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DESCRIPTION OF ORAL CAVITY MICROBIAL CULTURE IN DENTAL CARIES SUFFERERS

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ABSTRACT
Background*: Dental caries is one of the most common oral health problems worldwide, affecting 573 million children and 2.5 billion adults. Dental caries is a disease that attacks the hard tissues of the tooth, such as cementum, dentin, and enamel. This causes bacterial invasion, pulp death, and spread of infection to periapical tissues, which can cause pain.* ***Purpose****: The purpose of this study was to determine the microbial culture picture of the oral cavity of patients with dental caries.* ***Methods****: This research is descriptive and the samples in this study amounted to 7 samples of patients with dental caries at Dr. Sitanala Hospital, Tangerang City. The sampling technique in this study used purposive sampling technique.* ***Results****: The results of this study found 1 sample (14.3%) of Candida albicans fungus in fungal culture. In addition, 9 samples (64.3%) of Staphylococcus Sp. bacteria, 3 samples (21.4%) of Klebsiella Sp. bacteria, and 2 samples (14.3%) of Bacillus Sp. bacteria were found in the bacteria culture.* ***Conclusion****: It has been proven that the oral cavity of dental caries sufferers contains various kinds of microbes, such as the fungus Candida albicans, the bacteria Staphylococcus Sp., the bacteria Klebsiella Sp., and the bacteria Bacillus Sp..*

Keywords*: Bacteria, Fungi, Dental caries, Oral cavity*
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INTRODUCTION

Dental caries is one of the most common oral health problems worldwide, affecting 573 million children and 2.5 billion adults. As a result, it puts pressure on public health care systems. Despite efforts by oral health care providers, the prevalence of dental caries has remained unchanged for the past 25 years.

The high frequency of dental caries shows that research against dental caries has an impact.29 Dental caries is a disease that attacks the hard tissues of the teeth, such as cementum, dentin, and enamel. It is caused by microorganisms that consume fermentable carbohydrates. Demineralization of the hard tissues of the teeth is a characteristic feature of this disease. This leads to bacterial invasion, pulp death, and the spread of infection to the periapical tissues, which can cause pain.[15]

Tooth decay can be prevented in several ways, including maintaining good oral and dental hygiene (removing bacteria and plaque in the mouth), strengthening teeth with fluoride solution, avoiding foods that are too sweet and sticky, brushing teeth before bed and after eating, using a soft toothbrush, using water and fruit as a mouthwash, and visiting a dentist every six months.[18] There are various types of bacteria that live in the complex microbiota ecosystem in the oral cavity, namely aerobic bacteria (requiring oxygen) and anaerobic bacteria (not requiring oxygen).[1] Aerobic bacteria that dominate the oral cavity are established when a person is one year old, such as *Streptococcus, Lactobacillus, Actinomyces, Neisseria*, and *Veillonella*. These microorganisms have the ability to settle on surfaces that are not lost when the teeth begin to erupt. After each tooth erupts, more surfaces are available for colonization. The gingival fissure is formed in response to colonization by periodontal microbes. Different microbial colonies can form in different locations on the tooth, such as pits and fissures, where plaque accumulates. This process leads to the development of a diversity of microbial species.

When a person ages and loses all of his teeth, his oral flora resembles that of children before the teeth erupt.[7] Although bacteria are considered the main cause of dental caries, fungi can also be found in the development of this disease, including species of the genus Candida. Candida albicans is the main agent responsible for the development of oral candidiasis. Bacteria and fungi interact by forming biofilms as one of the main steps to defend themselves from the immune response.[11]

According to the research results of Priya Nimish Deo and Revati Deshmukh (2019), with 392 taxa having at least one reference genome and nearly 1500 total genomes in the oral cavity. Around 700 species of prokaryotes have been identified in the oral cavity. These species are spread across 185 genera and 12 phyla, of which around 54% have official names, 14% are unnamed but have been cultivated, and 32% are only known as uncultivated phylotypes. The 12 phyla are SR1, Synergistetes, Gracilibacteria (GN02), Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Spirochaetes, and Saccharibacteria (TM7). Meanwhile, the results of other research according to Khusnul and Sri Jamilah Muta'aly (2018), regarding the identification of fungi in dental caries in children under 10 years of age, as many as 15 samples (75%) of fungi Candida albicans, 2 samples (10%) of Candida stellatoidea fungus, 2 samples (10%) of Aspergillus Flavus fungus, and 1 sample (5%) was not identified.

