AUCTORES
Globalize your Research

Dentistry and Oral Maxillofacial Surgery
Hassan A. Al-Shamahy *

Open Access

Research Article

In Vitro Antifungal Susceptibility of Candida Albicans Isolated from Yemeni Patients with Denture Stomatitis

Mohammed Mohammed Lutf AlKhawlani [1], Mohammed Mohammed Ali Al-Najhi [3,4], Khaled A AL-Haddad [3], Abdulwahab Ismail Mohamed Al-Kholani [2], Hassan Abdulwahab Al-Shamahy [4,5*] and Mohammed Mohammed Ali Alsamhari [1]

[1] Department of conservative dentistry, Faculty of Dentistry, Genius University for Sciences & Technology, Dhamar city, Republic of Yemen.

[2] Department of conservative dentistry, Faculty of Dentistry, Sana'a University, Republic of Yemen.

[3] Orthodontics, Pedodontics and Prevention Department Faculty of Dentistry, Genius University for Sciences, Technology, Dhamar city, Republic of Yemen.

[4] Department of Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen.

[5] Medical Microbiology department, Faculty of Medicine, Genius University for Sciences & Technology, Dhamar city, Republic of Yemen.

***Corresponding Author:** Hassan A. Al-Shamahy, Department of Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen.

Received date: May 14, 2022; Accepted date: May 30, 2022; Published date: June 13, 2022

Citation: Mohammed M. A, Mohammed A. A, Khaled A. A, Hassan A A, Mohammed M. (2022). In Vitro Antifungal Susceptibility of Candida Albicans Isolated from Yemeni Patients with Denture Stomatitis. *J Dentistry and Oral Maxillofacial Surgery*, 5(3); DOI: 10.31579/2643-6612/034

Abstract

Background and aims: *Candida*-associated denture stomatitis (CADS) is a common fungal infection in people who wear dentures. The main objectives of this study were to identify the causative agents of CADS and *in vitro* antifungal susceptibility testing (AFST) for *Candida albicans* in Yemeni patients with denture stomatitis.

Methods: A total of 88 *Candida* spp. obtained from patients with denture stomatitis. *Candida* spp were identified using standard microbiological methods. The *in-vitro* antifungal susceptibility of *Candida albicans.* to fluconazole (FCZ), itraconazole (ICZ), voriconazole (VCZ), and amphotericin B (AMB) was evaluated using the E test strips. Interpretive sensitivity criteria for antifungal breakpoints were adapted from the Clinical and Laboratory Standards Institute (CLSI).

Results: Overall, *C. albicans* was the most commonly isolated species (n = 60; 68.2%), followed by *C. glabrata* (n = 9; 10.2%), *C. tropicalis* (n = 7; 8%), and *C. parapsilosis* (n = 3; 3.4%). Voriconazole had the lowest geometric mean minimum inhibitory concentration which was 0.0418 μg/ml for MIC50, and 0.957 μg/ml for MIC90; followed by amphotericin B (AMB) in which MIC50 was 0.518 μg/ml and for MIC90 was 1.06 μg/ml.

Conclusion: Our study showed that *Candida albicans* was the most prevalent *Candida* species in Yemeni patients with CADS and was susceptible to both azoles and amphotericin B. In addition, voriconazole could be a suitable alternative to antifungal agents currently used in the treatment of CADS, as well as in the treatment of recurrent Candidasis.

Keywords: *Candida albicans*; *Candida*-associated denture stomatitis (CADS); *in vitro* antifungal susceptibility testing (AFST); Yemen

