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Identification of *Candidia Albicans* associated with Angular Cheilitis by Polymerize Chain Reaction and Culture media

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Abstract

Background: The *candida albicans* is most possible causative factor of angular cheilitis, this type of microorganism diagnosed by different laboratory tests (Culture and sugar fermentation); with polymerase chain reaction more accurate detectable tool.

Aims of the study: Laboratory detection of*candida albicans* related to angular cheilitis.

Subjects, Materials and Methods: Isolating and identifying *candida albicans* by Culture media, detecting and confirming *Candida albicans* by Polymerase Chain Reaction technique and to distinguish *Candida albicans* by germ tube test, then finally; noticing *Candida albicans* by sugar fermentation test.

Results: Diagnosis of patients with angular cheilitis was in cooperation with oral medicine specialists as team work in the oral medicine clinic. The Sabouraud dextrose agar, HiChrome Candida differential agar, Sugar fermentation medium agar, DNA ladder marker.

Conclusions: Both cultures and polymerase chain reaction techniques are the most famous principles for growth of *candida albicans*

Keywords: culture media, polymerase chain reaction, *candida albicans*

Introduction

Candida species is valid polymorphic organisms that produce yeast form, "*pseudohyphea*", and/or true *"hyphae*", but they no dependent on temperature.

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Candidas are endemic in most, if not all, intensive care units. These fungi organism rank among the top incidence of hospital-acquired infections and are the most common cause of nosocomial fungal infections **(Hardy, 2002; Walsh *et al.*, 2004; Pfaller and Diekema, 2007).**There are more than one-hundred types of *Candida* organisms; however, only "*C albicans"* is isolated from humans regularly, either as from ill patients or normal flora **(Hardy, 2002; Salerno, 2011)**. In the oral cavity, there are isolated about fivetypes of *Candida species*, "*Candida albicans"; "Candida Tropicalis"; "Candida Krusei"; "Candida Parapsilosis"* and finally *Candida Guilliermondi* **(Gravina *et al.*, 2007).***"Candida albicans"* typically cultures and grows as oval to spherical budding yeast cells (3-5 × 5-10) μm in size. These yeast phase cells also named blastospores **(WHO, 2003; Mitchell, 2007; Samaranayake, 2012)**.**Soll, 2002 and Hube, 2004** stated that the candida infections in superficial mucosal sites and skin caused by interplay between host defenses and fungal virulence. Pathogenesis process needs virulence factors in differential expression nature at each new stage of the process, rapid alteration propensity of the expressed phenotype in "*C. albicans"* may be a significant factor in forming comparatively high pathogenic potential **(Hardy, 2002)**. Host (tissue environment and immune system) detects the balancing between pathogenicity and commensalism processes.The *C. albicans* have the ability to infect such diverse host niches are supported by a wide range of virulence factors and fitness attributes including **(Höfs *et al.*, 2016; Mayer *et al.*, 2013)**:

1. Adherence on the oral epithelial surface.

   A-hydrophobicity of cell surface {reversible adherence}

   B-Expression of the cell surface adhesins (Als3, Hwp1, etc.)

2. The ability of Formation biofilm

   -Failure of antifungal treatment.

3. The capacity of evasion the host defenses

   A-Phenotypic switching

   B-Binding to the complement

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C-The resistance to the phagocytic stresses {oxidative and nitrosamine stress response}

D- Proteolytic degradation of host immune factors {antimicrobial peptides, antibodies etc.}

4. Invasion of host tissue and destruction the tissue.

A-Hyphal formation and thigmotropism (tissue penetration)

B-Secretion of hydrolytic enzymes: - phospholipases, secreted aspartyl proteinases (SAPs), lipases (tissue degradation)

C-Secretion of hypha-specific toxin (candidalysin)

D-Degradation of the E-cadherin

E-Induction of endocytosis

In order for the Candida to cause infection, it has to be retained in the mouth. The effect of swallowing and salivary flow is an important factor in host defense against Candida attachment to mucosa and overgrowth. Ability of Candida to resist these removal mechanisms can be considered as a key virulence factor **(Lewis and Williams, 2017).**