Based on the background explained above, the researcher is interested in conducting a study on the Description of Oral Cavity Microbial Culture of Dental Caries Patients.

METHODS

This study is descriptive in nature, namely to determine the presence of Gram-positive and Gram-negative bacteria, as well as fungi in the oral cavity of patients with dental caries at Dr. Sitanala General Hospital, Tangerang. The study was conducted in February - April 2024 at the Microbiology Laboratory, Department of Medical Laboratory Technology, Health Polytechnic, Ministry of Health, Banten.

The population of this study were dental caries patients at Dr. Sitanala General Hospital, Tangerang City who were willing to participate in this study. The sample used in this study was dental caries patients at Dr. Sitanala General Hospital, Tangerang, totaling 7 respondents. The sampling technique in this study used purposive sampling technique according to specific criteria that must be met by the samples used in this study. Inclusion Criteria: Aged ≥ 19 years, Number of caries ≥ 1 tooth, Willing to be a respondent. Exclusion Criteria: Aged <19 years, No dental caries.

The tools used in this study were analytical balance, Erlenmeyer flask, measuring cup, beaker, watch glass, spatula, hotplate, magnetic stirrer, test tube, autoclave, oven, sterile cotton swab, scissors, sterile eppendorf, petri dish, incubator shaker, incubator, staining rack, object glass, ose puncture, ose round, dropper pipette, marker, bunsen, match, microscope, tweezers. Meanwhile, the materials used are Nutrient Broth (NB), Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), distilled water, cotton plugs, tissue, crystal violet, gram's iodine (lugol), 96% alcohol, safranin, 3% H2O2 reagent, Voges Proskauer (VP), Citrate, citrate plasma, immersion oil, kovac, Methyl Red (MR), α-naphthol, 40% KOH, oxidase test strip, LPCB, chloramphenicol.

All glassware used is dry sterilized by placing it in an oven at 170°C - 180°C for 2 hours. Furthermore, for liquid materials, it is wet sterilized in an autoclave at 121°C for 15 minutes at a pressure of 1-2 atm.[6]

The preparation of the media used is done by weighing the media powder according to the instructions on the packaging, the weighted media powder is dissolved with distilled water in an Erlenmeyer flask, the media solution is heated and homogenized on a hotplate, the media solution is sterilized in an autoclave at a temperature of 121°C, a pressure of 1-2 atm for 15 minutes, the media is poured into a suitable container. Biochemical test media are distributed into test tubes, while agar media for plating are poured into petri dishes with a diameter of 60 x 15 mm.

Sampling

The patient rinses his mouth with water to remove food residue left in the mouth, the patient opens his mouth and the sample is swabbed on the tooth surface (occlusal, axial, and proximal surfaces) twice using a sterile cotton swab for approximately 1 minute, the sample for fungal culture is transferred into a sterile eppendorf and the sample for bacterial culture is transferred into an eppendorf containing 1 mL of Nutrient Broth.[16] Nutrient Broth (NB) media is an enrichment media used for culture cultivation. NB is made with carbon and nitrogen sources to obtain the nutrients needed by bacteria.[25]

Fungal Culture

Samples in sterile eppendorf were taken

using tweezers and inoculated using the streak plate method on Sabouraud Dextrose Agar (SDA) media. Sabouraud Dextrose Agar (SDA) was incubated for 5 to 7 days at room temperature of 25ºC - 30ºC.[20]

Bacterial Culture

Samples in eppendorf containing 1 mL of Nutrient Broth (NB) were incubated using a shaker incubator overnight at 37oC, samples were taken using tweezers and inoculated using the streak plate method on Nutrient Agar (NA) media, Nutrient Agar (NA) was incubated using an incubator overnight at 37ºC, single colonies on Nutrient Agar (NA) media were taken using an ose and subcultured on a new Nutrient Agar (NA) media plate, Nutrient Agar (NA) was incubated using an incubator overnight at 37ºC.[2]

Glycerol Stock Preparation

The subculture results were rejuvenated in Luria Broth (LB) media for 6 hours at 37oC, then put into an eppendorf containing 500 µl (1:1) of 50% Glycerol, the eppendorf was homogenized 5-6 times until completely mixed, the eppendorf was placed in a freezer at -20°C.[13]

Bacterial Rejuvenation

Eppendorf is taken from the freezer, the bacteria in the Eppendorf is taken using ose and inoculated on Nutrient Agar (NA) media, Nutrient Agar (NA) was incubated using an incubator overnight at 37ºC.