Introduction

Wearing dentures and inadequate denture hygiene, especially constantly wearing dentures rather than removing them during sleep, is another risk factor for both oral candidiasis and *Candida* carriage. Dentures offer a relatively acidic, moist, and anaerobic environment for the reason that the mucous membrane covered by the dentures is protected from oxygen and saliva, which prevents or limits the growth of germs. Loose and poor-fitting dentures may also cause minor mucosal trauma which is thought to increase mucosal permeability and increase the ability of *C. albicans* to invade tissue. Dentures may therefore become covered with biofilm and act as reservoirs of infection, resulting in persistent re-infection of the mucosa [1-4]. *Candida*-associated denture stomatitis (CADS) is a chronic atrophic complication of the oral cavity that mainly affects people who wear removable dentures. Some have reported that up to 65% of denture wearers have this condition to some degree. Although this condition is also known as 'denture sore mouth", there is rarely any pain [5]. *Candida* is associated with approximately 90% of denture-related stomatitis cases [6]. Numerous evidence-based studies have revealed that *Candida albicans* is the major causative agent of denture stomatitis (DS), goes along by *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, or other species as *Candida*

stellatoidea, *Candida pseudotropicalis*, *Candida famata*, *Candida rugosa*, *Candida geotrichium*, *Candida dubliniensis*, and *Candida guilliermondii* [1-4, 7-9]. The untimely diagnosis of pathogenic fungal agents and the detection of their susceptibility to antifungal drugs are vital to the treatment of infection and the development of preventive healthcare-associated strategies [1,2,10,11].

Management of CADS is based on a broad-choice treatment strategy [12], which consists of detecting and eradicating potential significant risk factors, avoiding systemic *Candida* infection, and dipping any associated inconveniences. Use of oral preparations of antimicrobial agents, such as amphotericin B (AMB), nystatin (NYS), and miconazole (MIC), and systemic drugs, such as fluconazole (FCZ), voriconazole (VCZ), posaconazole (POS), itraconazole (ICZ), and ketoconazole (KTZ), has been revealed to be efficient in the treatment of CADS [13–16]. Echinocandins, such as caspofungin (CAS), are a class of antifungal drugs that appear to be highly effective against all species of *Candida*, including those that are less sensitive or are resistant to FCZ and/or ICZ [15]. Nevertheless, earlier studies have described recurrence and clinical relapse of CADS after treatment [17, 18]. Obtain sufficient information about the antifungal susceptibility testing (AFST) from *Candida* spp. participation in CADS may aid in the selection of alternative antifungal treatments for recurrent oral candidiasis.

Despite the lack of research on dental problems in Yemen in the past, there is a growing interest in dental problems nowadays as many studies have been conducted in cases of oral bacterial and fungal infections, drug resistance, tooth decay, causes of permanent tooth extraction, chemical drugs and Oral herbal [1-4, 19-33]. In Yemen, there have been several studies discussing biofilm formation, sensitivity to antifungals, and isolation of *Candida* from the oral cavity of denture wearers and free

dentures members, but no study has been found in determining MIC50 and MIC90 antifungal drugs for *Candida albicans* [1-4]. Therefore, in the current study, we identified the causative agents of CADS and an *in vitro* antifungal susceptibility test (AFST) to determine the MIC in *Candida albicans* and the rate of resistance to 5 selected antifungal drugs among Yemeni patients with CADS.

Materials and Methods

Study site and patient selection

The patients were selected from dental clinics in Al-Thawra Hospital, Al-Jumhouri Hospital, and some private dental centers in Sana'a, Yemen. The inclusion criteria for selecting the subject were healthy individuals with no clinical signs of *Candida* infection and no systemic disease. In addition, individuals who smoked or were currently taking antifungals, steroids, antibiotics, or immunosuppressive drugs in the past six months were excluded.

Sample Collection Process

After examination of the oral cavity, denture samples were obtained by scraping sterile swabs across the inner surface of the denture. In a 6-month period (1st January to end of June 2020), a total of 88 clinical isolates were collected from 79 patients aged 39–76 years with DS. For patients with stomatitis caused by *Candida albicans* (CADS) contained 13 (21.7%) males and 47 (78.3%) females, their ages ranged between 39-74 years (Table 1). All samples were streaked on Sabouraud dextrose agar and incubated at 35°C. for 7 days. All suspected colonies were detected by CHROM Agar *Candida*. *Candida* species were identified by the color of the colonies using the color reference guide provided by the manufacturer. When color determination was unclear, fermentation assay for sucrose, maltose, glucose, lactose-galactose was performed. *Candida* species has also been identified by the ability to produce Chlamydia spores on glutinous rice agar [33].