Clinically, to make the proper or possible diagnosis, the clinical examination should be taken in adequate manner to each subject, these principles includes oral hygiene practices, the patient's dental status then finally occupational condition.It is important to ask the patients about medically compromised diseases or illnesses, such as diseases leading to immunodeficiency and anemia; smoking ; chewing tobacco use; drinking of alcohol; cutaneous disease (lichen planus, psoriasis, and atopic dermatitis);" allergic disorders" like (asthma and eczema and the use of any type of medications). Generally it is supposed (that those who wear oral appliances like dentures and oral applied prostheses are more prone to have *Candida* species seeded in their oral flora) **(Lamey and Lewis, 1989)**.Many microbiological ways can be used to detect relationship of *Candida albicans* with angular chielitis like swab of the lesion which is a simple method for detecting *Candida albicans* growth (**Ax´ell *et al.*, 1985).** The most frequently used primary isolation medium for Candida is Saboraud Dextrose Agar (SDA) which, although permitting growth of Candida, suppresses the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 3080 – 3094
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growth of many species of oral bacteria due to its low PH. Incorporation of antibiotics into SDA will further increase its selectivity. Candida develops as cream, smooth, pasty convex colonies on SDA and differentiation between species is rarely possible **(Baveja, 2010).**The immunocompromised host's physiological conditions can induce dimorphism in *Candida albicans* to a hyphal state of growth. These growth conditions can be created in the laboratory and stimulate *Candida albicans* grows as yeast only or short term germ tubes {hyphal state} **(Raghunath *et al.*, 2014).** This can be achieved by manipulating the concentrations and the type of carbohydrates and amino acids in culture media **(Makwana *et al.*, 2012).**

The Polymerase Chain Reaction (PCR); it is a technique employed widely in the basic and the biomedical sciences. (PCR); is a laboratory technique use to amplify specific segments of DNA, utilized for a wide range of laboratory and/or clinical applications. Building on the work of (Panet and Khorana's) successful amplification of DNA in-vitro, Mullis and coworkers developed PCR in the early 1980s, having been met with a Nobel Prize only a decade later. In dentistry, as early as 1992, PCR was used to identify DNA from human tooth pulp tissue for use in forensic dentistry **(Pötsch *et al.*, 1992).**

Recent developments in molecular methods have revolutionized the detection and characterization of microorganisms in a broad range of medical diagnostic fields, including virology, mycology, parasitology, microbiology and dentistry. Among these methods, Polymerase Chain Reaction (PCR) has generated great benefits and allowed scientific advancements. PCR is an excellent technique for the rapid detection of pathogens, including those difficult to culture **(Valones *et al.*, 2009).**

Subjects, Materials and Methods

Forty five subjects with age range (25-68 years) participated in this clinical trial study; five of them were excluded from the study due to negative culture results on Sabouraud dextrose agar and they were referred to oral medicine specialists.

Forty samples collection was from Babylon University / College of Dentistry / Oral Medicine clinic and Specialized Center for Dentistry in Diayla Province.

Inclusion Criteria: Involving patients with angular cheilitis.

Exclusion Criteria:

1) Patients with signs and symptoms of any medical diseases.
2) Patients with negative culture results on Sabouraud dextrose agar.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 3080 – 3094
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Agars:

1) Sabouraud dextrose agar (SDA)
2) HiChrome Candida differential agar

Oral Sample collection: Oral samples were obtained at baseline for both treatment groups. By a sterile cotton tip applicator, swab was collected from the lesion at the angle of the mouth by weeping all lesion area then plated immediately in transport media. Then it was taken for lab for microbiological study and culturing on sabouraud dextrose agar and selective media.

Sabouraud dextrose agar (SDA) was prepared according to the instructions of the manufacturing company .Sixty- two grams of Sabouraud dextrose powder was added to 1000 ml of distilled water in a large flask cupped with plug, then was heated to boiling to dissolve the medium, then the flask was placed in autoclave at 121^0 C for 15 minutes. After sterilization, the molten agar allowed cooling to 50^0 C, and then 20 mg of Chloramphenicol was added, mixed well, and then poured into Petri dishes. It was used for primary growth of Candida.

