

Fungal Infection Rate and Composition Identification of Fungal Species Isolated From the Mouth of Dental Prostheses Patients in Nam Dinh Province, Viet Nam, In 2019-2020

Nguyen Huu Ban^{1*}, Le Ngoc Tuyen², Dinh Tuan Duc³

¹PC11- Nam Dinh Provincial Public Security Department, Nam Dinh Province, Viet Nam

²National Hospital Of Odonto-Stomatology, Ha Noi City, Viet Nam

³National Institute Of Malariology - Parasitology And Entomology, Ha Noi City, Viet Nam

ABSTRACT

Objectives: Determination of fungal infection rate and composition identification of fungal species isolated from the mouth of a dental prosthesis patient in Nam Dinh province, Vietnam, in 2019-2020. **Subjects and research methods:** Cross-sectional descriptive studies with analysis were performed with 132 patients with dentures in Nam Dinh province in Vietnam, using random sampling. **Results:** 74 patients are male (accounting for 56.1%), 58 patients are women (accounting for 43.9%), there are 86 patients with oral thrush, accounting for 65.2%. 100% of patients with oral thrush infection are yeast type. Single-infected status is 43 patients, accounting for 50% and multi-infected status is 43 people, accounting for 50%. Identifying the composition of the fungus species, 11 species of fungi were identified, of which *C. Albican* species accounted for the highest proportion, with 43 patients (accounting for 29.25%), two less common fungi were: *C. asahii* with 01 disease people (accounting for 0.68%), *C. Polymorpha* with 01 patient (accounting for 0.68%). **Conclusion:** The percentage of patients with dentures infected with oral thrush is quite high (accounting for 65.2%), of which the main infection is *C. Albican* (accounting for 29.25%). Communication and education on oral health, oral hygiene, dental hygiene, and periodic examination should be strengthened in patients with dentures.

Keywords:

Oral thrush, prosthodontics, dentures, Nam Dinh province

1.Introduction

Oral disease is a common disease of all ages. According to the survey of many authors around the world as well as in the country, it shows that people with oral diseases account for a very high rate, such as tooth decay, gingivitis, periodontitis, tooth loss, oral mucosa disease, tongue, oral thrush, ..., oral diseases have the highest prevalence, in some countries there may be over 90% of the population suffering from this disease. ^{[1], [2], [3]} Stomatitis in dental prostheses (prosthodontics) is the most common and long-term problem in people with dentures. The causes of denture stomatitis are multifactorial and complex, prosthodontics induce changes in the oral environment and adversely affect the integrity of oral tissues. Occurrence of fungi in the mouth with an abnormality in denture materials and control of infection in dental clinics, denture labo, poor oral hygiene and some factors of body protection are common cause for the onset of fungal stomatitis. ^{[3], [4], [5]} Some studies in the world have shown that: *Candida* fungus isolated from the oral cavity is 45% in infants (from 45 to 60%), in healthy children (from 30 to 45%), in adults healthy (from 50 to 60%), in people with dentures (from 65 to 88%). ^{[4], [6]} In Viet Nam, there is a study that shows that 64.52% of patients with removable dentures have determined the presence of oral thrush. ^[7]

There have been many studies on oral thrush in different population groups in the community, but studies on oral thrush infection rates and fungal species composition identification in dental prostheses patients are quite limited. Therefore, the determination of infection rate and composition identification of fungal species isolated from the mouth in dental prostheses patients is a necessary and highly practical issue. Objectives of the study:

Determination of fungal infection rate and composition identification of fungal species isolated from the mouth of a dental prosthesis patient in Nam Dinh province, Viet Nam, in 2019-2020

II. SUBJECTS AND RESEARCH METHODS

2.1. Research subjects

- Including patients who are wearing dentures to the examination and treatment of oral diseases in Nam Dinh province.
- Including samples taken from the mouth in patients who are wearing dentures.
- Selection criteria
 - + Including patients who bring dentures to the examination and treatment of oral diseases in Nam Dinh province.
 - + Including men and women
 - + Regardless of age
 - + Voluntarily, agree to participate in research.
 - + Specimens (patient samples) are taken in accordance with the technical process and the process of storing and transporting specimens.
- Exclusion criteria
 - + Including patients who did not cooperate in the study
 - + Including patients who do not wear dentures
 - + Patients with acute oral disease, diabetes, cancer, chronic kidney failure, HIV infection, and mental illness
 - + Subjects who did not agree to participate in the study
 - + Specimens are not taken correctly according to the technical process and the storage and transporting process.