Characters	Number	Percentage
Sex		
Male	13	21.7
Female	47	78.3
Age groups		
≤40 years	2	3.3
41-50 years	16	26.7
51-60 years	35	58.3
>60 years	7	11.7
Total	60	100
Mean age	53 years	
SD	9.5 years	
Min	39 years	
Max	74 years	

Table 1: *The age and sex distribution of patients with Candida albicans-associated denture stomatitis (CADS) at a selected dental clinic in the city of Sana'a*

Antifungal susceptibility testing

The *in vitro* activity of the antifungal agents against each isolate was determined by E-test (HiMedia, Mumbai, India) according to the manufacturer's instructions. E-test strips for fluconazole (FCZ; 0.016 ~ 256 µg/mL), itraconazole (ICZ; 0.002 ~ 32 µg/mL), voriconazole (VCZ; 0.002 ~ 32 µg/mL), and amphotericin B (AMB; 0.002 ~ 32 µg/mL) were used [34]. Interpretive sensitivity criteria for antifungal breakpoints were adapted from the Clinical and Laboratory Standards Institute (CLSI), [35]. The breakpoints used in *C. albicans* are: FCZ (S=2; SDD = 4; R ≥ 8); VCZ (S ≤ 0.12; R ≥ 1), ICZ (S= 0.12; R ≥ 1) and AMB (S =2; R > 2). For quality control, *C. albicans* (ATCC 10231) was used as a reference strain and tested simultaneously with clinical isolates.

Results

Overall, *C. albicans* was the most commonly isolated species (n = 60; 68.2%), followed by *C. glabrata* (n = 9; 10.2%), *C. tropicalis* (n = 7; 8%), and *C. parapsilosis* (n = 3; 3.4%). 78.3% of the participants are females and only 21.7% are males. The age average ± SD for participants was 53 ± 9.5 years. Most of the subjects covered were in the age group 51-60 years (58.3%) followed by 41-50 years (26.7%). Table 3 shows, the In-*vitro* antifungal susceptibility of 4 antifungal agents against 60 *Candida albicans* isolated from *Candida*-associated denture stomatitis. Voriconazole had the lowest geometric mean minimum inhibitory

concentration which was 0.0418 μg/ml for MIC50, and 0.957 μg/ml for MIC90; followed by amphotericin B (AMB) in which MIC50 was 0.518 μg/ml and for MIC90 was 1.06 μg/ml.

Antifungal drugs	Minimum inhibition concentration (MIC μg/mL)			
	MIC range	Geometric MIC50	Geometric MIC90	Resistant rate
Amphotericin B (AMB; 0.002 ~ 32 μg/mL)	0.025 – 3.0	0.518	1.06	3 (5%)
Fluconazole (FCZ; 0.016 ~ 256 μg/mL	0.35-16	5.56	4.12	11 (18.3%)
Itraconazole (ICZ; 0.002 ~ 32 μg/mL),	0.025 – 9	1.273	1.73	9 (15%)
Voriconazole (VCZ; 0.002 ~ 32 μg/mL),	0.015-3.0	0.418	0.957	7 (11.7%)

MIC_{50} = Minimum Inhibitory Concentration required to inhibit the growth of 50% of organisms. MIC_{90} = Minimum Inhibitory Concentration required to inhibit the growth of 90% of organisms. MIC range is the range of the lowest and highest MIC values obtained from 60 *C. albicans* isolates tested. Percentage resistance is the percentage of isolates resistant to a specific antifungal dru

Table 3: *In vitro antifungal susceptibility of 4 antifungal agents against 60 Candida albicans isolated from Candida-associated denture stomatitis.*

