ORIGINAL RESEARCH

Evaluation of Different Techniques to Assess Oral Candidal Carriage in Various Intra-Oral Locations in Healthy Subjects

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ABSTRACT

Background: The aim of this study was to determine the optimal sampling site and the most sensitive technique for oral candidal isolation and also to determine the normal oral candidal carriage in healthy individuals.

Material and Methods: The study included 60 healthy adult subjects (30 males, 30 females) with no known underlying systemic disease and good oral hygiene (subjects obtaining a score of 0-1.2 using the simplified oral hygiene index). Subjects were stratified into 4 groups based on their age and gender. Oral rinse, swab, smear and imprint culture techniques were employed for isolation of Candida from the oral cavity and samples were obtained on a daily basis. All samples obtained were processed and plated on SDA. The culture plates were then incubated at 37˚C for 72 hours and the candidal colonies manually counted.

Results: Significant differences observed between candidal colonies obtained by the various sampling techniques and oral rinse was found to be the most optimal technique followed by swab, smear and imprint. Similarly significant differences were observed between candidal colony counts obtained from the various sites. Dorsal surface of the tongue was found to be the most sensitive site followed by palate, labial vestibule and buccal mucosa.

Conclusions: Age and gender do not influence the oral candidal carriage in case of healthy individuals. Oral rinse was found to be the most superior sampling technique and tongue the most optimal sampling site for candidal isolation from the oral cavity of healthy subjects.

Keywords: Oral candidal carriage, Healthy adult subjects, Candida albicans, Isolation techniques.

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INTRODUCTION

Candida is a yeast-like fungus and is a normal commensal present in the human oral cavity, skin, gastrointestinal and urogenital tracts. The reported rates of yeast carriage in the human mouth vary widely from 25 – 75%, depending on the population sampled and sensitivity of the sampling technique. The most commonly isolated species from the human oral cavity is Candida albicans.[1]

Many studies involving the oral cavity concentrate on the colonization of *Candida albicans* as a surrogate marker of immune status or as a direct marker of oral infection. Various methods are used in the isolation of candidal organisms and these include smears, swabs, oral rinse and imprint cultures.[2,3] Each of these available sampling techniques have some advantages and disadvantages which together with the diverse topography of the oral cavity may put a clinician or researcher in a dilemma as to the optimal technique or site to be utilized in the study of candidal organisms. Therefore, the aim of this study was to determine the optimal sampling site and the most sensitive technique for candidal isolation in healthy subjects so as to standardize the sampling site and technique for future studies, and also to determine the normal oral candidal carriage in healthy individuals.

MATERIAL AND METHODS

The study included 60 healthy adult subjects (30 males, 30 females) with no known underlying systemic disease and good oral hygiene (subjects obtaining a score of 0-1.2 using the simplified oral hygiene index). Informed consent was obtained from the patients in the study sample as per the regulations of the Institutional Ethics Committee (MCODS, Mangalore).

Subjects for the study were categorized into four groups of 15 individuals each, based on their age and gender. Group 1 consisted of subjects in the age range of 20-30 years, Group 2 consisted of subjects in the age range of 30-40 years, Group 3 consisted of subjects in the age range of 40-50 years and Group 4 consisted of subjects in the age range of 50-60 years.

METHODOLOGY

Day 1 – oral rinse sample of the subject was obtained

Day 2 – swab samples were obtained from four representative sites (buccal mucosa, tongue, palate and labial vestibule) of the same subject.

Day 3 – smear samples were obtained from the same four representative sites of the same subject.

Day 4 – imprint samples were obtained from the same four representative sites of the same subject.

Oral rinse technique

10 ml of sterile saline was given to the subject and requested to rinse the mouth for 60 seconds, following which the subject was asked to return the rinse in a sterile broad-mouthed container, which was transferred to the laboratory. The sample was centrifuged for 10 minutes, supernatant discarded, deposit re-suspended in 1 ml of sterile saline and 100 microlitre of sample thus obtained was plated on SDA.[3]

Swab technique

Four moist sterile cotton swabs were taken and one swab each was rubbed against four representative sites (buccal mucosa, dorsum of the tongue, vestibular sulcus and palate) in the oral cavity of the subject, following which they were kept in sterile containers, containing 1 ml of sterile saline and transported to the laboratory. The containers were then vibrated using a vortex mixer, so that candidal organisms are dislodged from the swab. Following this, the sample was centrifuged for 10 minutes, supernatant discarded and the deposit re-suspended in 1 ml of sterile saline and 100 microlitre of the sample thus obtained was plated on SDA.[3]

