

Comparison of *Candida* sp. Colonies in Gargling-volume Culture from Subject Wearers of Heat-cured and Self-cured Acrylic Resin Removable Partial Dentures

¹Zaki Mubarak, ²Liana Rahmayani, ³Abdillah I Nasution, ⁴Pratiwi Bunjamin

ABSTRACT

Introduction: *Candida* is a normal microflora commonly found in subjects wearing denture compared with nonwearing denture. Altogether, removable partial denture (RPD) is manufactured by heat-cured and self-cured acrylic resins.

Aim: The aim of this study is to observe the comparison of the colonies of *Candida* sp. in gargling-volume culture from subject wearers of heat-cured and self-cured acrylic resin RPDs. This study was conducted in the Microbiology Laboratory of Veterinarian Faculty, Syiah Kuala University (Unsyiah).

Materials and methods: This is an analytical study with an experimental research design. Subjects were obtained by purposive sampling technique and were willing to fill in the informed consent. The gargling volume of six subjects wearing heat-cured and self-cured acrylic resin RPD was inoculated into the Sabouraud 4% dextrose agar (SDA) and incubated at 37°C for 24 to 48 hours. The colonies of *Candida* sp. were calculated with colony counter.

Results: Only 4 of 6 samples (45% precision) had shown colonic growth of *Candida* sp. However, the *t*-test analysis shows that the colonies of *Candida* sp. displayed no significant difference between subjects wearing heat-cured and self-cured acrylic resins RPD ($p > 0.05$).

Conclusion: It was concluded that there was no difference in the number of colonies of *Candida* sp. in the subject wearers of heat-cured acrylic resin compared with self-cured acrylic resin RPDs.

Clinical significance: Clinically, the results of this study showed differences in the number of colonies of *Candida* sp. In the subjects of RPD users of heat-cured acrylic resins, they contained fewer colonies of *Candida* sp. than the subjects of RPD self-cured acrylic resin users.

Keywords: Acrylic resin, *Candida* sp., *Candida albicans*, Heat-cured, Removable partial denture, Self-cured.

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INTRODUCTION

Candida species is a fungus, which is commonly found in healthy oral cavity¹ and usually persists inside the oral cavity and constituting 25 to 50% of normal microflora.² Commensal *Candida* may become parasitic with predisposing factors to help *Candida* proliferate and cause oral candidiasis.^{1,3} The most common *Candida* species found in the human oral cavity is *Candida albicans*. Furthermore, other species, such as *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *Candida dubliniensis*, and *Candida parapsilosis* are also found in the oral cavity.¹ *Candida* is more common in women, people with blood type O, denture wearers, smokers, and immunocompromised patients.^{4,5} Factors that are capable of increasing the prevalence of *Candida* and causing candidiasis are high carbohydrate intake, xerostomia, corticosteroid use, immunosuppressive, long-term broad-spectrum antibiotics,^{1,5} antihistamines,⁶ and mouth breathing.⁷ Some other factors, such as physiological conditions, nutritional deficiencies,^{5,6} hematinics,⁴ diabetes mellitus,^{1,4,5,8} and the effects of chemotherapy and radiotherapy.⁶ Denture-related stomatitis associated with *Candida* is the most common form of candidiasis,⁹ which is of about 60 to 65% in a subject wearing denture.¹⁰

The results of a study in 2007 had shown greater number of *Candida* (66.7–73.3%) in denture-wearer subjects of 40 to 59 years old compared with nonwearer denture subjects (39.3–34.1%).⁷ Several factors affecting the attachment of *Candida* to the surface of the denture are isolated hydrophobicity,¹¹ porosity,^{12,13} water absorption of the material, surface free energy, and surface roughness.¹²

Acrylic resins are the most common synthetic polymers used in the making of artificial teeth.¹⁴ The *C. albicans* are often detected in acrylic resin dentures.