2. Research Methods

- Time and place to study
 - + Time for data collection: From September 2019 to June 2020.
 - + Research location: In Nam Dinh province, Department of Parasitology of Viet Nam Military Medical University, Department of Molecular Biology of National Institute of Malariology - Parasitology And Entomology.
- Study design: Cross-sectional description and analysis of oral thrush from patient samples isolated from the mouths of these patients.
- Sample size and sample selection
 - + Sample size: Apply the sample size formula to estimate a ratio:

$$n = Z_{1-\alpha/2}^2 \frac{p(1-p)}{d^2}$$

Inside:

- $Z_{1-\alpha/2} = 1.96$ (with reliability 95%)
- $d = 0.1$ expected error is 10%
 - $p = 0.65$ The rate of removable dentures with *Candida albicans* fungal infection was 64.52%.^[7]

Instead of the formula we calculate $n = 90$, to ensure that the sample size is not reduced in the study, we take an increase of 20%, so the sample size needed for the study will be $n = 110$. In fact, we collected 132 study participants, $n = 132$.

- + How to select a research sample:
 - Select research subjects: Randomly select an oral clinic, from clinics select at random from the list of patients wearing dentures and invite re-examination.

- Select specimens: Patient samples were selected isolated from the mouths of 132 patients wearing dentures

3. Process and techniques for data collection and processing

- Information collection techniques: Information collection includes 04 steps: (1) Develop research records and technical procedures for patient sampling, (2) test and complete research records and technical procedures for patient sampling, (3) training on research records and technical procedures for patient sampling, (4) Investigating and collecting data with interviews, examinations and patient samples.
- Technical procedure for patient sampling, storage and transportation of patient samples: Patient samples are isolated from the patient's mouth, soft specimens are used with a cotton swab and put in a cotton swab test tube with culture medium or growth medium, for hard specimens such as tartar plaque, put them in a test tube with physiological saline solution. Specimens are stored in a cold storage tank and then transferred to Labo, Department of Parasitology of Viet Nam Military Medical University and Department of Molecular Biology of National Institute of Malariology - Parasitology and Entomology for testing to determining the infection rate and analysis of oral fungal species composition identification.
- Data collectors include 05 medical doctors and 02 nursing bachelors specializing in dentistry, well trained before data collection.
- Data analysis and processing: Data were checked and entered using Epidata 3.1 software and analyzed using Stata 14.2 software.

4. Materials research

- Machine and tools: Molecular biology testing system, including 03 parts (extraction unit, reaction setting unit and realtime PCR analyzer), centrifuge, scanner and gel imaging electrophoresis, electrophoresis sets, microscopes.^[8]
- Biochemistry: Culture medium with Sabouraud Dextrose Agar (SDA) agar, EB extraction buffer (NaCl 5M- EDTA 0.5M pH 8- TrisHCl 1M pH 8.6- Sucrose 0.16 M- SDS 10 %), restriction enzyme solution MspI (Thermo), 8M Kac solution, 99% Ethanol alcohol, 70% Ethanol alcohol, TE solution (10 mM TrisHCl pH 8- 1 mM EDTA), deionized water.^[8]
- Primers for PCR reaction: ITS1 5.8S (5'-TCC GTA GGT GAA CCT GCG G-3'), ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') according to SH Mirhendi et al, 2001, IDT synthesizes and provides.^[8]

5. Research ethics

The research was conducted in accordance with the regulations on medical ethics in medical research. Accredited by the Ethical Council in Biomedical Research of National Institute of Malariology - Parasitology and Entomology. According to Decision No. 303 / QD-VSR of the Director of National Institute of Malariology - Parasitology and Entomology, dated March 26, 2019. The subjects were introduced to the purpose of this study and request for supply. grant written consent if they agree to participate and they have signed a notified consent form. Participants can withdraw from the study at any time. Their information remains confidential. Oral thrush patients receive free counseling, examination and treatment or referral to hospital for examination and treatment.

III. RESULTS

Table 1: General information about study subjects (n=132)

Content		n	%
Gender	Male	74	56.1
	Female	58	43.9
Age group	< 16	1	0.75
	16 - 34	29	21.98
	35 - 44	18	13.64
	45 - 60	55	41.67
	> 60	29	21.97
Marital status	Married	105	79.5
	Single and unmarried	27	20.5
Educational level	Below high school level	51	38.6
	High school	40	30.3
	Professional intermediate/Graduate/	41	31.1
	Postgraduate		
Occupation	Farmer	26	19.7
	Worker	20	15.2
	Pupils, students	6	4.5
	Old age, retirement	25	18.9
	Other	55	41.7
Personal history of oral thrush	Yes	1	0.8
	No	131	99.2
Personal history of wearing dentures	Yes	119	90.2
	No	13	9.8
Systemic medical history	Yes	1	0.8
	No	131	99.2
Type of prosthodontics	Fixed prosthodontics	91	68.9
	Removable prosthodontic	6	4.5
	Both fixed and removable prosthodontic	35	26.5
Personal income	Depends on family	11	8.3
	< 3 million VND	25	18.9
	≥ 3 million VND	96	72.7
Smoke	Yes	28	21.2
	No	104	78.8
Status of drinking alcohol and beer	Yes	34	25.8
	No	98	74.2
Be wearing dentures	Yes	102	77.3
	No	30	22.7
Currently treating oral diseases	Yes	20	15.2
	No	112	84.8
Currently using topical drugs, oral cleaning solutions	Yes	37	28.0
	No	95	72.0