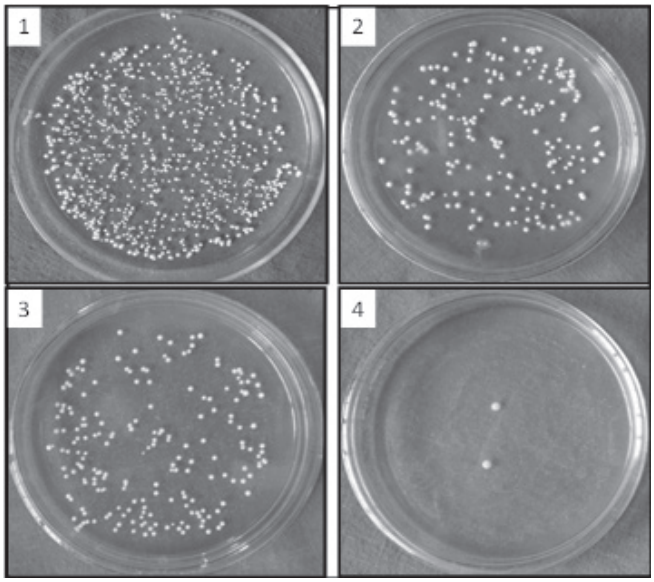
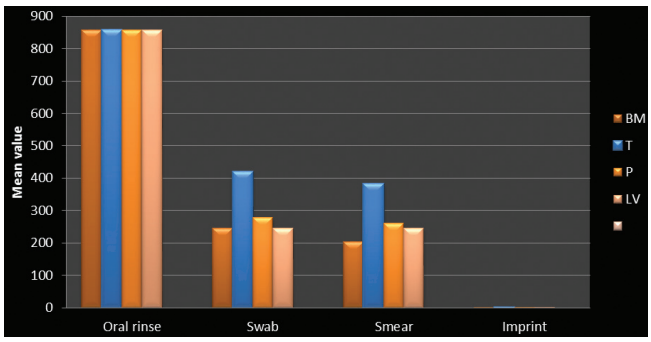


Figure-1: Shows numerous colonies of *Candida albicans* growing on Sabouraud's Dextrose Agar (SDA) obtained by oral rinse technique; **Figure-2:** Shows less numerous colonies obtained by swab technique; **Figure-3:** Shows colonies obtained by smear technique; **Figure-4:** Shows few colonies obtained by imprint technique



Bar Chart-1: Comparison of candidal colony counts with sampling techniques for each site

SMEAR TECHNIQUE

Four moistened tongue blades were taken and smears obtained from the same above-mentioned four representative areas for each patient using five-six strokes for each site. The tongue blade was rinsed with 1ml of sterile saline and the sample thus obtained was stored in a sterile, broad- mouthed container, following which the sample was centrifuged for 10 minutes and re-suspended in 1 ml of sterile saline. 100 microlitre of the sample was then plated on SDA.[3]

IMPRINT CULTURE TECHNIQUE

Sterile plastic foam pads (2x2 cms) were taken, dipped in sterile peptone water and placed with minimal pres-

sure upon each of the above-mentioned representative sites for 60 seconds. The foam pads were pressed on to the SDA medium, after which they were removed and the plated SDA was incubated at 37 degree celsius for 72hours.[3]

All samples obtained on each day were processed and individually plated on Sabouraud's Dextrose Agar (SDA). The plates were incubated at 37˚C for 72 hours and the candidal colonies manually counted. Candidal carriage in 1 ml of the sample was calculated by multiplying the number of colonies counted on the plate by a factor of 10 (dilution factor).[4]

Statistical Analysis

The statistical analysis was done using Kruskal-Wallis test, Mann Whitney test, Friedman test, Wilcoxon