^{1,3}Department of Oral Biology, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia

²Department of Prosthodontics, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia

⁴Dental Education Program, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia

Corresponding Author: Zaki Mubarak, Department of Oral Biology, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia, Phone: +06517555183, e-mail: zakimubarak54@yahoo.com

The amount of *Candida* on the acrylic resin dentures varies by over 80%, and the contamination of the maxillary prosthesis is of 76%. The most commonly isolated species are *C. albicans* (78%), *C. glabrata* (44%), and *C. tropicalis* (19%).¹⁵ Another study had also mentioned that *Candida* adhesion is a complex multifactorial process because it depended on the host and characteristics of each *Candida*.¹

The most commonly used acrylic resins are heat-curing and self-curing resins.¹⁶ The installation of acrylic removable partial dentures on dental worker (66.3%) was higher than to the dentists (33.7%) that described at a study in 2011. Dwi Andhira's research (2011) shows that fixed denture made by a dental worker does not meet the procedural requirements of making fixed denture because the denture looks like a removable denture but is glued to the tooth through a self-curing process. In the same year, another research showed the surface of the self-cured acrylic resin is more porous and rougher than the heat-cured acrylic resin.¹⁷ A study in 2003 had found that residual monomers in self-cured acrylic resins were higher (up to 4.5%) than in heat-cured acrylic resins (2.2%).¹⁸ In general, the fungus tends to stick to the self-cured acrylic resin rather than to the heat-cured acrylic resin.⁵ *In vitro* study in 2011 also showed that *Candida* is less attached to heat-cured acrylic resins compared with self-cured acrylic resins.¹³ Based on the above description, authors became interested in engaging in research to see the comparison of colonies of *Candida* sp. in gargling-volume culture from users of heat-cured and self-cured acrylic resins RPD.

MATERIALS AND METHODS

The type of this research is analytic research, with experimental research design to compare *Candida* sp. colonies from gargle volume cultures of users of heat-cured and self-cured acrylic resins RPD. The research was conducted at the Microbiology Laboratory of the Faculty of Veterinary Medicine of Syiah Kuala University, Indonesia. The population of this study was all partial denture users made by dental workers in Banda Aceh. The subjects of this study were users of heat-cured and self-cured acrylic resin RPDs. The subjects of the research were obtained by purposive sampling technique with certain considerations made by the researcher himself based on the characteristics or properties of the population that were formerly known. The sample was *Candida* sp. derived from oral specimens of subjects with RPD of heat-cured and self-cured acrylic resins. Based on the preresearch data for 3 days in six dental workers in Banda Aceh, there were 11 people wearing acrylic resin RPD, that included four heat-cured acrylic resin RPDs and seven self-cured acrylic resin RPDs. To acquire the minimum sample, this study used Slovin's formula, so that the minimum sample is 3.

Inclusion Criteria

The inclusion criteria were

- Subjects wearing RPDs of *heat-cured* and *self-cured* acrylic resin by dental worker in Banda Aceh.
- Subjects were in good health condition .
- Subjects had not shown any clinical signs of *Candida* infection.
- Duration of wearing RPD is more than 3 months and <1 year.
- Willing to be subjects of the research.

Exclusion Criteria

The exclusion criteria were

- Subjects who were smokers
- Subjects who had been taking any medications (antibiotic, antifungal, corticosteroid, immunosuppressive, or antihistamine) within past 6 months

The tools and materials for the research included an informed consent sheet, questionnaire, sterile gloves, sterile mask, oral glass, measuring cup, plastic sterile container, cooling thermo, centrifugate (Kokusan H-9R), reaction tube, micropipet, paper, cotton, digital scales (Ohaus), Erlenmeyer (Duran), glass cup (Duran), hot plate stirrer and heater (Heidolph MR 3001 K), magnetic stirrer, Petri dish (9 cm diameter), glass spreader, object glass, autoclave (ALP), Spiritus, Ose, incubator (Memmert), refrigerators (Toshiba), colony counter (Suntex 570), microscopes (Olympus CX21), digital cameras (Sony), notebooks, and stationery. Materials included phosphate-buffered saline (PBS) sterile solution (0.01 M PBS solution, pH 7.2), SDA Germany Merck (Table 1), Gentamicin 0.1/100 mL, crystalline violet, Lugol and safranin, 96% alcohol, emerson oil, carbohydrate solution consisting of glucose, maltose, sucrose, lactose, and mannitol, and distilled water.