HiChrome Candida differential agar was prepared according to the instructions of the manufacturing company. A size of 42.72 g was suspended in 1000 ml distilled water, then was heated to boiling to dissolve the medium completely, then was cooled to 50^0 C and pour into sterile Petri plate.

It was used for differentiation between species of Candida according to color. Green colony indicates presence of Candida albicans. Then multiple green colonies were carved by swab and were saved in pipet tube with 0.5 cc DNA rehydration and refrigerated **(Baradkar *et al.*, 2010).**

The DNA was isolated from fungal growth according to protocol of ABIOpure Extraction .

Results

This study included 40 patients with angular cheilitis presented with mean age of 49.5±10.8 yrs; 20% of patients were in the age group <40 yrs, 25% of them in age group 40-49 yrs, 35% of them were in the age group 50-59 yrs and 20% of them were in age 60 yrs and more. Male patients with angular cheilitis were equal to female patients with angular cheilitis; as shown in **Table (1), Figures (1 and 2).**

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Table (1): Demographic characteristics of patients with angular cheilitis

Variable	Number of Patients	Percentage %
Age **Mean±SD (49.5±10.8 years)**		
<40 years	**8**	**20.0**
40-49 years	**10**	**25.0**
50-59 years	**14**	**35.0**
≥60 years	**8**	**20.0**
Total	**40**	**100.0**
Gender		
Male	**20**	**50.0**
Female	**20**	**50.0**
Total	**40**	**100.0**

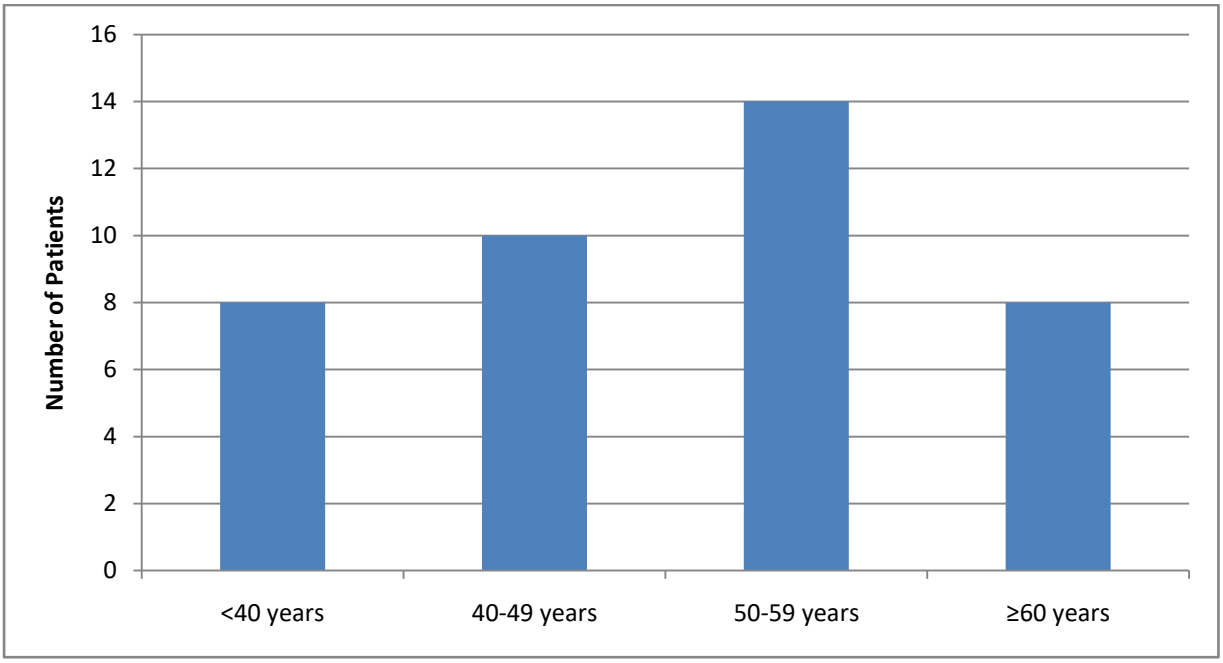

Figure (1): Age distribution.

