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Assessment of Oral Sample Collection Technique for the Isolation of *Candida albicans* from Patients having Denture Stomatitis

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Abstract: ***Candida associated denture stomatitis remains the more frequent form of oral candidiasis and specifically seen among denture wearers. Denture stomatitis is inflammatory lesion of the denture bearing area caused by Candida albicans. Direct laboratory methods such as microscopy provide preliminary information about the microbial involvement in an infection, but microbial growth is usually required for definitive identification and characterization. There are number of methods used in the sample collection. This investigation has assessed the techniques used in the collection of the fungal specimen. Thirty seven patients who had Candida induced denture stomatitis were selected clinically according to Newton`s criteria. For each patient, three methods of sample collection were done to investigate the presence of Candida colony. The methods were colony forming unit, swab from palatal mucosa, swab from saliva and salivary culture. The samples were cultured in Sabouraud dextrose agar for 48 hours, then semiquantitive and quantitive measure of the colonies were assessed. The results were high significant relation between the three methods. So that, any of the three methods of oral sample collection can be used to determine oral Candida count.***

Keywords: oral, Candida, microbiology

1. Introduction

Candida associated denture stomatitis is a common inflammatory process affecting about 60% of the subjects carrier of a prosthesis [1], with preferential localization to the palatal mucosa. Among the predisposing local factors the main one is the accumulation of microbial plaque especially *Candida albicans* which has the highest adhesion capacity to oral epithelial cells and on the surface of the denture in contact with the mucosa [2], [3].

The clinical diagnosis of any of the forms of oral candidiasis is based on recognition of the lesions, which can be confirmed by the microscopic identification of *Candida* in the oral samples and/or isolation in culture, among other diagnostic methods [4]-[7]. Although the clinical diagnosis of oral candidiasis is relatively simple, confirmation of the diagnosis can be made by the microscopic morphological criteria observation of *Candida* in oral lesion samples which can be made with fresh samples using special stains and by isolation in culture (macroscopic morphological criteria) [7], [8].

Specimen collection is the process taking microbes from the infection site (in *vivo*) by some means of collection. After that, growing them in the artificial environment of the laboratory (in *vitro*). Once grown in culture, most microbial populations are easily observed without microscopy and are present in sufficient quantities to allow laboratory identification procedures to be performed [9]. The objective of inoculating the specimen or a culture of fungus on to a solid medium is to obtain discrete colonies of organisms after appropriate incubation. Diagnostic microbiology involves the study of specimens taken from patients suspected of having infections. The end result is a report that should assist the dentist or clinician in reaching a definitive diagnosis and a decision on antimicrobial therapy. Hence, dentists should be acquainted with the techniques of taking specimens for laboratory analysis [6], [9]-[11].

A standard technique has been followed. Solid media are a useful media as it facilitate observation of colonial characteristics and helpful in identification of organisms, it also facilitate quantification of organisms as in colony forming unit (CFU) [6], [9], [11].

In culture, a scrape can be cultivated on Sabouraud Dextrose agar (SDA) which contains glucose and modified peptone (pH 7.0), then incubated for 48-72 hours. When *Candida albicans* appears as cream-coloured large convex colonies, the result is expressed as colony forming units per milliliter (CFU/mL). This method is a valuable adjunct in the diagnosis of erythematous candidiasis and denture stomatitis as these infections consist of homogeneous erythematous lesions [12]-[14].

Salivary culture techniques are used in parallel with other diagnostic methods to get an adequate quantification of Candida colonies [15]-[18]. Saliva is collected and number of candidal colonies counted after culture on Sabouraud agar [19].

2. Patients, Materials and Methods

The sample of the study were 37 patients recruited from Baghdad College of Dentistry clinics and from specialist dental health centers in Baghdad city. Patients fulfilled the clinical selection criteria of oral candidiasis with *C. albicans* positive culture have entered the clinical trial. Approval was obtained from the local authorities to conduct the trials. Clinical signs consistent with oral candidiasis in palatal

mucosa were categorized according to Newton`s criteria [20] as follows:
0: no inflammation; Grade 1: pinpoint hyperaemia; Grade 2: generalized erythematous type and Grade 3: hyperplastic granular type.