RESEARCH PROCEDURES

This study was conducted in compatible subjects in accordance with inclusion and exclusion criteria and who were willing to participate in the study by signing the informed consent sheet and filling in the questionnaire. Furthermore, subjects were asked to rinse for 1 minute with 10 mL of sterile PBS (0.01 M PBS solution, pH 7.2) and the gargled specimen was filled in as much as possible into the sterile container. Samples are immediately stored

Table 1: Media composition of 4% SDA

Media composition: Peptone from casein	5 g/L
Peptone from meat	5 g/L
D(+)-glucose	40 g/L
Agar	15 g/L

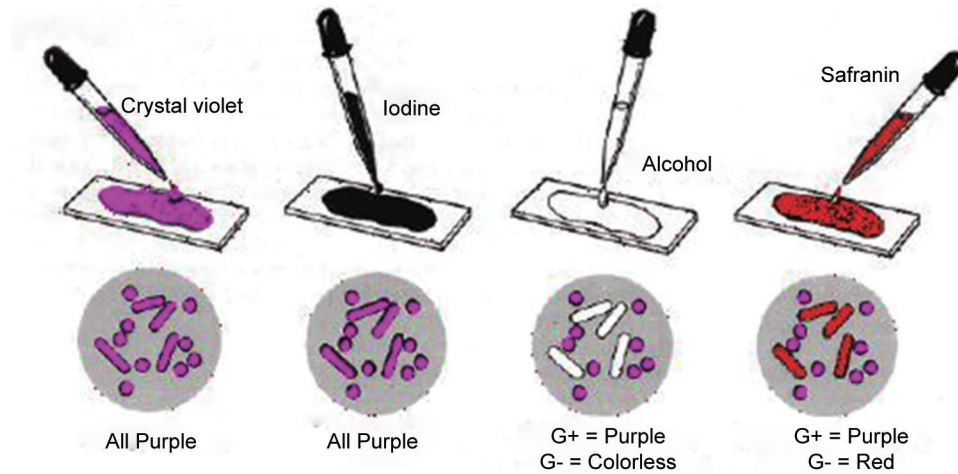


Fig. 1: Gram-staining procedure²⁰

in a cooling thermos to be brought to a microbiology laboratory. The samples were then centrifuged at 3500 rpm at room temperature for 10 minutes to form supernatant and pellet. Supernatant was removed and the pellet was taken. The pellet was then homogenized using 1 mL PBS.¹⁹ Erlenmeyer tube, test tube, magnetic stirrer, Petri dish, and ose were sterilized with autoclave at 121°C at 15 psi for 20 minutes.⁷ Breeding on SDA media began from making SDA media first, and the cultured on SDA Media. Culture was done by inoculating 0.5 mL of the homogenized gargle volume on the SDA. Calculation of *Candida* colonies was done after the colony had grown. Each dish was counted using a colony counter and after that identification was done by Gram staining. The cells of the microorganisms that appeared dark blue/purple are called Gram-positive, while the red ones are called Gram-negative (Fig. 1).²⁰

Identification by Fermentation Test and Assimilation

Cultures of 24 to 48 hours on solid medium are taken by ose, and then inoculated into each carbohydrate solution consisting of glucose, maltose, sucrose, lactose, and mannitol. Fermentation is considered positive when the medium color changes from purple to yellow and the formed gas trapped in the fermented tube/Durham tube. Fermentation is considered negative if there is no

Table 2: Characteristics of isolated *Candida* sp. from clinical specimen on fermentation media²¹

Species	Fermentation				
	Glucose	Maltose	Sucrose	Lactose	Galactose
<i>C. albicans</i>	+	+	-	-	+
<i>C. tropicalis</i>	+	+	+	-	+
<i>C. parapsilosis</i>	+	-	-	-	-
<i>C. krusei</i>	+	-	-	-	-

discoloration on the media after 21 days. The determination of *Candida* species was done by matching the fermentation pattern formed with Table 2.²¹