Gram Staining

Bacterial culture on Nutrient Agar (NA) media was taken using an aseptic ose, then flattened in the center of the object glass, the preparation was fixed on a bunsen burner, the preparation was stained with crystal violet for 1 minute. Crystal violet was washed using distilled water, the preparation was stained with lugol's solution and left for 1 minute, the lugol's solution was washed with 96% alcohol and washed using distilled water, then the preparation was stained with safranin and left for 1 minute, the preparation was washed using distilled water, dried and examined under a microscope (100x magnification) by adding immersion oil.[12]

Biochemical Test of Gram Positive Bacteria

In the catalase test, H_2O_2 reagent is dropped onto a glass object, one loop of bacterial colonies is taken and homogenized.[14] In the coagulase test, plasma is dropped onto a glass object, one loop of bacterial colonies is taken and homogenized.[14]

Biochemical Test of Gram Negative Bacteria

Starch test, iodine solution is dripped onto the object glass, 1 loop of bacterial colony is taken and homogenized, then the color change is observed.[21] Oxidase test, 1 loop of bacterial colony is taken and smeared on the oxidase test strip, then the color change is observed.[8]

IMVIC test, bacterial colonies are inoculated on SIM media by piercing the center of the media using a piercing loop, bacterial colonies are inoculated on MR and VP media by homogenizing using a round loop, bacterial colonies are inoculated on SCA media by scratching the slope of the media using a piercing loop, media (SIM, MR, VP, and SCA) are incubated for 24 hours at 37oC, Kovac's reagent is added to SIM media, Methyl Red reagent is dropped as much as 5 drops on MR media, 40% KOH reagent and α-naphthol are dropped as much as 5 drops on VP media, media (SIM, MR, VP, and SCA) are observed for color changes.[9]

Data Analysis

Data from the results of the description of the oral cavity microbial culture of dental caries patients at Dr. Sitanala General Hospital, Tangerang City were analyzed descriptively and presented in the form of tables and figures.

RESULTS

The results of the study were conducted in February - April 2024 at the Banten Health Polytechnic Bacteriology Laboratory, obtained 7 samples of patients with dental caries. This study aims to determine the presence of Gram-positive and Gram-negative bacteria, as well as fungi in the oral cavity of dental caries sufferers.

The results of observations of dental caries cultures planted on Sabouraud Dextrose Agar (SDA) media using the germ tube test can be seen in the table below.

Table 1. Results of fungal identification on Sabouraud Dextrose Agar (SDA) media

Sample Codes	Medium SDA	Macroscopic	Microscopic	Fungal Suspect
1	Ungrowth	-	-	-
2	Ungrowth	-	-	-
3	Ungrowth	-	-	-
4	Ungrowth	-	-	-
5	Ungrowth	-	-	-
6	Ungrowth	-	-	-
7	Growth	Medium round, white yellowish, even edge, convex elevation, unsheen colony aspect	Oval to round in shape, thin bone yeast cells	*Candida albicans*

Based on Table 1, it shows the results of dental caries culture planted on Sabouraud Dextrose Agar (SDA) media, with 6 samples showing no fungal growth and 1 other sample showing fungal growth.