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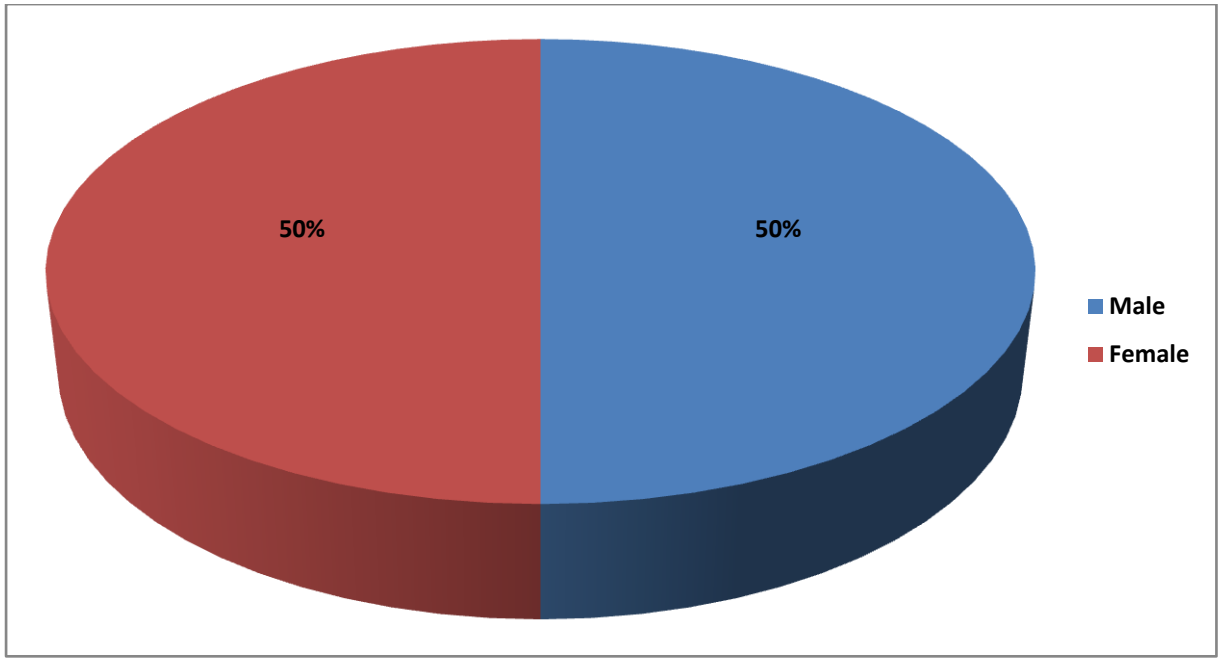

Figure (2): Gender distribution.

The culture findings of angular cheilitis revealed positive results of *Candida Albicans* in all of patients and PCR also revealed positive results of *Candida Albicans* in all of patients with angular cheilitis, as shown in **Table (2).**

Table (2): Culture and PCR of patients with angular cheilitis

Variable	Number of Patients	Percentage %
Culture		
Positive	**40**	**100.0**
Negative	**0**	**-**
Total	**40**	**100.0**
PCR		
Positive	**40**	**100.0**
Negative	**0**	**-**
Total	**40**	**100.0**

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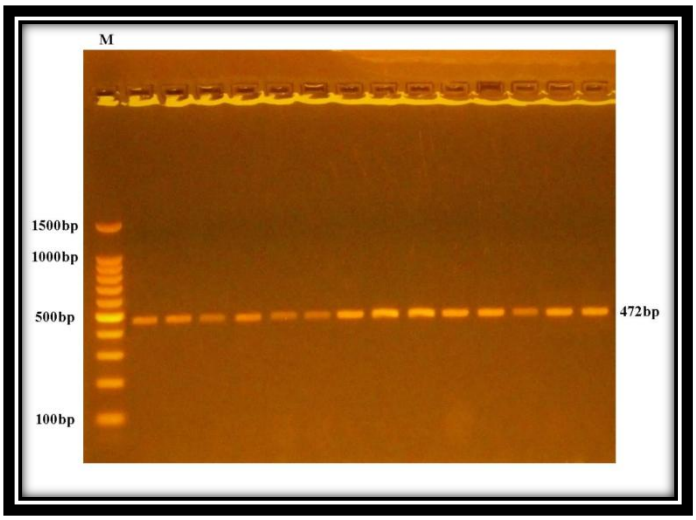