	Site	Age	Minimum	Maximum	Mean	Std. Deviation	Median	Kruskal-Wallis Test	p value
Swab	BM	20 - 30	0	640	210.67	238.970	120.00	3.845	.279
		30 - 40	0	700	155.33	235.216	30.00		NS
		40 - 50	0	1940	344.67	538.409	100.00		
		50 - 60	0	700	274.67	244.478	180.00		
	T	20 - 30	50	1030	395.33	280.048	340.00	2.824	.420
		30 - 40	80	1040	467.27	359.001	370.00		NS
		40 - 50	0	1610	366.11	415.949	160.00		
		50 - 60	70	1080	470.00	263.944	390.00		
	P	20 - 30	0	660	168.00	207.061	90.00	3.959	.266
		30 - 40	20	850	378.18	288.299	300.00		NS
		40 - 50	0	1340	302.22	356.957	170.00		
		50 - 60	0	1090	291.25	360.349	135.00		
	LV	20 - 30	20	400	167.67	149.557	116.00	3.325	.344
		30 - 40	20	1230	380.00	406.873	265.00		NS
		40 - 50	0	1610	298.42	489.890	80.00		
		50 - 60	0	760	144.12	188.748	110.00		
Smear	BM	20 - 30	0	1440	248.00	365.615	130.00	1.671	.184
		30 - 40	0	1020	309.33	281.868	300.00		sig
		40 - 50	0	570	116.67	196.457	.00		
		50 - 60	0	700	139.33	213.624	30.00		
	T	20 - 30	10	1500	402.00	388.885	240.00	2.243	.523
		30 - 40	20	1340	462.73	504.620	210.00		NS
		40 - 50	0	1790	431.67	489.685	220.00		
		50 - 60	0	1250	252.50	349.142	125.00		
	P	20 - 30	20	860	188.33	268.379	80.00	7.088	.069
		30 - 40	0	860	250.00	333.647	80.00		NS
		40 - 50	0	1230	383.89	351.537	295.00		
		50 - 60	0	1610	196.25	404.341	35.00		
	LV	20 - 30	0	760	149.75	218.297	75.00	.829	.842
		30 - 40	10	1260	279.17	388.434	75.00		NS
		40 - 50	0	1610	330.53	474.394	70.00		
		50 - 60	0	1080	196.71	300.012	80.00		
Imprint	BM	20 - 30	0	5	.87	1.356	.00	1.978	.577
		30 - 40	0	3	.73	1.100	.00		NS
		40 - 50	0	4	.47	1.125	.00		
		50 - 60	0	3	.60	.910	.00		
	T	20 - 30	0	5	1.07	1.792	.00	.738	.864
		30 - 40	0	4	.82	1.601	.00		NS
		40 - 50	0	4	.56	1.294	.00		
		50 - 60	0	4	.50	1.033	.00		
	P	20 - 30	0	6	.80	1.568	.00	2.583	.062
		30 - 40	0	2	.64	.674	1.00		NS
		40 - 50	0	1	.06	.236	.00		
		50 - 60	0	1	.19	.403	.00		
	LV	20 - 30	0	2	.33	.651	.00	6.668	.083
		30 - 40	0	3	.75	.965	.50		NS
		40 - 50	0	1	.11	.315	.00		
		50 - 60	0	3	.35	.862	.00		

Table-1: Comparison of candidal colony counts with the various age groups

Signed Ranks test and Bonferroni test. The p value of < 0.05 was considered statistically significant.

RESULTS

The normal oral candidal carriage in healthy individuals was found to be in the range of 0 – 1940 CFU/ml. Candidal colonies obtained were different for each age group (Table-1), however the differences were not statistically significant. Upon comparison of the various sampling techniques (Table-2), differences were observed between the candidal colonies obtained and the differences were statistically significant (p<0.0001). It was inferred that *oral rinse is the most optimal technique followed by swab, smear and imprint*. On comparing the various representative intraoral sites (Table-3), variations were found in candidal colony counts. The difference was significant (p<0.0001). *Dorsal surface of the tongue* was found to exhibit the maximum candidal carriage followed by *palate, labial vestibule and buccal mucosa*. However, palate was the best site for candidal isolation by the imprint technique but the difference was non-significant.

Bar chart compares the mean candidal colony counts with the various sampling techniques employed and shows that tongue has the highest sensitivity in determining candidal colonization.

DISCUSSION

Candida albicans is a commensal yeast normally present in small numbers in the oral flora of a large proportion of humans, but mere presence of this fungus is not sufficient to produce the disease. According to the present study, the normal oral candidal carriage in healthy individuals was found to be in the range of 0 – 1940 CFU/ml. Similar results were obtained by Oliver and Shillitoe in a study conducted on one hundred healthy Caucasian subjects.[5] The importance of creating a normal candidal carriage baseline is that it can serve as an indicator of immunosuppression or any other underlying systemic disease in an individual. A very fine line of separation exists between commensalism and disease as *Candida* is a highly opportunistic commensal which can quickly turn pathogenic.