Assimilation is considered positive when there is turbidity in the media and is considered negative if there is no turbidity or the solution remains clear after 21 days. In the test tube, the solution should remain clear until the last day of incubation. The determination of *Candida* species is done by matching the assimilation pattern formed with Table 3.²¹

Management and Data Analysis

Data were collected through an experimental test at the Microbiology Laboratory of the Veterinary Faculty of Syiah Kuala University. Recording system is processed and analyzed by Statistical Package for the Social Sciences program to see the distribution of data in the form of tabulation and described. Statistical analysis was done using unpaired t-test.

Table 3: Characteristic of isolated *Candida* sp. from clinical specimen on assimilation media²¹

Species	Assimilation										
	Glu	Mal	Suc	Lac	Gal	Mel	Sel	Ino	D-xyl	Raf	Tre
<i>C. albicans</i>	+	+	+	-	+	-	-	-	+	-	+
<i>C. tropicalis</i>	+	+	+	-	+	-	+	-	+	-	+
<i>C. glabrata</i>	+	-	-	-	-	-	-	-	-	-	+
<i>C. parapsilosis</i>	+	+	+	-	+	-	-	-	+	-	+
<i>C. krusei</i>	+	-	-	-	-	-	-	-	-	-	-

RESULTS

This study was conducted on a patient with RPD made by dental worker in Banda Aceh. The sampling of the research was done using gargling technique with six research subjects.

Characteristics of Demography of Subjects

The demography data show that 6 people (100%) are the subjects of the study with age ranging from 43 to 51 years. Male subjects are 4 (66.67%) and female subjects are only 2 in number (33.33%). The data are displayed in Table 4.

Clinical Examination

According to the clinical examination, it is shown that all the subjects had not shown any sign of oral *Candidiasis* clinical features (100%). The data are displayed in Table 5.

Prosthesis Evaluation

Based on prosthesis evaluation, it is shown that heat-cured RPD users were 3 (50%) and self-cured were 3 in number (50%). All the RPDs were made by dental workers in Banda Aceh (100%). Duration of using RPD is >3 months and <1 year (100%) (Table 6).

Candida sp. Culture and Breeding Results on SDA Media

Results of *Candida* sp. colony culture show a slightly convex, smooth, slippery, and round surface on SDA

media. Colonies are yellowish-white and smell sour like that of a aromatic yeast. Breeding of *Candida* sp. on SDA medium was performed with incubation for 24 to 48 hours at 37°C (Fig. 2).

Counting the Colonies of *Candida* sp.

Calculation of number of *Candida* sp. colonies was done using a colony counter. Four dishes show *Candida* sp. growth, while the other two dishes show the growth of fungi instead of *Candida* sp. The numbers of *Candida* sp. found in users of heat-cured acrylic resins removable partial denture, which is in subject 1 obtained 27 colonies and 282 colonies numbers of subject 3. The results are seen in Table 7.

Results of Identification with Gram Staining

The results of identification of four samples on SDA media showed *Candida* sp. growth, which morphologically showed the cells of microorganisms to appear as dark blue/purple under a microscope (×100). After Gram staining, these cells are termed as Gram-positive microorganisms (Fig. 3).

Result of Identification using Fermentation Test and Assimilation

The results of identification of four culture samples on SDA media aged 24 to 48 hours showed growth of *Candida* sp. Morphologically, they showed the presence

Table 4: Frequency distribution of subjects based on age and gender

Variable	Total n (%)
*Age	
43–51 years	6 (100)
*Gender	
Male	4 (66.67)
Female	2 (33.33)

*Significant for gender, between male and female

Table 5: Frequency distribution of subjects based on clinical feature of oral *Candidiasis*

Variable	Total n (%)
*Clinical feature of <i>Candidiasis</i>	
Oral cavity	
Yes	0 (0)
No	6 (100)