Figure (3) Results of PCR

Positive Germ tube was detected in 100% of patients with angular cheilitis, also, positive sugar fragmentation was present among 100% of patients with angular cheilitis, as shown in **Table (3)**

Table (3): Germ Tube and Sugar Fragmentation of Patients with Angular Cheilitis

Variable	Number of patients	Percentage %
Germ Tube		
Positive	40	100.0
Negative	0	-
Total	40	100.0
Sugar Fragmentation		
Positive	40	100.0
Negative	0	-
Total	40	100.0

Discussion

A total of 40 patients with angular cheilitis were included in the current study. The results of the current study showed that the mean age of patients with AC in was 49.5±10.8 years; 20% of patients were in age group <40 years, 25% of them were in

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age group 40-49 years, 35% of them were in age group 50-59 years and 20% of them were in age 60 years and more. The finding of this study regarding to age agrees with Öhman and Jontell **(1988)** who found that the mean age of patients with angular cheilitis was 55.8 years.

Also these results were concord with that reported by Griffith **(2016)** who mentioned that the peak incidence of angular cheilitis (AC) is in the 6th decade of life. This may be due to that AC is associated with factors leading to fold in the skin deeper than usual like edentulous patient or due to damage in elastic tissue.

The finding in the current study disagrees with the results of Cross *et al.,* **(2010**) who noticed that patients with AC were with mean age 14.7 years; and were only patients with orthodontic appliances that included who are usually young.

The gender percentage in the present study showed that 50% of patients with angular cheilitis were male and 50% were female.

In this study;the result of culture of angular cheilitis showed positive results of Candida Albicans in all of patients and PCR also revealed positive results of Candida Albicans in all of patients with angular cheilitis.

Different species of candida microorganism found in the human body as a commensal type of microorganism that behave as opportunistic pathogen, eighty percent of candida species present in healthy individuals are Candida Albicans Williams and Lewis **(2011)**;this study was agreed with the present study.

This study was accordance with the result of Raju and Rajappa, **(2011)** who mentionedthat Saboraud dextrose agar (SDA); which the most famous media for culturing of candida that allow growth of candida and suppression of other species due to low PH. Also this study used "HiChrome Candida differential agar" as a selective media used to differentiate Candida species from other type of species can found in the same type of lesion.

Candida develops as cream, smooth, pasty convex colonies on SDA and differentiation between species is rarely possible. Ten percent of oral samples contain more than one Candida species and in recent years the ability to detect nonalbicans species has become increasingly important **(Baveja, 2010).** Other differential media have been developed that permit isolation of certain Candida species based on colony appearance and color following primary culture. The benefit of such media is that the presence of multiple Candida species in a single infection can be determined which can be useful in selecting subsequent treatment options. Example of such media is

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HiChrome Candida differential agar which can differentiate species of Candida based on the color of the colony on the agar. Candida albicans will appear green on HiChrome Candida differential agar which has specificity of 95 % to diagnose Candida albicans **(Williams and Lewis, 2000).**

Öhman *et al.*,**(1986);** Öhman and Jontell, **(1988)** showed that seventy percent of patients with angular cheilitis (AC) had positive culture for Candida albicans; these results were agreed with this current study.

The present study was accept with the results of Scully *et al.*,**(2002)** who revealed that positive culture for candida albicans; but differed in percentage of positive results (77%) while this percentage in the present study was (100%). In their study, they revealed that angular chielitis caused by Candida albicans is more prevalent in patients with Down syndrome and this may be due to immunological defect which predispose to candidiasis or may be due to the presence of macroglossia which lead to drooling of saliva.

Krishnan and Kannan, **(2013)** reported that (67%) positive culture of this species (candida albicans); this study was agreed in positive results with the present study.

