stored at +4ºC until analysis. Analysis was done within 6 hours. While taking the samples, a questionnaire of 20 questions was applied to the participants simultaneously.

Microbiological analyses

First, the yeast loads of the samples were determined by two different methods. In the dilution method, serial dilutions of the samples up to 10-4 were prepared and inoculated on Sabouraud Dextrose Agar medium containing chloramphenicol (SCAF) according to the spread plate method (100 µL). For each sample, two petri dishes were inoculated from each dilution, and 8 SCAF media were used [9,11,12]. In the centrifuge method, the samples were first centrifuged at 4000 rpm for 20 minutes. After centrifugation, the supernatant was discarded 8. 400 µL of sterile pH:7 PBS was added to the pellet and vortexed for 15 seconds. Then, it was inoculated into Sabouraud Dextrose Agar (SDA) and SCAF medium using the spread plate method (100 µL). A total of 4 media were inoculated for each sample, including 2 SDAs and 2 SCAFs [9,11]. The petri plates were incubated at 37°C for 48 hours in both methods. After incubation, firstly, counting was made, and then isolates were taken from counting petri plates. Each sample count plate was pooled together and evaluated visually for isolation. In the visual evaluation, isolates were taken by considering colony structure criteria such as colony colour, colony margin structure, and colony height. The isolated isolates were first purified by inoculation into petri plates containing SDA. Stock from single-drop colonies was transferred to tubes containing Sabouraud Dextrose Broth (SDB). Whether the isolates were yeast or not was determined by slide-lamella preparation.

Identification of Yeast Isolates

A germ tube test (GTT) was performed to identify *C. albicans* or *C. dubliniensis* species from the isolates. For GTT, 10 µL of pure culture from SDB growing fresh culture was added to 0.5 ml of human serum. Then, after incubation at 37°C for 2 hours, germ tube formation was determined by lam-lamella preparation [13].

MALDI–TOF MS analysis was performed as described in Taban and Numanoğlu Çevik (2021) [14]. Bruker Microflex (Bruker, Biotyper; Bruker Daltonics, Bremen, Germany) Flex Control 3.0 software was used for yeast identification.

Statistical Analysis

All data were evaluated using SPSS 23 program with 95% confidence interval.

RESULTS

Two different methods were used in this study. Samples were taken from 29 people in the dilution method and from 31 people in the dilution method. The five participants also participated in each sampling method. For this reason, the study was carried out with 55 people.

In the dilution method used to determine the oral yeast load of the participants, samples were taken from 29 people. It was determined that there were four colonies in one person and one colony in two participants, and only three participants had yeast load in the SCAF medium. Yeast load was determined in 12 participants in SCAF medium, out of 31 participants by centrifugation method. While yeast load could not be determined by the dilution method of 5 participants participating in two samplings, yeast load was determined in two of them by centrifugation method.

It was determined that the yeast load in the SCAF medium ranged from 0.00±0.01 (1 CFU) to 1.87±0.01 (74 CFU) log CFU. However, it was determined that the total load of bacteria and yeast in the SDA medium from the same samples varied between 1.79±0.0 and >3.00 log CFU (Table 1).

Table 1. Microbial load determined in participants by centrifugation method.