Discussion

A complete denture (also known as a full denture or plate) is a removable device used when all the teeth inside the jaw are missing and need to be replaced with prosthetics. Unlike a partial denture, a full denture is created when there are no more teeth left in the arch and is therefore an exclusively tissue-reinforced prosthesis. In the current study, the mean age ± SD of the participants was 53 ± 9.5 years and ranged from 39 to 74 years. This result differs from that reported from Iran where the mean age of DS patients was 65 ± 7.5 years and the patients' ages ranged from 55 to 84 years. The younger age of Yemenis in these findings could be explained by the exposure of Yemeni patients to factors of tooth loss, such as: dental caries, periodontal disease, trauma, congenital disorders (such as ergogenesis imperfecta, hypomineralization of molar incisors) and functional impairment more than the Iranian population [36]. Dentures in the oral cavity serve as a reservoir of *Candida spp*. and, thus, it considered a predisposing factor for DS in denture patients, as well as a possible origin for re-infection [37]. In the current study, the dominant *Candida* species isolated from the mouth was *Candida albicans* with 58%. Other species were also isolated from the mouth; *Candida glabrata* from 9 patients (10.2%), *Candida tropicalis* from 7 patients (8%), and *Candida parapsilosis* isolated from 3 patients (3.4%). These results are similar to that reported by Samaranayake *et al.* and Lee *et al*. in which the most frequently identified species is *C. albicans*, although *C. glabrata*, *C. guilliermondii*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis* are less commonly seen [38,39]. However, Bouquot *et al.* reported that *C. tropicalis* was the most common non-*Candida albicans Candida* (NCAC), followed by *C. glabrata*, while *C. parapsilosis* were rare isolated in oral colonization or as a cause of stomatitis [40]. As it is known *Candida* species may be able to metabolize ethanol and convert it into the carcinogen acetaldehyde, and can thus progress oral and upper gastrointestinal tract cancer. This means that our studied individuals under risk of oral and upper gastrointestinal tract cancer. Consequently, , greater emphasis should be placed on diagnosis and treatment of oral *Candida albicans* infections, also on other *Candida* species than *C. albicans* as recommended to prevent this development [41,42]. In agreement with other studies, the current research found that *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* caused CADS [1, 2, 43-45] and recommended drugs in CADS patients without an underlying disease commonly includes a NYS suspension or a clotrimazole tablet. However, topical application of anazole, such as Fluconazole (FCZ or Itraconazole (ICZ), can also be used to prevent persistent or chronic fungal infections in the patients [34, 46]. Several studies reported the emergence of antifungal resistance to azoles, which has been associated with multiple episodes of recurrence [16, 47-49]. In the current study, 18.3% of *C. albicans* (11/60) was observed to be resistant to Fluconazole (FCZ). In contrast with the current study data, Abaci and Haliki-Uztan (2011) reported that 59.4% of *C. albicans* were resistant to Fluconazole (FCZ) [50].

***Candida* species**	**Number**	**Percentage**
Candida albicans	51	58
Candida glabrata	9	10.2
Candida tropicalis	7	8
Candida parapsilosis	3	3.4
Candida albicans+ Candida glabrata	5	5.7
Candida albicans+ Candida tropicalis	4	4.5
Total *Candida albicans*	60	68.2
Total Candida species	88	100

Table 2: *Distribution of different types of Candida species among Candida-associated denture stomatitis (CADS) isolated from 79 patients.*

AMB, also used in the management of CADS, proved effective against *Candida* spp. [51]. Besides, the findings obtained in the present study were in agreement with the results of Wingeter *et al.* [52] regarding the susceptibility of oral *Candida* strains to AMB. In the current study, 5% of *C. albicans* (3/60) was observed to be resistant to Amphotericin B (AMB) as AMB-resistant C. *albicans* isolates were reported from several previous studies [50,53]. In the current study, 11.7% of *C. albicans* (7/60) was observed to be resistant to Voriconazole (VCZ). However, the