Sharmila and Muralidharan, **(2015)**; Oza and Doshi, **(2017)** showed low percentages of positive culturing for candida albicans were (25%) and (15%) respectively.

The results of PCR in patients with angular cheilitis in this current study revealed a positive finding of Candida albicans for these patients; this harmonized with the results of **Imabayashi *et al.*, (2016)** that showed one-hundred percent of involved patients with pseudomembranous oral candidiasis (thrush) were positive growth for Candida albicans by polymerase chain reaction (PCR).

Polymerase chain reaction is a laboratory technique utilized to amplify specific segments of DNA for a wide range of laboratory and/or clinical applications.

In addition, the present study in compact with the results of Liguori*et al.*,**(2009);** Ieda*et al.*,**(2014),** but the results dissimilar with the results of Liguori *et al.*,**(2010).**

Finally, all patients with negative culturing and negative PCR results for candida albicans were excluded in the present study.

The results of the present study showed that 100% Positive Germ tube and 100% was detected in sugar fermentation in patients with angular cheilitis.

The results of the current study matched with Giolo and Svidzinski, **(2010)** who revealed that a continuous prolongation of original or mother cell formed at the

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beginning of process of filamentation process of the yeast C. albicans and is being as a transitional form between the mycelium and the yeast.

Moreover, the data of this study matched with the results of Baradkar *et al.*,**(2010)** showed that one hundred percent of candida albicans were positive for the test of germ tube.

Jeddy *et al.*,**(2011);** Souza *et al.*,**(2015);** Oza and Doshi, **(2017)** were matched with the results of present study that stated (all candida albicans isolated from the patients with angular cheilitis were positive for germ test tube).

Candida albicans exhibits two developmental programs that provide a portion of its phenotypic plasticity, the bud-hypha transition and high-frequency phenotypic switching. Transition to a hyphal growth form provides Candida albicans with the capacity to penetrate the tissue and disseminate, and the mutants of C. albicans do not form hyphae that exhibit a reduction in virulence **(Jeddy *et al.*, 2011).**

Furthermore, Saigal *et al.*,**(2011)** revealed that 80% of isolated Candida albicans were positive for germ tube which is in accordance with the results of the present study.

The result ofNayak *et al.*,**(2012)** was not matched with the present study that concluded only 48% was isolated as positive for germ tube test. This difference is due to the fact that patients in their study did not have candidiasis.

Sugar fermentation test which is a conventional test uses dextrose, maltose, sucrose and lactose. Samples positive for germ tube and which show positive fermentation for dextrose and maltose indicate presence of Candida albicans which harmonize with this study **(Nayak *et al.*, 2012).**

The results of Jeddy *et al.*,**(2011);** Oza and Doshi, **(2017)** were agreed with the results of data of the present study in positively findings of sugar fermentation in patients with angular cheilitis.

Conclusions

In this current study; positive results in all AC patients for both cultures and PCR techniques. The Polymerase chain reaction is accurate laboratory technique used to perform specific segments of DNA for wide circles of clinical and/or laboratory applications.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 3080 – 3094
Received 05 March 2021; Accepted 01 April 2021.