Table 2. Frequency distribution of fungal identification results on Sabouraud Dextrose Agar (SDA) media

Sample Codes	N	Growth		Ungrowth	
		Frequency	Percentage	Frequency	Percentage
7	1	1	14,3%	0	0%
1, 2, 3, 4, 5, 6	6	0	0%	0	85,7%

Table 3. Results of fungal identification on Nutrient Agar (NA) media

Sample Codes		Gram Staining										Biochemistry Test						Bacterial Suspect
		Form	Color	Edge	Elevation	Colony Aspect	Form	Arrangement	Characteristic	Catalase	Coagulase	Starch	Oxsidase	SIM	MR	VP	SCA	
1	1.1	Sr	Wy	E	F	D	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
	1.2	Sr	Y	E	Sc	S	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
	1.3	Lr	W	Ue	C	S	b	Cl	Gn	X	X	-	-	-	-	+	+	*Klebsiella Sp.*
2	2.1	Sr	Wy	Ue	F	Ss	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
3	3.1	Sr	Wy	Ue	F	D	b	Ic	Gp	+	-	X	X	X	X	X	X	*Bacillus Sp.*
	3.2	Mr	Wy	Ue	Sc	Ss	b	Ic	Gp	+	-	X	X	X	X	X	X	*Bacillus Sp.*
	3.3	Lr	W	Ue	C	D	b	Cl	Gn	X	X	-	-	-	-	+	+	*Klebsiella Sp.*
4	4.1	Sr	Wy	Ue	F	D	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
5	5.1	Mr	W	E	Sc	S	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
	5.2	Mr	Y	E	Sc	S	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
6	6.1	Sr	Wy	Se	F	D	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
	6.2	Mr	Wy	Ue	F	D	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
7	7.1	Sr	Wy	E	F	D	c	Cl	Gp	+	-	X	X	X	X	X	X	*Staphylococcus Sp.*
	7.2	Mr	Wy	E	Sc	S	b	Cl	Gn	X	X	-	-	-	-	+	+	*Klebsiella Sp.*

Annotation:
Sr: Small round, Mr: Medium round, Lr: Large round, Wy: White yellowish, Y: Yellow, W: White, E: Even, Ue: Uneven, Se: Slightly even, F: Flat, Sc: Slightly convex, C: Convex, D: Dull, Ss: Slightly sheen, S: Sheen, c: Coccus, b: Basil, Cl: Clustered, Ic: Interchain, Gp: Gram positive, Gn: Gram negative, +: Positive, - : Negative, X : Not tested.

Based on Table 3, it can be seen that all the results of dental caries culture on Nutrient Agar (NA) media showed bacterial growth. The next examination is the identification of gram-positive bacteria, starting from Gram Staining, then continued with biochemical tests to find out what bacteria can identify patients with dental caries. The results of gram staining with 9 sample codes (1.1, 1.2, 2.1, 4.1, 5.1, 6.1, 6.2, 7.10 showed positive coccus clusters, 2 sample codes (3.1, 3.2) showed gram-positive chained bacilli, and 3 sample codes (1.3, 3.3, 7.2) showed gram-negative clustered bacilli. Furthermore, a Gram Positive biochemical test was carried out, where 11 sample codes (1.1, 1.2, 2.1, 3.1, 3.2, 4.1, 5.1, 5.2, 6.1, 6.2, 7.1) showed a Positive catalase test (+) and a Negative Coagulase test (-). Meanwhile, the Gram Negative biochemical test, where 3 sample codes (1.3, 3.3, 7.2) showed a Negative starch test (-), positive oxidase (+), negative SIM, negative MR (-), positive VP (+), positive SCA (+). So it can be concluded with the code 9 samples (1.1, 1.2, 2.1, 4.1, 5.1, 5.2, 6.1, 6.2, 7.1)

indicate Staphylococcus Sp bacteria. 3 sample codes (1.3, 3.3, 7.2) indicate Klebsilla Sp bacteria. and 2 sample codes (3.1, 3.2) indicate *Bacillus, Sp.* bacteria.

Table 4. Frequency distribution of bacterial identification results on Nutrient Agar (NA) media

Sample Codes	N	*Staphylococcus Sp.*		*Klebsiella Sp.*		*Bacillus Sp.*	
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.1, 1.2, 2.1, 4.1, 5.1, 5.2, 6.1, 6.2, 7.1	9	9	64,3%	0	0%	0	0%
1.3, 3.3, 7.2	3	0	0%	3	21,4%	0	0%
3.1, 3,2	2	0	0%	0	0%	2	14,3%

DISCUSSION

The study was carried out by planting on SDA media for fungal culture. The results of macroscopic observations of 7 samples showed that only 1 sample had colony growth, microscopic observations were carried out using germ tube testing. In line with the research of Indrayati (2018) which stated that for further identification of fungi, it was carried out using the germ tube test. The germ tube testing technique is carried out by taking 0.5 ml of serum which is put into an eppendorf tube and adding colonies to the SDA media which is taken with a round ose and incubated for 1-2 hours in the incubator. Then 1 drop of colony is taken which is dropped on the glass object. After that, it is observed under a microscope at 40x magnification.