No significant differences were observed in the candidal colony counts obtained in the different age groups and between males and females of the studied age group be-

Site	N	Minimum	Maximum	Std. Deviation	Median	Friedman Test	p
BM							
Oralrinse	60	0	1670	483.086	800.00		
Swab	60	0	1940	332.731	105.00	117.428	p<0.000I HS
Smear	60	0	1440	277.281	70.00		
Imprint	60	0	5	1.115	0.00		
T Oralrinse							
Swab	60	0	1670	483.044	800.00		
Smear	60	0	1610	331.892	370.00	114.719	p<0.000I HS
Imprint	60	0	1790	430.983	205.00		
	60	0	5	1.415	0.00		
P Oralrinse							
Swab	60	0	1670	423.001	800.00		
Smear	60	0	134.0	314,898	145.00	122.733	p<0.0001
Imprint	60	0	1610	346.758	90.00		HS
	60	0	6	0.904	0.00		
LV Oralrinse							
Swab	60	0	1670	482.959	800.00		
Smear	60	0	1610	355.933	110.00	124.518	p<0.000l HS
Imprint	60	0	1610	387.884	80.00		
	60	0	3	0.732	0.00		
Total Oralrinse Swab							
Smear	240	0	1670	479.981	800.00		
Imprint	240	0	1940	341.247	150.00	473.520	p<0.000l HS
	240	0	1790	363.755	100.00		
	240	0	8	1.078	.00		

Table-2: Comparison between the sampling techniques

Site	N	Minimum	Maximum	Std. Deviation	Median	Kruskal-Wallis Test	p
Swab							
BM	60	0	1940	338.731	105.00		
T	60	0	1610	331.892	370.00	21.746	0.000 HS
P	60	0	1340	314.896	145.00		
LV	60	0	1610	355.933	110.00		
Total	240	0	1940	341.247	150.00		
Smear							
BM	60	0	1440	277.261	70.00		
T	60	0	1790	430.983	205.00	7.940	0.047 sig
P	60	0	1610	346.758	90.00		
LV	60	0	1610	367.684	80.00		
Total	240	0	1790	363.755	100.00		
Imprint							
BM	60	0	5	1.115	0		
T	60	0	5	1.415	0	3.394	0.335 NS
P	60	0	6	0.904	0		
LV	60	0	3	0.732	0		
Total	240	0	6	1.078	0		

Table- 3: Comparison between the sites

tween 20 to 60 years. This indicates that age and gender of individuals are not the confounding factors for *Candida* colonization, although further studies in these aspects will be required with specific age and sex related comparisons. Zaremba et al[6] conducted a study in which the incidence rate of *Candida species* in the oral cavity of middle aged and elderly subjects was studied and no significant differences were noted in the incidence of *Candida species* between middle aged and elderly subjects. However, Lockhart et al demonstrated that frequency, intensity of carriage and multispecies carriage all increase as a function of age, independent of denture use. Possibly, could be due to the advanced age range of 60-93 years of the subjects. Age related changes in the oral cavity which may be responsible for increased colonization is reduced salivary flow, leading to a more acidic environment predisposing to increased candidal carriage. Also natural yeast inhibitors in saliva like histatin, defensin, secretory immunoglobulin A and lactoferrin levels show decrease with age. The phagocytic activity of salivary neutrophils is also reduced; thereby their Candida killing activity is also suppressed.[7]

Samarnayake et al.[8] compared the sensitivity of the rinse culture and imprint culture methods and showed that oral rinse is the best technique for candidal isolation. In the present study, Oral rinse was found to be most sensitive technique in estimating the oral candidal colonization in healthy individuals, followed by swab, smear and imprint techniques (decreasing order of colony counts).[3]

Arendorf and Walker[9] reported that in the dentate, dorsum of the tongue was the most densely colonized region. In our study also, dorsal surface of the tongue exhibited maximum candidal carriage, followed by palate, labial vestibule and buccal mucosa. This could be attributed to the irregular surface topography of the tongue and keratinisation. Similar results have been obtained by Scully et al[10] and Cannon and Chaffin.[11]

The oral cavity presents many niches for Candida albicans colonization, and the yeast is able to adhere to a plethora of ligands which include epithelial cells, endothelial cells, soluble factors, extracellular matrix proteins (laminin, collagen, fibrinogen, fibronectin and entactin) and inert materials implanted in the body of the host. Saliva molecules (salivary pellicle) adsorbed to many oral surfaces promotes Candida albicans adherence.[11] Certain other factors like Als protein, Hwp1p and candidal proteinases-secreted aspartic proteinases (SAPs) also have been found to play a significant role in adhesion to oral epithelial cells.[12,13]

CONCLUSIONS

The present study revealed that no significant difference exists in the candidal colony counts obtained between the various age groups and between males and females. Also it was found that oral rinse was the most superior sampling technique and tongue was the most optimal sampling site for candidal isolation.

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