References

1. Ax´ell, T., Simonsson, T. & Birkhed, D. (1985). Evaluation of a simplified diagnostic aid (Oricult-N) for detection of oral candidoses. *Scandinavian Journal of Dental Research*, 93(1), 52–55.
2. Baradkar, V. P., Mathur, M. & Kumar, S. (2010). Hichrom candida agar for identification of candida species. *Indian J Pathol Microbiol*, 53, 93-95.
3. Baveja, C. (2010). Medical mycology, in Text Book of Microbiology for Dental Students, pp. 322–323, *Arya Publications, Delhi, India, 3rd edition*.
4. Cross, D., Eide, M. L. & Kotinas, A. (2010). The clinical features of angular cheilitis occurring during orthodontic treatment: a multi-centre observational study. *J Orthod*, 37(2), 80-86.
5. Giolo, M. P. & Svidzinski, T. I. (2010). Pathophysiology, epidemiology and laboratory diagnosis of candidemia. *J Bras Patol Med Lab*. 46, 225–234.
6. Gravina, H. G., De Morán, E. G., Zambrano, O., Chourio, M. L., De Valero, S. R., Robertis, S. & Mesa, L. (2007). Oral Candidiasis in children and adolescents with cancer Identification of Candida spp. *Med Oral Patol Oral Cir Bucal*, 12(6), 419-423.
7. Griffith, R. S. (2016). A Triad of Dermatologic Dilemmas. *Mo Med*, 113(4), 288–292.
8. Hardy, S. P. (2002). Human Microbiology. *Taylor & Francis Inc. NY, USA*.
9. Höfs, S., Mogavero, S. & Hube, B. (2016). Interaction of Candida albicans with host cells: Virulence factors, host defense, escape strategies and the microbiota. *J. Microbiol*, 53, 149–169.
10. Hube, B. (2004). From commensal to pathogen: stage and tissue specific gene expression of Candida albicans. *Curr Opin Microbiol*, 7, 336–341.
11. Ieda, S., Moriyama, M., Takashita, T., Maehara, T., Imabayashi, Y., Shinozaki, S., Tanaka, A., Hayashida, J. N., Furukawa, S., Ohta, M., Yamashita, Y. & Nakamura, S. (2014). Molecular Analysis of Fungal Populations in Patients with Oral Candidiasis Using Internal Transcribed Spacer Region. *PLOS ONE* 9(6).
12. Imabayashi, Y., Moriyama, M., Takeshita, T., Ieda, S., Hayashida, J. N., Tanaka, A., Maehara, T., Furukawa, S., Ohta, M., Kubota, K., Yamauchi, M., Ishiguro, N., Yamashita, Y., & Seiji Nakamura, S. (2016). Molecular analysis

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 3080 – 3094
Received 05 March 2021; Accepted 01 April 2021.

of fungal populations in patients with oral candidiasis using next-generation sequencing. *Sci Rep*, 6, 28110.

13. Jeddy, N., Ranganathan, K., Devi, U. & Joshua, E. (2011). A study of antifungal drug sensitivity of Candida isolated from human immunodeficiency virus infected patients in Chennai, South India. *J Oral Maxillofac Pathol,* 15, 182-186.
14. Krishnan, P. A. & Kannan, R. (2013). Comparative study on the microbiological features of angular cheilitis in HIV seropositive and HIV seronegative patients from South India. *J Oral Maxillofac Pathol*, 17(3), 346–350.
15. Lamey, P. J. & Lewis, M. A. (1989). Oral medicine in practice: angular cheilitis. *Br Dent J,* 167(1), 15–18.
16. Lewis, M. & Williams, D. (2017). Diagnosis and management of oral candidosis. Br. Dent. J, 223, 675–681.
17. Liguori, G., Di Onofrio, V., Gallé, F., Lucariello, A., Albano, L., Catania, M. R. & Guida, M. (2010). Candida albicans identification: comparison among nine phenotypic systems and a multiplex PCR. *J prev med hyg,* 51, 121-124
18. Liguori, G., Di Onofrio, V., Lucariello, A., Gallé, F., Signoriello, G., Colella, G., Amora, M. D. & Rossano, F. (2009). Oral candidiasis: a comparison between conventional methods and multiplex polymerase chain reaction for species identification. *Oral Microbiol Immunol*, 24, 76–78.
19. Makwana, G. E., Gadhavi, H. & Sinha, M. (2012). Comparison of germ tube production by Candida albicans in various media. *NJIRM*, 3, 6.
20. Mayer, F. L., Wilson, D. & Hube, B. (2013). Candida albicans pathogenecity mechanisms. *Virulence*, 4, 119–128.
21. Mitchell, T. G. (2007). Medical mycology, chapter 45 in Jawetz, Melnick, & Adelberg's Medical Microbiology; 24th edition. *McGraw Hill Medical, NY, USA*. p:621-658.
22. Nayak, S., Kavitha, B., Sriram, G, Saraswathi, T. R., Sivapathasundharam, B. & Dorothy, A. L. (2012). Comparative study of Candida by conventional and CHROMagar method in non-denture and denture wearers by oral rinse technique. *Indian J Dent Res,* 23(4), 294-297.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 3080 – 3094
Received 05 March 2021; Accepted 01 April 2021.