Participants No	SCAF Log CFU	Yeast İsolates Number of SCAF	SDA Log CFU	Yeast İsolates Number of SDA	Bacteria Isolates Number of SDA
1	1.87±0.01*	9	1.95±0.04	6	4
5	-	0	>3.00±0.01	0	15
7	1.33±0.18	5	1.79±0.04	8	-
20	-	0	>3.00±0.01	0	15
25		0	>3.00±0.01	0	15
30	-	0	>3.00±0.01	0	15
31	-	0	>3.00±0.01	0	15
32	0.95±0.11	7	>3.00±0.01	11	0
33	-	0	>3.00±0.01	0	16
34	-	0	>3.00±0.01	0	7
35	-	0	>3.00±0.01	1	8
36	1.16±0.06	5	>3.00±0.01	4	2
37	-	0	>3.00±0.01	0	15
38	-	0	>3.00±0.01	0	11
39	-	-	>3.00±0.01	0	10
40	-	-	>3.00±0.01	0	4
41	-	-	>3.00±0.01	0	16
42	0.78±0.01	5	>3.00±0.01	5	0
43	-	-	>3.00±0.01	0	10
44	1.32±0.03	5	>3.00±0.01	4	6
45	-	-	>3.00±0.01	0	16
46	0.00±0.01	1	>3.00±0.01	0	16
47	1.71±0.01	8	>3.00±0.01	8	2
48	-	-	>3.00±0.01	0	7
49	1.68±0.01	7	>3.00±0.01	7	3
50	-	-	>3.00±0.01	0	14
51	0.90±0.42	5	>3.00±0.01	4	1
52	0.72±1.01	5	>3.00±0.01	5	0
53	0.75±0.64	5	>3.00±0.01	4	1
54	-	-	>3.00±0.01	0	4
55	-	-	>3.00±0.01	0	12

-: microbial load could not be determined. *Results are given as mean + standard deviation.

It could not be evaluated statistically because of the dilution method's results (1 CFU = 0.00 ± 0.01 log CFU and no yeast growth = 0.00 ± 0.01 log CFU) obtained. For this reason, yeast loads and questionnaire results of 31 participants who participated in the centrifuge method were evaluated statistically. Demographic characteristics and oral hygiene information of 31 participants are given in Table 2. It was determined that there was no statistically significant difference between yeast loads and the demographic and oral hygiene of the participants (P=0.05).

Table 2. Association of demographic characteristics of study populations and yeast load

Variables	n	%	Yeast load	p-value
Sex				
Female	27	87.1	0.49 ± 0.13	0.107*
Male	4	12.9	0.00 ± 0.01	
Age				
21	5	16.1	0.49 ± 0.33	0.864†
22	16	51.6	0.39 ± 0.16	
23	8	25.8	0.40 ± 0.20	
Marital Status				
Married	2	6.5	0.66 ± 0.66	0.647*
Single	29	93.5	0.41 ± 0.12	
What is the frequency of brushing your teeth?				
Once a day	3	9.7	0.26 ± 0.26	
Two per day	24	77.4	0.39 ± 0.12	0.735†
After each meal	2	6.5	0.86 ± 0.86	
No	0	0	0.00 ± 0.01	
How often do you go to the dentist?				
Once a month	1	3.2	0.00 ± 0.01	
Quarterly	5	16.1	0.53 ± 0.35	0.799†
Once a year	10	32.3	0.36 ± 0.20	
Never without dental distress	13	41.9	0.43 ± 0.18	
Do you use dental floss?				
Yes	8	25.8	0.63 ± 0. 27	0.314*
No	21	67.7	0.32 ± 0.12	
Do you use mouth rinse water?				
Yes	12	38.7	0.58 ± 0.20	0.287*
No	17	54.8	0.29 ± 0.14	
Do you still have health problems with your teeth?				
Yes	12	38.7	0.57 ± 0.18	0.089*
No	17	54.8	0.30 ± 0.15	
Do you have fillings in your teeth?				
Yes	20	64.5%	0.50 ± 0.14	0.105*
No	9	29.0%	0.21 ± 0.21	
Do you have dentures in your teeth?				
Yes	3	9.7	0.57 ± 0.57	0.870*
No	26	83.9	0.39 ± 0.12	
How often do you chew gum?				
Once a day	0	0	0.00 ± 0.01	
Two per day	2	6.5	0.84 ± 0.84	0.500†
As it comes to my mind	22	71.0	0.43 ± 0.13	
No	5	16.1	0.15 ± 0.15	
How often do you change your toothbrush?				
Once in a month	3	9.7	0.56 ± 0.56	
Quarterly	14	45.2	0.23 ± 0.10	0.457†
Once in a six month	9	29.0	0.65 ± 0.27	
As it comes to my mind	3	9.7	0.39 ± 0.39	
Do your gums bleed when you brush your teeth?				
Yes	11	35.5	0.45 ± 0.16	0.408*
No	17	54.8	0.41 ± 0.17	
Have you pulled your wisdom teeth?				
Yes	5	16.1	0.60 ± 0.26	0.395*
No	23	74.2	0.39 ± 0.13	