geometric MIC90 was 0.957 μg/mL which is the lowest MIC90 for all tested drugs; the good *in vitro* activities of Voriconazole (VCZ) in the current study have been previously reported against *Candida* spp. obtained from oral candidacies patients [54-56]. As shown in Table 4, Voriconazole (VCZ) was the most effective drug in vitro with GMMICs of 0.957 μg/ml, for *C. albicans*, Omran *et al.* (2018) in Iran previously showed that the GM-MIC90 for Voriconazole (VCZ) were 0.25 μg/ml for *C. albicans* less than our findings [36]. Several other studies also demonstrated that Voriconazole (VCZ) was strong antifungal agents against *Candida* spp. [36, 54-57]. Other studies have shown that ITC is useful for treating patients with DS [37, 58, 59]. Dorocka-Bobkowska and Konopka (2007) reported that AMB, FCZ, and ICZ were effective against 100%, 88.7%, and 87.3% of *C. albicans* and less sensitivity rates equal to 79.6%, 71.4%, and 79.6% respectively for other *Candida* strains [12]. Our results showed that the tested antifungal showed good efficacy for most of the isolates. However, the observed variability between some of the isolates and drug resistance highlights the need for the AFST as a monitor to administer the therapeutic procedure.

Conclusion

In conclusion, *Candida albicans* was the most prevalent *Candida* species in Yemeni patients with CADS and was susceptible to both azoles and amphotericin B. In addition, Voriconazole could be a suitable alternative to antifungal agents currently used in the treatment of CADS, as well as in the treatment of recurrent Candidasis.

Also, the increasing rates of NCAC strains among CADS patients in Yemen should viewed as both novel and alarming. Extensive observational studies should be performed on all clinical specimens yielding significant growth of *Candida* spp. and the effect of resistance pattern on ICZ. As a consequence of selective pressure, emergence of drug resistance is inevitable. Therefore, future studies should focus on the emergence of drug-resistant *Candida* strains and their frequencies.

Acknowledgments

The authors thank the Faculty of Dentistry, Sana'a University, Sana'a, Yemen for their generous support in providing working space and materials.

Ethical approval

We have obtained written consent from all cases. Consent was obtained from the participants prior to sample collection. The study proposal was evaluated and approved by the Ethics Committee of the Faculty of Medicine and Health Sciences, Sana'a University.

Conflict Of Interest

No conflict of interest associated with this work.

Author's Contributions

All authors co-wrote the article and reviewed the results. Laboratory parts and data analysis were performed by Hassan Abdelwahab Al Shamahy.