23. Öhman, S. C. & Jontell, M. (1988). Treatment of angular cheilitis: The significance of microbial analysis, antimicrobial treatment, and interfering factors, *Acta Odontologica Scandinavica*, 46(5), 267-272
24. Öhman, S. C., Dahlen, G., Moller, A. & Öhman, A. (1986). Angular cheilitis: a clinical and microbial study. *J Oral Pathol*, 15, 213-217.
25. Oza, N. & Doshi, J. J. (2017). Angular cheilitis: A clinical and microbial study. *Indian J Dent Res*, 28, 661-5.
26. Pfaller, M. A. & Diekema, D. J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev*, 20, 133–163.
27. Pötsch, L., Meyer, U., Rothschild, S., Schneider, P. M. & Rittner, C. (1992). Application of DNA techniques for identification using human dental pulp as a source of DNA. *Int J Legal Med,* 105, 139-143.
28. Raghunath, P., Seshu Kumari, K. & Subbannayya, K. (2014). SST broth, a new serum free germ tube induction medium for identification of Candida albicans. *World Journal of Microbiology and Biotechnology*, 30(7), 1955–1958.
29. Raju, S. B. & Rajappa, S. (2011). Isolation and Identification of Candida from the Oral Cavity. ISRN Dentistry.
30. Saigal, S., Bhargava, A., Mehra, S. K. & Dakwala, F. (2011). Identification of Candida albicans by using different culture Medias and its association in potentially malignant and malignant lesions. *Contemp Clin Dent*, 2(3), 188–193.
31. Salerno, C., Pascale, M., Contaldo, M., Esposito, V., Maurizio, B., Milillo, L., Guida, A., Petruzzi, M. & Serpico, R. (2011). Candida associated denture stomatitis. *Med Oral Patol Oral Cir Bucal*, 16 (2), 139- 143.
32. Samaranayake, L. (2012). Diagnostic microbiology and laboratory methods in Essential microbiology for dentistry, 4th edition. *Churchill, Livingstone Elsevier,* p: 49-65.
33. Scully, C., Van Bruggen, W., Diz Dios, P., Casal, B., Porter, S. & Davison, M. F. (2002). Down syndrome: lip lesions (angular stomatitis and fissures) and Candida albicans. *British Journal of Dermatology*, 147, 37–40.
34. Sharmila, R. & Muralidharan, N. P. (2015). Angular Chelitis in Complete Dentures. *J. Pharm. Sci. & Res,* 7(8), 598-599.

35. Soll, D. R. (2002). Phenotypic switching. In: Claderone R (ed.) Candida and candidiasis, *ASM, Washington DC,* p: 123–142.
36. Souza, M. N., Ortiz, S. O., Mello, M. M., Oliveira, F. M., Severo, L. C. & Goebel, C. S. (2015). Comparison between four usual methods of identification of candida species. *Rev Inst Med Trop Sao Paulo*, 57(4), 281–287.
37. Valones, M. A, Guimarães, R. L, Brandão, L. A., Souza, P. A., Carvalho, A. A. & Crovela, S. (2009). Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. *Braz J Microbiol*, 40(1), 1–11.
38. Walsh, T. J., Grol, A., Hiemenz, J., Fleming, R., Roilides, E. & Anaissie, E. (2004). Infections due to emerging and uncommon medically important fungal pathogens. *Clin Microbiol Infect*, 10(1):48–66.
39. Williams, D. & Lewis, M. (2011). Pathogenesis and treatment of oral candidosis. *J. Oral Microbiol*, 2011, 3.
40. Williams, D. W. & Lewis, M. A. O. (2000). Isolation and identification of Candida from the oral cavity. *Oral Diseases*, 6(1), 3–11.
41. World Health Organization. (2003). Basic laboratory procedures in clinical Bacteriology, 2nd. edition. Editors: Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner P., Piot, P. & Heuck, C. Geneva, Switzerland.