Cont. Table 2

Do you get dental calculus cleaning?				
Yes	12	38.7	0.32 ± 0.14	0.612*
No	17	54.8	0.48 ± 0.17	
Do you pay attention to the contents of the toothpaste you use?				0.685*
Yes	17	54.8	0.44 ± 0.14	
No	12	38.7	0.37 ± 0.18	
Have you ever used braces?				
Yes	4	12.0	0.19 ± 0.19	0.447*
No	25	80.6	0.44 ± 0.13	
When was the last time you used antibiotics?				
in the last month	5	16.1	0.91 ± 0.38	
in the last three months	9	29.0	0.11 ± 0.10	0.265†
in the last six months	6	19.4	0.25 ± 0.16	
over a year ago	9	29.0	0.54 ± 0.24	
How often do you use alcohol?				
everyday	0	0	0.00 ± 0.01	
once a week	4	12.9	0.43 ± 0.20	0.254†
once in a month	11	35.5	0.00 ± 0.01	
no	14	45.2	0.51 ± 0.18	
Do you use cola or acidic beverages?				
Yes	22	71.0	0.37 ± 0.13	0.559*
No	7	22.6	0.55± 0.27	

*Mann-Whitney U-test. †Kruskal Wallis Test

Four hundred isolates were taken from counting petri dishes. The isolates were first evaluated with slide-lamella preparation, which is one of the traditional methods, and it was determined that 140 of them were yeast. Bacterial isolates were eliminated, and work with yeast isolates was continued. Possible *C. albicans* or *C. dubliniensis* species were determined by performing GTT on yeast isolates. The isolates were finally identified using the MALDI–TOF MS method. Identification of the isolates according to the traditional and MALDI–TOF MS method is given in Table 3.

Table 3. Identification of isolates

Participant number	Isolate Code	GTT (+/ -)	Identification According to Traditional Methods	MALDI-TOF MS Identification	MALDI-TOF MS Score*
1	101	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.427
1	102	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.900
1	103	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.828
1	104	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.723
1	105	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.809
1	106	+	*C. albicans or C. dubliniensis*	*C. albicans or C. africana*	1.676
1	107	+	*C. albicans or C. dubliniensis*	*C. albicans or C. africana*	1.471
1	108	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.744
1	109	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.703
1	111	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.674
1	112	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.964
1	114	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.74
1	116	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.601
1	117	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.422
1	119	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.736

Cont. Table 3

7	120	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.825
7	121	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.590
7	122	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.773
7	123	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	2.169
7	124	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.861
7	125	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.765
7	126	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.536
7	127	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.751
7	128	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.728
7	129	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.565
7	130	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.797
7	131	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.506
7	132†	-	-	*C. dubliniensis*	1.666
36	133	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.755
36	134	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.937
36	135	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.808
36	136	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	2.004
36	137	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.821
36	138	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.598
36	139	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.561
36	140	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.679
36	143	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.478
42	144	-	-	*Pichia manshurica*	1.919
42	145†	+	*C. albicans or C. dubliniensis*	*C. parapsilosis*	1.650
42	146	-		*C. parapsilosis*	1.887
42	147	-		*Pichia manshurica*	1.871
42	148	-		*C. parapsilosis*	1.687
42	149	-		*C. parapsilosis*	1.753
42	150	-		*Pichia manshurica*	2.110
42	151	-		*C. parapsilosis*	1.652
42	152	-		*C. inconspicua*	1.774
42	153	-		*C. parapsilosis*	1.903
44	154	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.859
44	155	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.828
44	156	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.703
44	157†	-		*C. albicans*	1.964