References

1. Al-Shamahy HA, Al-labani MA, Al-akwa AA (2020). Biofilm formation and antifungal susceptibility of *Candida* isolates from oral cavity of denture wearer and free denture individuals. EC Dental Sci; 19(10):58-66.
2. Al-Haddad KA, Al-dossary OE, Al-Shamahy HA (2018). Prevalence and associated factors of oral *non-candida albicans Candida* carriage in denture wearers in Sana'a city- Yemen. Universal J Pharm Res. 3(4):7-11.
3. Al-Kebsi A, Othman A, Al-Shamahy HA, *et al.* (2017). Oral *C. albicans* colonization and non-*Candida albicans Candida* colonization among university students, Yemen. Universal J Pharm Res. 2(5):1-6.
4. Al-Sanabani N, Al-Kebsi AA, Al-Shamahy H, Abbas A (2018). Etiology and risk factors of stomatitis among Yemeni denture wearers. Universal J Pharm Res. 3(1):1-6.
5. Salerno, C; Pascale, M; Contaldo, M; et al. (2011). "*Candida*-associated denture stomatitis" (PDF). Medicina Oral, Patologia Oral y Cirugia Bucal. **16**(2):139–143.
6. Scully, Crispian. (2008). Oral and maxillofacial medicine: the basis of diagnosis and treatment (2nd ed.). Edinburgh: Churchill Livingstone. 191-199.
7. Martins M, Henriques M, Ribeiro AP *et al.*, (2010). "Oral *Candida* carriage of patients attending a dental clinic in Braga, Portugal," Revista Iberoamericana de Micolog´ıa. 27(3):119-124.
8. Marcos-Arias C, Vicente JL, Sahand IH *et al.* (2009). "Isolation of *Candida dubliniensis* in denture stomatitis," Archives of Oral Biolog. 54(2):127-131.
9. Zomorodian K, Haghighi NN, Rajaee N *et al.*, (2011). "Assessment of *Candida* species colonization and denture-related stomatitis in complete denture wearers," Medical Mycology. 49(2):208-211.
10. Kanafani ZA, Perfect JR (2008). "Resistance to antifungal agents: mechanisms and clinical impact," Clinical Infectious Diseases. 46 (1):120-128.
11. Badiee P, Hashemizadeh Z. (2014). "Opportunistic invasive fungal infections: Diagnosis & clinical management," Indian Journal of Medical Research. 139:195-204.
12. Dorocka-Bobkowska B, Konopka K (2007). "Susceptibility of Candida isolates from denture-related stomatitis to antifungal agents in vitro," International Journal of Prosthodontics; 20(5):504-506.
13. Mart´ınez-Beneyto Y, L´opez-Jornet P, Velandrino-Nicol´as A, Jornet-Garc´ıa V (2010). "Use of antifungal agents for oral candidiasis: results of a national survey.," International Journal of Dental Hygiene; 8(1):47–52.
14. Lyon JP, Moreira LM, Cardoso MAG, Saade J, Resende MA. (2008). "Antifungal susceptibility profile of *Candida* spp. oral isolates obtained from denture wearers," Brazilian Journal of Microbiology 39(4):668–672.
15. Sanit´a PV, De OliveiraMima EG, Pavarina AC, *et al.* (2013). "Susceptibility profile of a Brazilian yeast stock collection of Candida species isolated from subjects with Candida-associated denture stomatitis with or without diabetes," Oral Surgery, Oral Medicine, Oral Pathology Oral Radiology, and Endodontology. 116 (5):562–569.
16. Marcos-Arias C, Eraso E, Madariaga L *et al.* (2012). "InVitroActivities of New Triazole Antifungal Agents, Posaconazole and Voriconazole, Against Oral *Candida* Isolates from Patients Suffering from Denture Stomatitis," Mycopathologia. 173(1):35-46.
17. Figueiral MH, Fonseca P, Lopes MM *et al.* (2015). "Effect of denture-related stomatitis fluconazole treatment on oral *Candida albicans* susceptibility profile and genotypic variability," The Open Dentistry Journal. 9(1):46–51.
18. Bissell V, Felix DH, Wray D (1993). "Comparative trial of fluconazole and amphotericin in the treatment of denture stomatitis," Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 76(1):35-39.
19. Alsamhari MMA, AlKhawlani MML, Al-Kholani AIM, Al-Najhi MMA, Al-Shamahy HA, Al-Sharani AA, Al-dossary OAI, AL-Haddad KA, Al-labani MA (2022). Antimicrobial activity of Sodium hypochlorite, nano Silver and Chlorhexidine against mono-species biofilms of selected microorganisms of

oral sources. Universal Journal of Pharmaceutical Research. 7(1):11-16.