*Significant for clinical feature of candidiasis

Table 6: Frequency distribution of subjects based on the manufacture of removable partial denture, maker of removable partial denture, and duration in using removable partial denture

Variable	Total n (%)
<i>Manufacture</i>	
Heat-cured acrylic resin	3 (50)
Self-cured acrylic resin	3 (50)
<i>Maker</i>	
Dentist	0 (0)
Dental worker	6 (100)
<i>Duration</i>	
>3 months–<1 year	6 (100)
<3 months	0 (0)

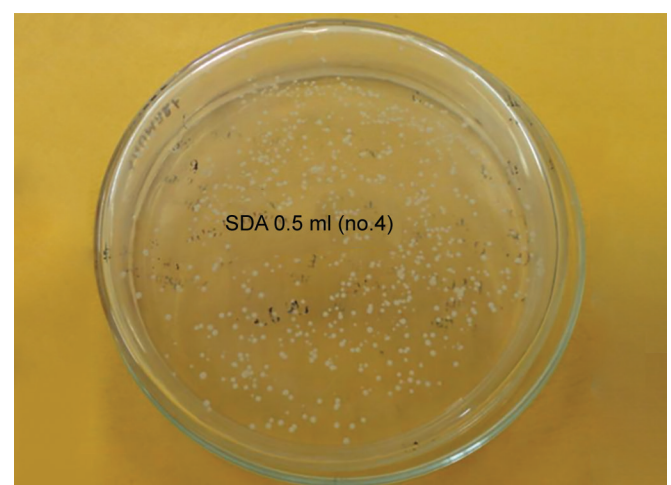


Fig. 2: Culture of *Candida* sp. on SDA

Table 7: Results of *Candida* sp. colonies counting on SDA after incubation for 24 to 48 hours

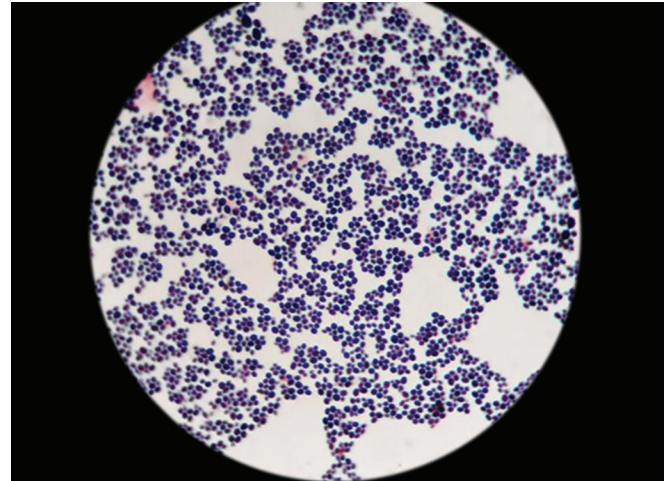
Denture	Colonies (CFU/mL)	Morphology characteristic
<i>Heat-cured acrylic resin</i>		
Subject 1	27	<i>Candida</i> sp.
Subject 3	282	<i>Candida</i> sp.
Subject 5	1	Non- <i>Candida</i> sp.
<i>Self-cured acrylic resin</i>		
Subject 2	3	Non- <i>Candida</i> sp.
Subject 4	230	<i>Candida</i> sp.
Subject 6	171	<i>Candida</i> sp.

of turbidity in carbohydrate solution according to the characteristics of each *Candida* species through assimilation test. In addition, there was also a change in the color of the carbohydrate solution from purple to yellow and a gas was trapped in the Durham tube according to the characteristics of the *Candida* species in the fermentation test. The results of this fermentation and assimilation test can be seen in Table 8.

DISCUSSION

The RPD is a removable prostheses that replace the loss of one or more teeth and surrounding tissue in some part of the jaw arch.²² Recently, the use of acrylic resins is widely applied in dentistry, one of which is used as the basis of RPD. Acrylic resins commonly used in the manufacture of denture are heat-cured and self-cured.²³ *Candida* sp. is an important pathogenic fungus that not only causes localized intraoral *Candida* infections but also is capable to initiate systemic effects.