Based on Table 1. shows the results, where 6 samples (85.7%) showed no fungal growth and 1 sample (14.3%) showed *Candida albicans* fungal growth. Previous research conducted by Khusnul and Sri Jamilah Muta'aly (2018), on the identification of fungi in dental caries in children under 10 years of age, 15 samples (75%) of *Candida albicans* fungi, 2 samples (10%) of *Candida stellatoidea* fungi, 2 samples (10%) of *Aspergillus Flavus* fungi, and 1 sample (5%) was not identified. It can be concluded that the most common fungus in the oral cavity is *Candida albicans*, which can be found in 40% of adults even in those who do not show any symptoms at all.[26] *Candida albicans* is a normal flora that is mostly found in the vagina, digestive tract, respiratory tract, skin, mucous membranes, and oral cavity. Oral candidiasis (moniliasis) is a disease caused by *Candida albicans*.[17] *Candida albicans* can also increase its colony numbers on dirty dentures, causing inflammation of the oral mucosa known as denture stomatitis.[28]

SMeanwhile, in the examination of bacterial culture, culture and subculture were carried out on NA media. The use of NA media as a universal medium for bacterial growth.[24] The results of the NA media culture were subjected to the Gram staining process, the purpose of gram staining is to distinguish between gram positive and gram negative, namely by using a differential staining procedure.[5] Furthermore, biochemical tests were carried out to determine the characteristics of bacteria through biochemical reactions. Identifying Gram Positive bacteria is done by catalase and coagulase tests. The catalase test is used to distinguish Staphylococcus bacteria from Streptococcus bacteria, while the coagulase test is used to distinguish Staphylococcus aureus bacteria species from other Staphylococcus species.[3]

Identifying Gram Negative bacteria is done by SIM, MR, VP, SCA, starch test, and oxidase test. SIM (Sulfur, Indole, Motility) test is used to see bacterial growth, indole production, and H_2S gas formation.[3] MR is used to produce mixed acids from glucose fermentation contained in MR medium. VP is used to produce neutral products such as acetyl methyl carbinol (acetoin) based on glucose metabolism results that will produce acid. SCA is used to see the ability of bacteria to use citrate as the only most abundant carbon source.[9] Starch test is used to determine the presence of polysaccharide carbohydrate content.[21] Oxidase test is used to see the presence of oxidase enzymes in bacteria.[4]

Based on Table 3. shows the results of bacterial growth, where as many as 9 samples (64.3%) of *Staphylococcus Sp.* bacteria, 3 samples (21.4%) of *Klebsiella Sp.* bacteria, and 2 samples (14.3%) of *Bacillus Sp.* bacteria. Previous research conducted by Yonesta (2018), on identifying normal microflora in cavities before and after using a toothbrush, found that the bacteria that were successfully identified were *Bacillus Sp., Klebsiella Sp.,* and *Streptococcus Sp. Staphylococcus Sp.* bacteria are normal flora bacteria in the oral cavity that have the potential to turn into pathogens and cause diseases, such as tooth decay and tartar.[22] *Klebsiella Sp*. are bacteria that are commonly found in the human mouth, nose, and intestines that can cause infections in a number of organ systems, such as lung infections (pneumonia), urinary tract infections, skin, and digestive tract.[23] *Bacillus Sp*. are bacteria found in the oral

cavity as normal flora, but under certain conditions can become pathogens or cause disease.[19]

Based on the results, it can be concluded that in the oral cavity of dental caries sufferers, various types of microbes are proven to be present, such as *Candida albicans* fungus, *Staphylococcus Sp. bacteria, Klebsiella Sp. bacteria,* and *Bacillus Sp. bacteria.*

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