Cont. Table 3

44	158	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.773
44	161†	-		*C. dubliniensis*	2.169
44	164†	+	*C. albicans or C. dubliniensis*	*C. inconspicua*	1.706
44	167	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.908
44	168†	+	*C. albicans or C. dubliniensis*	*C. parapsilosis*	1.785
47	169	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	2.004
47	170	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.447
47	171	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.905
47	172†	+	*C. albicans or C. dubliniensis*	*C. inconspicua*	1.720
47	173	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.920
47	174	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.908
47	175†	+	*C. albicans or C. dubliniensis*	*C. parapsilosis*	1.785
47	176	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.905
47	177	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.972
47	178	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.886
47	179	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.848
47	181	+	*C. albicans or C. dubliniensis*	*C. albicans or C. africana*	1.911
47	182	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.761
47	184	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.888
47	185	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.682
47	186	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.601
49	187	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.722
49	188	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.769
49	189	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.806
49	190	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.578
49	191	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.750
49	192	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.847
49	193-1	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.641
49	193-2	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.896
49	194	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.783
49	197	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.903
49	198	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.938
49	199	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.784
49	201	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.761
49	202	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.654
51	203	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.606
51	204	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.438
51	205	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.765
51	206	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.330
51	207	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	2.025
51	209	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.659
51	210	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	2.189

Cont. Table 3

51	211	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.617
51	212	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.867
52	213	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.728
52	214	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	2.123
52	215	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.685
52	216†	+	*C. albicans or C. dubliniensis*	*C. parapsilosis*	1.882
52	217	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.584
52	218	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.689
52	219	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.842
52	220	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.665
52	221	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.808
52	222	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.946
53	223	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.837
53	224	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.899
53	225	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.757
53	226	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.944
53	227†	-	-	*C. albicans*	2.072
53	228†	-	-	*C. albicans*	1.690
53	229	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.983
53	230	+	*C. albicans or C. dubliniensis*	*C. albicans*	2.017
53	231	+	*C. albicans or C. dubliniensis*	*C. albicans or C. africana*	1.694
32	301	-	-	*C. parapsilosis*	1.844
32	302	-	-	*C. parapsilosis*	1.615
32	303	-	-	*C. parapsilosis*	2.007
32	304	-	-	*C. parapsilosis*	1.706
32	305	-	-	*C. parapsilosis*	1.933
32	306	-	-	*C. parapsilosis*	2.036
32	307	-	-	*C. parapsilosis*	1.917
32	308	-	-	*C. parapsilosis*	1.504
32	309	-	-	*C. parapsilosis*	1.971
32	310	-	-	*C. parapsilosis*	1.842
32	311	-	-	*C. parapsilosis*	1.895
32	312	-	-	*C. parapsilosis*	1.812
32	313	-	-	*C. parapsilosis*	1.849
32	314	-	-	*C. parapsilosis*	1.836
32	315	-	-	*C. parapsilosis*	1.761
32	316	-	-	*C. parapsilosis*	1.827
32	317	-	-	*C. parapsilosis*	2.043
32	318	-	-	*C. parapsilosis*	1.862
35	342	-	-	*Wickerhamomyces subpelliculosus*	1.959
46	400†	+	*C. albicans or C. dubliniensis*	*C. parapsilosis*	1.808
12	469	+	*C. albicans or C. dubliniensis*	*C. dubliniensis*	1.902

Cont Table 3

18	470	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.660
18	471	+	*C. albicans or C. dubliniensis*	*C. albicans or C. africana*	1.443
18	472	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.766
18	473	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.871
21	474	+	*C. albicans or C. dubliniensis*	*C. albicans*	1.886

*2.300-3.000 High probability species identification; 2.000-2.299 Safe genus identification, possible species identification; 1.700-1.999 possible breed identification; 0.000-1.699 Unreliable identification, †The traditional method and MALDI-TOF MS result are different.