Comments: The study showed that 74 male patients (accounting for 56.1%), 58 female patients (accounting for 43.9%). Contents accounting for the highest proportion were patients with no history of oral thrush and patients with no history of systemic disease were all 131 patients (accounting for 99.2%). Contents accounting for the lowest percentage are patients with a history of oral thrush and patients with a history of systemic disease are 1 patient (accounting for 0.8%).

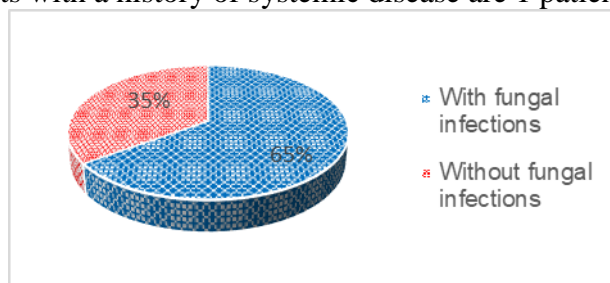


Figure 1. Prevalence of oral thrush in patients with dentures

Comments: In the study of 132 patients with dentures, there were 86 patients with oral thrush (accounting for 65.2%) and 46 patients without oral thrush (accounting for 34.8%).

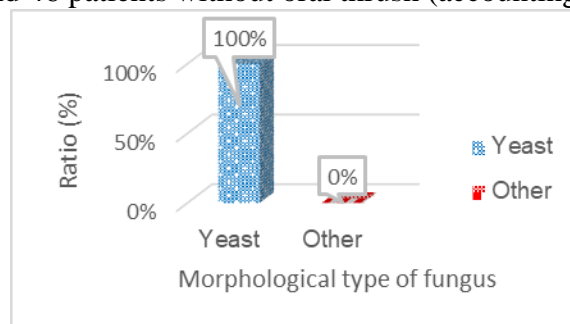


Figure 2. Classification of fungi by morphology

Comments: 100% of oral thrush patients are yeast type

Table 2. Status of single and multiple fungal infections in the mouth of patients with dentures (n = 86)

Status	n	%
Single fungal infections	43	50
Multiple fungal infections	43	50
Total	86	100

Comments: Research shows that: 86 patients wearing dental prostheses infected with oral thrush, the single-infection status is 43 patients (accounting for 50%) and the multi-infection status is 43 patients (accounting for 50%)

Table 3. Identification of the fungal species composition of the mouth of the patient with dentures

Rank	Species of fungi	n	%
1	C. albicans	43	29.25
2	C. krusei	24	16.32
3	C. glabrata	23	15.64
4	C. parapsilosis	21	14.28
5	C. tropicalis	19	12.95

6	C. ohmeri	06	4.08
7	C. mesorugosa	04	2.72
8	C. guilhermondii	03	2.04
9	C. lusitaniae	02	1.36
10	C. asahii	01	0.68
11	C. polymorpha	01	0.68

Note: C is the abbreviation of Candida

Comments: Patients wearing dental prostheses often have 05 types of fungal infections: C.Albican accounts for the highest rate, with 43 patients (accounting for 29.25%), C. Krusei with 24 patients (accounting for 16.32 %), C. Glabrata with 23 patients (accounting for 15.64%), C. Parapsilosis with 21 patients (accounting for 14.28%), C. Tropcalis with 19 patients (accounting for 12.95%). Two less common fungi are: C. asahii with 01 patient (accounting for 0.68%), C. Polymorpha with 01 patient (accounting for 0.68%).