20. Abbas AM, Al-Kibsi TAM, Al-Akwa AAY, AL-Haddad KA, Al-Shamahy HA, Al-labani MA (2020). Characterization and antibiotic sensitivity of bacteria in orofacial abscesses of odontogenic origin. Universal J Pharm Res. 5(6):36-42.
21. Al-Akwa AA, Zabara A, Al-Shamahy HA, *et al*. (2020). Prevalence of *Staphylococcus aureus* in dental infections and the occurrence of MRSA in isolates. Universal J Pharm Res. 5(2):1-6.
22. AL-Haddad KA, Ali Al-Najhi MM, Al-Akwa AAY, Al-Shamahy HA, Al-Sharani AA, Al-labani MA (2021). AntimicrobialSusceptibility of *Aggregatibacter Actinomycetemcomitans* Isolated from Localized Aggressive Periodontitis (Lap) Cases. JDent Ora Heal Ad Re: JDOHAR. 103.
23. Alhasani AH, Ishag RA, Yahya Al-Akwa AAY, *et al.* (2020). Association between the *Streptococcus mutans* biofilm formation and dental caries experience and antibiotics resistance in adult females. Universal J Pharm Res. 5(6):1-3.
24. Al-Haddad KA, Al-Najhi MMA, Abbas AKM, Al-Akwa AAY, Al-Shamahy HA, Al-labani MA (2021). Clinical features, age and sex distributions, risk factors and the type of bacteria isolated in periodontitis patients in Sana'a, Yemen. Universal J Pharm Res. 6(1):1-8.
25. Alsamhari MMA, Al-Najhi MMA, Al-Shamahy HA, Al-dossary OAI (2021). Analysis of biofilms for *Streptococcus mutans* from dental root surfaces of adult patients with root caries. Universal J Pharm. 6(5):19-23.
26. Al-Shami IZ, Al-Shamahy HA, Abdul Majeed ALA, Al-Ghaffari KM, Obeyah AA (2018). Association between the salivary *Streptococcus mutans* levels and dental caries experience in adult females. On J Dent Oral Health. 1(1):1-6.
27. Shogaa Al-Deen SH, Al-Ankoshy AAM, Al-Najhi MMA, Al-Shamahy HA, *et al*. (2021). *Porphyromonas gingivalis*: biofilm formation, antimicrobial susceptibility of isolates from cases of Localized Aggressive Periodontitis (LAP). Universal J Pharm Res; 6 (4): 1-6.
28. Gylan EMA, Muharram BA, Al-Kholani AIM, AL-Haddad KA, Al-Akwa AAY, Al-Shamahy HA, Al-Hamzi MA, Al-labani MA (2022). *In-vitro* evaluation of the antimicrobial activity of five herbal extracts against *Streptococcus mutans*. Universal Journal of Pharmaceutical Research; 7(1):37-44.
29. Alhadi Y, Rassem AH, Al-Shamahy HA, Al-Ghaffari KM (2019). Causes for extraction of permanent teeth in general dental practices in Yemen. Universal J Pharm Res. 4(2):1-6.
30. Mutaher NJA, AL-Haddad KA, Al-Shamahy HA, *et al.* (2020). Prevalence and causes of traumatic dental injuries to anterior teeth among primary school children in Sana'a city, Yemen. Universal J Pharm Res. 5(3):38-43.
31. Dahaq WAM, Al-Kholani AIM, Al-Kibsi TAM, Al-Deen HS, Al-Shamahy HA, AL-Haddad KA, Al-Akwa AAY, Al-labani MA (2021). Tanaka and Johnston's mixed dentition validity: an analysis among Yemeni adults in Sana'a city. Universal J Pharm Res. 6(6):1-5.
32. Al-Sharani AA, Al-Hajj W, Al-Shamahy HA, Jaadan BM (2019). The effect of nanosilver and chlorhexidine mouthwash on anaerobic periodontal pathogens counts. Universal J Pharm Res. 4(5):1-6.
33. Staib P, Morschhäuser J (2007). Chlamydospore formation in *Candida albicans Candida dubliniensis* – an enigmatic developmental programme. Mycoses. 50:1-12.
34. Song YB, Suh MK, Ha GY, Kim H (2015). Antifungal susceptibility testing with Etest for *Candida* species isolated from patients with Oral Candidasis. Ann Dermatol. 27(6):715–720.
35. Clinical Laboratory Standards Institute (2008). *CLSI document M27-S3: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard*, CLSI, Wayne, PA, USA.
36. Omran SM , Dastjerdi MR, Zuashkiani M *et al*. (2018). *In Vitro* Antifungal Susceptibility of *Candida* Species Isolated from Iranian Patients with Denture Stomatitis. Hindawi, Bio-Med Research International. 6.
37. Cross LJ, Williams DW, Sweeney CP *et al.* (2004). "Evaluation of the recurrence of denture stomatitis and *Candida* colonization in a small group of patients who received itraconazole," *Oral* Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 97(3):351-358.
38. Samaranayake LP, Keung Leung W, Jin L (2009). "Oral mucosal fungal infections," Periodontology*;* 49(1):39-59.
39. Lee SH, Kim SW, Bang YG (2002). "A study on the distribution of oral candidal isolates in diabetics," Korean Journal ofMedical Mycology;7(3):139-148.
40. Bouquot, Brad W. Neville, Douglas D. Damm, Carl M. Allen, Jerry E (2002). Oral and maxillofacial pathology (2nd. ed.). Philadelphia: W.B. Saunders; 189–197.
41. Enfert C, Hube B (2007). *Candida*: Comparative and Functional Genomics. Caister Academic Press.
42. Manolakaki D, Velmahos G, Kourkoumpetis T *et al.*(2010). *Candida* infection and colonization among trauma patients. Virulence; 1(5), 367-375.
43. Silva S, Henriques MC, Hayes A, *et al.* (2011). "*Candida glabrata* and *Candida albicans* co-infection of an in vitro oral epithelium," Journal of Oral Pathology & Medicine *;* 40(5): 421–427.
44. Coco BJ, Bagg J, Cross LJ *et al.* (2008). "Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis," Oral microbiology and immunology; 23(5): 377–383.
45. Gendreau L, Loewy ZG (2011). "Epidemiology and etiology of denture stomatitis," Journal of Prosthodontics ; 20(4):251–260.
46. Niimi M, Firth NA, Cannon RD (2010). "Antifungal drug resistance of oral fungi," Odontology ; 98(1):15–25.
47. Carvalhinho S, Costa AM, Coelho AS *et al.* (2012). "Susceptibilities of *Candida albicans* mouth isolates to antifungal agents, essentials oils and mouth rinses," Mycopathologia; 174(1):69–76.
48. Koga-Ito CY, Lyon JP, De Resende MA (2008). "Comparison between E-test and CLSI broth microdilution method for antifungal susceptibility testing of Candida albicans oral isolates," Revista do Instituto de Medicina Tropical de S˜ao Paulo; 50(1): 7–10.
49. Bergendal T, Holmberg K, Nord CA (1979). "Yeast colonization in the oral cavity and feces in patients with denture stomatitis," Acta Odontologica Scandinavica; 37(1):37–45.
50. Abaci O, Haliki-Uztan A (2011). "Investigation of the susceptibility of *Candida* species isolated from denture wearers to different antifungal antibiotics," African Journal of Microbiology Research; 5(12):1398–1403.
51. Salerno C, Pascale M, Contaldo M *et al*.. (2011). *"Candida*-associated denture stomatitis," Medicina Oral Patolog´ıa Oral y Cirug´ıa Bucal; 16 (2): e139–e143.
52. Wingeter MA, Guilhermetti E, Shinobu CS *et al*. (2007). "Microbiological identification and *in vitro* sensitivity of *Candida* isolates from the oral cavity of HIV positive