With GTT, 108 isolates were probable *C. albicans* or *C. dubliniensis*. With MALDI–TOF MS, 38.57% (n= 54) of *C. albicans*, 3.57% (n=5) of *C. albicans* or *C. africana*, 32.86% (n=46) of *C. dubliniensis*, 20% (n=28) of *C. parapsilosis*, 2.14% (n=3) of *C. inconspicua*, 2.14% (n=3) of *Pichia manshurica*, and 0.72% (n=1) of *Wickerhamomyces subpelliculosus* has been defined. A difference was found between naming 12 isolates using the traditional method and naming them with MALDI–TOF MS. As a result of MALDI–TOF MS, out of 108 isolates thought to be *C. albicans* or *C. dubliniensis* by the traditional method; five were named as *C. parapsilosis* and two as *C. inconspicua*. In addition, because of MALDI–TOF MS, it was found that 2 of 32 isolates with negative GTT were *C. dubliniensis*, and 3 of them were *C. albicans*. There was a discrepancy between the GTT and MALDI–TOF MS results in identifying 12 out of 140 isolates.

The yeast load study conducted with 31 participants determined that 41.93% (13) of the participants were yeast carriers. The classification of the yeasts detected in the participants according to the species is given in Table 4. It was determined that *C. albicans* species alone was found in three participants (1, 49, 53), *C. dubliniensis* species alone in two participants (36, 51) and *C. parapsilosis* species in two participants (32, 46). While only one participant (7) was a carrier of both *C. albicans* and *C. dubliniensis*, another participant (52) was found to be a carrier of both *C. dubliniensis* and *C. parapsilosis*. It was determined that *C. parapsilosis, C. inconspicua* and *P. manshurica* in one participant (42), *C. albicans, C. dubliniensis, C. inconspicua*, and *C. parapsilosis* in one participant (44). In one participant (47), it was determined that the five *Candida* species identified coexisted. It was determined that the *C. inconspicua* species isolated from three individuals (9.48%) carried it together with *C. parapsilosis* in one of the carriers, and it was carried together with both *C. albicans* and *C. dubliniensis* in the others.

Table 4. The classification of the yeasts detected in the participants according to the species.

Participant number	*C. albicans*	*Candida albicans or C. africana*	*C. dubliniensis*	*C. parapsilosis*	*C. inconspicua*	*P. manshurica*	*W. subpelliculosus*
1, 53	x	x					
7	x		x				
32,46				x			
36, 51			x				
42				x	x	x	
44	x		x	x	x		
47	x	x	x	x	x		
49	x						
52			x	x			
35							x

DISCUSSION

Colonies with different colony morphology from counting plates were visually evaluated, and isolates were taken. It was determined that the isolates from the SCAF medium were yeast, while the majority of isolates from the SDA medium were bacteria. As a result, although the SDA medium seemed to determine the microbial load better, mostly bacteria grew in this medium. Therefore, if yeast load is to be determined in the oral yeast load study, it is recommended to use a medium including antibiotics. Samaranayake, MacFarlane and Williamson (1987) collected mouthwash samples from 150 individuals and inoculated them

on Pagano–Levin agar and SDA medium. As a result, they reported that Pagano–Levin agar medium was slightly superior to the SDA medium in obtaining yeasts [10]. This study shows that a more specific medium should be used for oral yeast load or isolation, as in our study.

Kleinegger and coauthors (1996) determined that *Candida* carriage was 40% in individuals aged 15-18 years and 53% in individuals aged 30-45 years living in the state of Iowa. Researchers have reported that *Candida* carriage increases with age [15]. Martins and coauthors (2002) collected samples from 68 healthy participants between the ages of 25 and 55 from São José dos Campos/UNESP dental clinics. They reported that they isolated *Candida* species from 42 individuals (61.76%). They reported that the most frequently isolated species were *C. albicans*, followed by *C. tropicalis* (20.42%), *C. glabrata* (6.12%) and *C. kefyr* (2.04%) [9]. Since the yeast load of young healthy individuals was determined in our study, the carrier rate with *Candida* species was determined as 35.48%.