IV. DISCUSSION

The study on 132 patients with dentures showed that: male patients are 74 people (accounting for 56.1%), female patients are 58 people (accounting for 43.9%), there are 86 patients with oral thrush (accounting for 65.2%) and 46 patients without oral thrush (accounting for 34.8%), of which 100% of patients with oral thrush were yeasts. Results of our research similarities with some research results in the country and around the world on the rate of fungal infection and 100% yeast infection.^{[7], [9], [10]} Contents accounting for the highest proportion were patients with no history of oral thrush and patients with no history of systemic disease were all 131 patients (accounting for 99.2%). Content with the lowest percentage is patients with a history of oral thrush and patients with a history of systemic disease are all 1 patient (accounting for 0.8%). Research shows that: 86 patients wearing dental prostheses infected with oral thrush, the single-infection status is 43 patients (accounting for 50%) and the multi-infection status is 43 patients (accounting for 50%). Patients wearing dental prostheses often have 05 types of fungal infections: C.Albican accounts for the highest rate, with 43 patients (accounting for 29.25%), C. Krusei with 24 patients (accounting for 16.32 %), C. Glabrata with 23 patients (accounting for 15.64%), C. Parapsilosis with 21 patients (accounting for 14.28%), C. Tropcalis with 19 patients (accounting for 12.95%). Two less common fungi are: C. asahii with 01 patient (accounting for 0.68%), C. Polymorpha with 01 patient (accounting for 0.68%). Results of our research similarities with some research results in the country and around the world on on fungal species composition and oral fungal infection rates of fungal species.^{[11], [12]}

V. CONCLUSION

The study on 132 patients wearing dental prostheses shows that: male patients are 74 people (accounting for 56.1%), female patients are 58 people (accounting for 43.9%), 100% of patients with oral thrush infection are type of yeast.Single-infection and multi-infection with equal rates. Species composition identification has identified 11 species of fungus, in which the percentage of patients with dentures infected with oral thrush is quite high (accounting for 65.2%), of which C. Albican infection is quite common (accounting for 29.25 %).

Recommendations

- Need to strengthen education and communication on oral health, especially oral thrush for patients with dentures

- Need to bring the health communication and education program on the occasion of periodic health check-ups for patients with dentures.
- It is necessary to maintain regular oral health examination every 06 months for patients with dentures.

Author Contributions

Nguyen Huu Ban is the main author and contact representative, data collection, data processing, journal writing. Authors Le Ngoc Tuyen and Dinh Tuan Duc, join in reading the article, commenting and editing the article

Conflict of interest

All the authors declare that they do not compete, conflict of interest.

ACKNOWLEDGMENT

To complete this research, our research team would like to express our sincere gratitude to:

- Institute leadership, Department of Science and Training, Department of Molecular Biology of National Institute Of Malariology - Parasitology And Entomology
- Department of Parasitology of Viet Nam Military Medical University.
- Board of Directors, Division of Operations, Medical Center of the Department of Health of Nam Dinh province.

REFERENCES

- [1] Peres M.A., Macpherson L.M.D., Weyant R.J., et al. (2019). Oral diseases: a global public health challenge. *Lancet Lond Engl*, 394(10194), 249–260.
- [2] WHO (2016). WHO | What is the burden of oral disease?. WHO, <https://www.who.int/oral_health/disease_burden/global/en/>, accessed: 02/14/2021.
- [3] Sharma D. and Sharma N. (2015). Denture Stomatitis – A Review. *IJOER*, 3(1), 5.
- [4] Pathmashri V.P. and Abirami (2016). A review on denture stomatitis. *Res J Pharm Technol*, 9(10), 1809.
- [5] Gleiznys A., Zdanavičienė E., and Žilinskas J. (2015). Candida albicans importance to denture wearers. A literature review. *Stomatol Balt Dent Maxillofac J*, 17(2), 13.
- [6] Akpan A. and Morgan R. (2002). Oral candidiasis. *Postgrad Med J*, 78(922), 455–459.
- [7] Dam Ngoc Lam and Luong N.M. (2013). Identifying The Presence Of Fungus In The Denture And In The Mouth Of A Patient With Removable Dentures. *J Pract Med*, 867(4), 48–53.
- [8] Mirhendi S.H., Kordbacheh P., and Kazemi B. (2001). A PCR-RFLP Method to Identification of the Important Opportunistic Fungi: Candida Species, Cryptococcus neoformans, Aspergillus fumigatus and Fusarium solani. *Iran J Public Health*, 30(3–4), 103–106.
- [9] Zomorodian K., Haghighi N.N., and Rajaei N. (2011). Assessment of Candida species colonization and denture-related stomatitis in complete denture wearers. *Med Mycol*, 49(2), 208–211.
- [10] Kilic K., Koc A.N., and Tekinsen F.F. (2014). Assessment of Candida Species Colonization and Denture-Related Stomatitis in Bar- and Locator-Retained Overdentures. *J Oral Implantol*, 40(5), 549–556.
- [11] Mai Anh Loi, Le Thanh Dong, and Nguyen Khac Luc (2017). Identification Of Fungal Species Collected From Oral Colonization Cancer Patients At 103 Hospital, 2015-2016. *J Pract Med*, 27(11), 357–364.
- [12] Khaled Y. and Pahuja B.K. (2019). Identifying the Different Kinds of Oral Candida Species in Denture Wearing Patients. *EC Dent Sci*, 7.