individuals," Journal of the Brazilian Society of Tropical Medicine; 40(3):272–276.

53. Ellis D (2002). "Amphotericin B: spectrum and resistance," Journal of Antimicrobial Chemotherapy; 49(1):7–10.
54. Quind´os G, Carrillo-Mu˜noz AG, Eraso E, *et al.* "In vitro antifungal activity of voriconazole: New data after the first years of clinical experience," Revista Iberoamericana de Micolog´ıa 2007; 24(3):198–208.
55. Vargas LOS, Eraso E, Carrillo-Mu˜noz AJ, *et al.* (2010). "*In vitro* activity of voriconazole against Mexican oral yeast isolates: Original article," Mycoses; 53(3):200–203.
56. Carillo-Mu˜noz AJ, Quind´os G, Ruesga M *et al*., (2005). "Antifungal activity of posaconazole compared with fluconazole and amphotericin B against yeasts from oropharyngeal candidiasis and other infections," Journal of Antimicrobial Chemotherapy ; 55(3): 317–319.
57. Carrillo-Mu˜noz AJ, Tur-Tur C, Hern´andez-Molina JM *et al*., (2010). "Antifungal activity of posaconazole against *Candida* spp. and non-*Candida* clinical yeasts isolates," Revista Espa˜nola de Quimioterapia; 23(3):122–125.
58. Martin-Mazuelos E, Aller AI, Romero MJ *et al.*, (1997). "Response to fluconazole and itraconazole of *Candida* spp. in denture stomatitis," Mycoses; 40(7-8): 283–289.
59. Cross LJ, Bagg J, Aitchison TC (2000). "Efficacy of the cyclodextrin liquid preparation of itraconazole in treatment of denture stomatitis: Comparison with itraconazole capsules,". Antimicrobial Agents and Chemotherapy; 44(2):425–427.

DOI: 10.31579/2643-6612/034