Mucosa swab from palate of the patients was taken. Mucosal oral samples were obtained by a sterile cotton tip applicator (figure 1), swab was collected from the palatal mucosa by weeping all palatal area then plated immediately in SDA then sent to microbiology laboratory for incubation and isolation of Candida.

Unstimulated Saliva Collection was made when subjects were instructed to remove their dentures, and whole unstimulated 2 ml of saliva was allowed to pool in a disposable sterilize plastic container. Other swab was taken from saliva and also cultured.

By micropipette, 10 µml of the sample was plated immediately to the SDA plate for colony forming unit (CFU) count.

For culturing and isolation of *Candida albicans*, swab of mucosa and saliva sample were streaked onto SDA (containing 40 mg/ml of Chloramphenicol) plates, semiquantitively struck out in four quadrants, the swab was applied by a dime-sized area. The inoculum is then crossed-struck with a loop. The loop is then flipped over or flamed and quadrant two was struck by pulling the loop through quadrant. The loop is then flamed again and quadrant three was streaked by going into quadrant two and streaking the rest of the area. Quadrant four was streaked by pulling the loop over the rest of the agar without further flaming. After that incubated aerobically at 37 °C for 48 hours.

For CFU, salivary fluid streak line was crossed-struck with a loop to produce isolated, countable colonies [9], [12].

The samples were identified using first culture characteristics by detecting the possibility of *Candida albicans* being present in the SDA, by describing shape, color and size of the yeast colonies grown on agar.

Gram stain and germ tube was done to examine microscopically. Candida count was done for each culture after 48 h of incubation period.

Statistical analysis: Data were translated into a computerized database structure. An expert statistical advice was sought for. Statistical analysis was computer assisted using SPSS version 21 (Statistical Package for Social Sciences).

The outcome quantitative variables in the current study were non-normally distributed. Such variables are described by median and interquartile range. The difference in median of a quantitative non-normally distributed variable between 3 groups was assessed by the non-parametric test Kruskal-Wallis.

The statistical significance, direction and strength of linear correlation between 2 quantitative variables were measured by Spearman's rank linear correlation coefficient. P value less than the 0.05 level of significance was considered statistically significant.

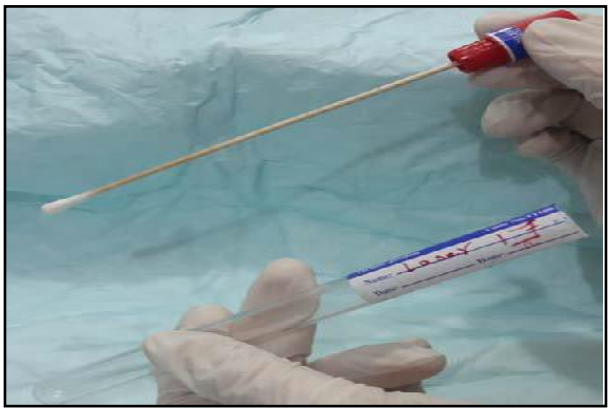

Figure 1: Swab

3. Results

Total of 37 patients have participated in this study, (12 males, 25 females), their age ranges between 30-75 years. The mean age ± SD was 55.7± 12.24.

Clinically, pin point erythema were 9 cases, diffuse erythema were 17 while papillary hyperplasia were 11 cases. Microbiologically, in macroscopic examination, colony characteristics were observed after 48 hours as creamy color with soft consistency and yeasty odor on media. The surface and margins of the colonies were smooth.

Counting of the colonies in mucosal (figure 2) and saliva plates was done for convenient presentation of the data, CFU/ml was adopted to plot the findings (figure 3).