Liguori and coauthors (2007) analyzed a total of 78 oral rinse solutions between 2004 and 2005. They determined that yeast was phenotypically present in 63 samples, and there was no yeast in 15 samples. The most frequently isolated species were *C. albicans* (48 strains, 76.2%), followed by *C. glabrata* (6 strains, 9.5%), *C. tropicalis* (5 strains, 7.9%), *C. krusei* (2 strains, 3.2%), *C. parapsilosis* (1 strain, 1.6%), and *C. famata* (1 strain, 1.6%) [16]. In our study, *C. albicans*, one of the two most frequently isolated species, was identified in 6 individuals and *C. parapsilosis* was isolated in 3 individuals, and other *Candida* species could not be obtained.

Ghannoum and coauthors (2010) identified fungi in the oral cavity in 20 healthy adults (aged 21–60) individuals by mouthwash with PBS using ITS primers. They determined 74 culturable and 11 non-culturable fungi species from the samples. They stated that the most frequently isolated genus was *Candida* (75%), followed by *Cladosporium* (65%), *Aureobasidium* (50%), *Saccharomycetales* (50%), *Aspergillus* (35%), *Fusarium* (30%) and *Cryptococcus* (20%) [8]. In our study, only yeasts were evaluated, and it was determined that 136 of the 140 yeast isolates that could only be cultured were *Candida*.

Monteiro–da–Silva and coauthors (2014) investigated the fungal load in the oral microbiota with 40 healthy students at the Faculty of Dentistry of the Portuguese University in their study. Sampling methods are largely similar to the method in our study, and researchers used the Sabouraud Glucose Agar medium. They incubated half of the inoculated petri dishes at 25°C and the other half at 37°C for 7 days. They used the API system (API/ID32C) (BioM'erieux. Marcy L'Etoile. France) to identify yeasts [3]. Like this study, they also determined that 20% of their participants had oral fungal load between 1.70-2.60 log CFU/mL. Researchers reported that they detected fungi better in incubation at 25°C compared to 37°C. They determined that 100% of the samples grew mold and 92.5% yeast when incubated at 25°C, while 42.5% grew mould and 45% yeast in the petri dishes incubated at 37°C. Researchers reported that they isolated *Candida* from 67.5% of the samples, and the species they isolated were *C. albicans, C. parapsilosis,* and *C. tropicalis*. They determined that variables such as oral hygiene or dental caries did not affect the prevalence of Candida in the oral cavity ($p<0.05$), but variables such as age, gender and use of alcohol did affect the prevalence ($p<0.05$) [3]. In this study, petri dishes were incubated at 37°C in accordance with the premise of this study. Two *Candida* species frequently isolated in the study were also frequently isolated in this study. However, no statistically significant difference was found in our study regarding yeast load, demographic characteristics of the participants and oral hygiene.

When the study is evaluated with the literature in general, there are differences between the results due to factors such as age group, demographic characteristics, oral health and hygiene of individuals, sampling methods and counting method differences.

Quiles–Melero and coauthors (2012) evaluated the MALDI–TOF MS system for rapid identification of *C. metapsilosis, C. orthopsilosis,* and *C. parapsilosis*. They identified 103 isolates (reference strain and clinical isolate) according to MALDI–TOF MS and ITS1 gene regions. As a result, they reported that the agreement between the two methods was 100% and could be useful in quickly and reliably distinguishing the species within the *C. parapsilosis* group [17]. Sendid and coauthors (2013) [18] compared MALDI–TOF MS and conventional methods to identify yeast isolated from clinical specimens. They identified a total of 1207 yeast isolates. They defined it with the ITS gene region as a reference method. As a result, 91.5% (1105) isolates were compatible in identification with the traditional method and MALDI–TOF MS, while 6.1% (74) reported that the isolate was misidentified. The correct identification of 73 of these 74 isolates by MALDI-TOF MS was confirmed by molecular identification. They declared that the compatibility between the two techniques had an accuracy of 98-100% in identifying clinically important species, including closely related species such as *C. parapsilosis-C. metapsilosis-C. orthopsilosis*. Researchers found that only 2.3% of the isolates of *Geotrichum* species or *C. magnoliae*, *C. lambica*, and *C. famata*, could be identified by MALDI–TOF MS and *Trichosporon* spp. reported that species could not be identified by MALDI–TOF MS. They reported that the MALDI–TOF MS method is reliable, fast and cost-effective in the identification of yeasts and can be an