Placement of the prosthesis in the oral cavity results in the change of environmental conditions in which the prosthesis becomes the site of colonization of oral microorganisms. Microbial plaque on removable prostheses that cover the palate area causes denture stomatitis in 69% of denture wearers.²⁴ According to this, a study was conducted to compare the colonies of *Candida* sp. in the subjects wearing RPD of heat-cured and self-cured acrylic resin. The results of this study showed that RPD is mostly made by the dental worker (100%). The number of subjects wearing RPD of the heat-cured acrylic resin type is 50%,

**Fig. 3:** Results of identification with Gram staining

and self-cured acrylic resin is 50%, with 45% research precision. This result is in accordance with the 2011 study, which showed the installation of RPD by dental worker (66.3%) was higher than by the dentist (33.7%). Data obtained from this study were analyzed using *t*-test paired with degree of freedom 4 and $p > 0.05$, and it was concluded that there is no significant difference in the number of colonies of *Candida* sp. in gargling-volume culture from subject wearers of heat-cured and self-cured acrylic resin RPD.

In the subject wearer of RPD of the heat-cured acrylic resin type, they contained fewer colonies of *Candida* sp. than the subjects wearers of RPD of the self-cured acrylic resin type. This is confirmed by *in vitro* research in 2011 which showed that *Candida* was less attached to heat-cured acrylic resins compared with self-cured acrylic resins.¹³ In the same year, the study showed the surface of the self-cured acrylic resin was more porous and rougher than the heat-cured¹⁷ acrylic resin that facilitates *Candida* retention.²⁵ Size of the surface area of heat-cured and self-cured acrylic resins of the RPDs is thought to affect the number of *Candida* sp. colonies. Number of colonies of *Candida* sp. in RPD manufactured by dental worker in Banda Aceh with the duration of use >3 months to <1 year was found as many as 710 colonies. Research in 2011 had shown that the installation of acrylic RPD by dental worker (66.3%) was higher than by dentists (33.7%). This is due to the public's assumption that the tools used

Table 8: Results of identification with fermentation and assimilation test (*gas formed in Durham tube)

Sample	Assimilation					Fermentation					<i>Candida</i> species
	Glucose	Maltosa	Sucrose	Lactose	D-mannitol	Glucose	Maltosa	Sucrose	Lactose	D-mannitol	
1	+	+	+	-	-	+	+	-	-	-	<i>C. albicans</i>
3	+	-	-	-	-	+	-	-	-	-	<i>C. krusei</i> / <i>C. glabrata</i>
4	+	-	-	-	-	+	-	-	-	-	<i>C. krusei</i> / <i>C. glabrata</i>
6	+	+	+	-	-	+	+	+	-	-	<i>C. tropicalis</i>

by the dental workers and dentists in the manufacture of acrylic RPD are the same and the lack of public knowledge about health being influenced due to the installation of RPD by the dental workers.

The subjects in this study were 43 to 51 years of age. However, the number of colonies of *Candida* sp. was higher in male than female subjects. This is not in accordance with a study conducted by Figueiral et al²⁶ of 128 subjects in Portugal who found that the amount of yeast is more prevalent in females. This is because iron deficiency often occurs in healthy women, which is an important predisposing factor in *Candida* infection. Iron deficiency is associated with the damage to natural immunity (innate) and cell-mediated immunity, contributing to increased risk of infections. Low iron levels result in neutrophil and macrophages dysfunction in killing pathogens.²⁷

CONCLUSION

The conclusion of this research shows that there is no significant difference in number of *Candida* sp. colonies from gargle volume culture user of heat-cured and self-cured acrylic resins RPD.

However, clinically, the results of this study showed differences in the number of colonies of *Candida* sp. In the subject of RPD users, heat-cured acrylic resins contain fewer colonies of *Candida* sp. than the subjects of RPD self-cured acrylic resin users.

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