The three different methods of microbiological measurements were correlated with each other, table 1 showed the difference in median of microbiological measurements mucosa and saliva swabs with CFU ordered categories with very strong correlation between salivary swab counting and salivary CFU methods (P< 0.001). Table 2 showed the difference in median mucosal colony count with saliva colony count in swab methods, the statistical results showed high significant relation between the two methods.

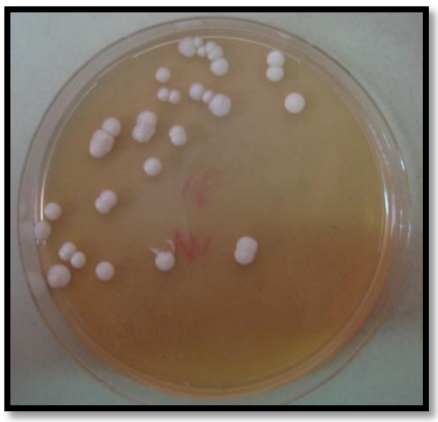

Figure 2: *Candida albicans* from palatal mucosa sample on SDA culture.

Figure 3: *Candida albicans* CFU from salivary sample on SDA culture.

Table 1: The difference in median of selected microbiological measurements (mucosa and salivary swabs) with CFU.

	CFU /ml quartiles			
	First (lowest) quartile (<= 2)	Average (3 - 125)	Fourth (highest) quartile (126+)	P
Mucosal colony count				0.032
Range	(0 to 13)	(0 to 151)	(0 to 283)	
Median	2	6	25	
Interquartile range	(0 to 5)	(1 to 8)	(6 to 37)	
N	10	18	9	
Mean rank	12.2	19.8	24.9	
r=0.54 P<0.001				
Saliva colony count				**<0.001**
Range	(0 to 2)	(1 to 120)	(11 to 150)	
Median	0	10	93	
Interquartile range	(0 to 0)	(4 to 35)	(80 to 137)	
N	10	18	9	
Mean rank	5.6	20.5	30.9	
r=0.938 P<0.001				

Table 2: The difference in median mucosal colony count with saliva colony count

	Saliva colony count quartiles			
	First (lowest) quartile (<= 1)	*Average (2 - 67)*	*Fourth (highest) quartile (68+)*	*P*
Mucosal colony count				**0.009**
Range	(0 to 13)	(0 to 151)	(0 to 283)	
Median	2	6	25	
Interquartile range	(0 to 5)	(1 to 8)	(6 to 37)	
N	10	18	9	
Mean rank	11.8	19	26.9	
r=0.611 P<0.001				

4. Discussion

The diagnosis of an infectious disease entails a number of decisions and actions by many people. The clinician or dentist collect an oral microbiological sample, send to laboratory and then the dentist receives the laboratory report and uses the information to manage the condition [6], [8]. The present study offers three methods of the sample collection of oral candidiasis, with the purpose of establishing when complementary microbiological techniques for the investigation of oral candidiasis should be used, and which techniques are most commonly employed in routine clinical practice in order to establish a definitive diagnosis.

The three different methods have been used in collecting microbiological sample. Swab from palatal mucosa; swab from saliva and salivary CFU) is easy and acceptable by the patient. The scraped methods are successfully used in detecting Candida [12], [13], and considered as a simplified diagnostic aid [4]. However, Terai and Shimahara, 2009 concluded that swab and culture test, which are currently used in the diagnosis of oral candidiasis, can yield false-negative results in 25% of cases [21].

In case of salivary method, many studies also confirm that salivary candidal shed can be used as a tool in predicting oral candidiasis [15], [16], [18]. Malamud and Rodriguez-Chavez in 2011 recommended that uses of saliva samples for Candida pathogen isolation for clinical diagnosis of fungal infections [17].

The higher significancy between three techniques indicated that all of these techniques can be used in determining Candida count. However, this research could not determine the superior method. Therefore additional study is needed to investigate the relation between the clinical criteria and candidal count.

5. Conclusion

The three different methods of collecting microbiological samples (swab from palatal mucosa; swab from saliva and salivary CFU) can be used in determining Candida count.

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