alternative to traditional identification methods [18]. Jamal and coauthors (2014) [19] compared the performance of MALDI–TOF MS and the VITEK 2 system in identifying clinical yeast isolates. They analyzed 188 clinical yeast isolates with Bruker Biotyper and VITEK MS. They reported that the correct identification percentages of VITEK 2, VITEK MS and Bruker Biotyper MS systems were 94.1% (177/188), 93.0% (175/188) and 92.6% (174/188), respectively. While three isolates were not identified by VITEK MS, nine *C. orthhopsilosis* isolates were misidentified as *C. parapsilosis*, which was due to the absence of this species in the database. They noted that eleven isolates were not identified or misidentified by Bruker Biotyper, and although 14 were more accurately described, they scored below <1.7 and the results were unreliable [19]. Taj-Aldeen and coauthors (2014) [20] conducted a retrospective study covering the period from January 1, 2004, to December 31, 2010 at a hospital in Qatar. They determined that 201 isolates obtained from 187 patients with candidemia gave the same results as molecular technique identification with MALDI–TOF MS. They reported that 21 isolates (10.4%) could only be identified at the genus level in identification with the traditional method [20]. In the above studies, it has been reported that the identification studies performed with MALDI–TOF MS are fast, inexpensive, and highly accurate.

CONCLUSION

As a result, oral yeast load of young healthy individuals aged 18–25 was determined. Oral yeast load was 0.00±0.01 to 1.87±0.01 log CFU in SCAF medium in young healthy individuals; It was determined that bacterial and yeast load in SDA medium ranged from 1.79±0.0 to >3.00 log CFU.

Two different sowing methods were used in our study. Serial dilutions of the mouthwashes taken in the first sampling were prepared and inoculated into two SCAF media from each dilution. But yeast load could not be obtained. The centrifugation method was used in the second sampling, and the collected mouthwashes were inoculated into two SCAF and two SDA media. As a result, counting results were obtained from petri dishes. In addition, when we evaluated the isolates taken from two media in the centrifugation method, it was determined that the isolates obtained from the SCAF medium were yeast, while the isolates obtained from the SDA medium were generally bacterial isolates. For this reason, it is recommended to use the centrifugation method to determine yeast load from oral samples and to use an antibiotic (such as SCAF) or specific (such as CHROMAgar Candida) medium for yeast isolation.

There was a discrepancy in the nomenclature of 12 isolates for identification with MALDI–TOF MS using conventional methods. However, 39 isolates identified by MALDI–TOF MS had a score of <1.7 and were within the unreliable definition. Further identification with both conventional and molecular techniques is required for the correct identification of isolates at the species level and the identification of unreliable labels. Despite the significant cost of the device and maintenance, MALDI-TOF MS has been used successfully in clinical laboratories because it is fast, inexpensive, easy and efficient. However, there is a need for research such as identifying closely related species and expanding databases.

Today, next-generation sequencing (NGS) studies have gained momentum in microbiome studies. Our study was carried out with a method based on traditional culture. Therefore, in our study, non-culturable species were missed in determining the oral yeast load. In addition, the studies in the literature were mostly carried out among individuals with the disease, and the age range is very wide. For this reason, it is recommended to conduct NGS studies with a narrow age range in which the characteristics of the participants can be determined more comprehensively.

Funding: This study was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit. Project No: BAP-TYL-2559.

Acknowledgements: Microbial count loads of centrifugation method and germ tube test results of this study were presented as an oral summary text in the 2ND INTERNATIONAL EURASIAN MYCOLOGY CONGRESS (EMC' 19) held in Konya, Turkey, on 6-9 September 2009. For her assistance in identification with MALDI-TOF MS, we would like to thank Dr. Yasemin NUMANOĞLU ÇEVİK.

Conflicts of Interest: The funders had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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