ORIGINAL ARTICLE

Bacterial growth assessment of extraction pliers following tooth extraction in culturing bacterial on various media: a quasi-experimental study

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ABSTRACT

Introduction: Tooth extraction injury potentially creating an entry point for bacteria that may cause infection. Dental extraction tools that come into contact with a patient's oral cavity contain many bacteria which can be opportunistic and pathogenic. The purpose of this study was to examine the bacterial growth on tooth extraction pliers following tooth extraction in culturing bacteria on various media. **Methods**: Type of study is a quasi-experimental research design, using pre and post-test analysis. It was conducted in September–October 2021 at the Teeth and Mouth Dentistry Hospital (RSGM), Universitas Syiah Kuala, focusing on the swab sampling of tooth extraction pliers. The sample examination stage was conducted at the Faculty of Dentistry Laboratory. The study involved culturing bacterial on various media, including NA (Nutrient Agar), MHA (Mueller Hinton Agar), TSA (Triptic Soy Agar), TYS20B (Trypticase Soy-Yeast 20% Sukrose with Bacitracin), and performing gram staining under a microscope. **Results**: NA media: out of 16 samples cultured, 8 were not covered by bacteria, while the other 8 were. MHA media: all samples were overgrown with bacteria, but each 8 had different characteristics. 9 samples of TYS20B medium were overgrown with bacteria, while the other seven did not show signs of bacterial overgrowth. On TSA media, 5 samples were not overgrown with bacteria, 9 samples were overgrown with bacteria exhibiting solid, yellowish white, and not slimy, and the other 2 samples contained bacteria that were soft, yellowish white, and slimy. **Conclusion**: *Bacillus sp., Diphtheroid basil sp.,* and *Streptococcus mutans* were found grown on the agar media. In general, MHA media is the most effective general growth medium, while TYS20B media is the best media for *Streptococcus mutans* growth.

KEYWORDS

bacteria, culture media, tooth extraction pliers

INTRODUCTION

The human oral cavity is estimated to be inhabited by more than 700 species of bacteria.[1] Bacteria in the oral cavity generally do not cause infection, but they can be pathogenic under certain conditions, such as age, immune system, and antibiotic use.[2] In recent years, the bacterial culture technique has been proven to be an effective approach/method for identifying bacteria consisting of liquid, solid, and semisolid media.[3]

Culture media is a growth tool that contains the nutrients needed by bacteria to grow.[4] These nutrients include water, carbon sources, nitrogen sources, and

some mineral salts. Besides these nutrients, bacterial growth requires additional growth factors in minimal amounts, such as vitamins, amino acids, antioxidants, and blood. Recent culture media are made similarly to bacteria's natural environment to make it easier for bacteria to grow.[5] Bacteria are a group of living things that do not have a nuclear envelope. Several types of bacteria are so pathogenic that they can cause disease or harm to their hosts, one of which is humans. Bacteria are small, so they can spread widely in the human environment.[6]

Tooth extraction is a common procedure in dentistry that is frequently performed in hospitals, public health centers (Puskesmas), and family dental care clinics is tooth extraction. It is the most frequently performed surgery.[7] The act of tooth extraction causes injury to the location of the extracted tooth, potentially creating an entry point for bacteria that may cause infection. These infections can prolong the healing process on the wound after tooth extraction.[8,9] This occurs when pathogenic bacteria enter the host's cell or tissue, where the bacteria attach to the host's cell or tissue, which can be the initial stage of infection. Studies regarding similar topic have been conducted in various locations and times.[10–15]

Dental extraction tools that come into contact with a patient's oral cavity contain many bacteria. These bacteria can be opportunistic and pathogenic, which pose a risk of cross-contamination, infection, and even cause systemic infection. Dentists face a high risk of cross-infection due to direct contact with the patient's mouth, saliva and blood. Contamination through blood can occur through wounds resulting from tooth extraction.

Based on the explanation provided, in purpose of expanding our understanding of this topic, the researchers conducted an examination regarding bacterial growth of extraction pliers following tooth extraction at the Teeth and Mouth Dentistry Hospital (RSGM) Universitas Syiah Kuala. The purpose of this study was to examine the bacterial growth on tooth extraction pliers following tooth extraction in culturing bacterial on various media.

METHODS

Type of study is a quasi-experimental research design, using pre and post test analysis. This research was conducted in September–October 2021. The sample size was determined by the calculation using the Slovin formula. Tooth extraction pliers were obtained from the Teeth and Mouth Dentistry Hospital (RSGM) Universitas Syiah Kuala. Sample of 16 tooth extraction pliers. The sample examination was carried out in the Faculty of Dentistry Laboratory.

Prior to this research, the primary requirement was to sterilize all instruments. Instruments that are resistant to high temperatures, such as loop needles, measuring cups, petri dishes, and test tubes, underwent thorough washing and drying. Following this process, the instruments were wrapped using HVS papers. Subsequently, they were sterilized at a temperature of 160° C 120 minutes in an oven.

The equipment used to produce transport media is sterile. In 100 mL of distilled water, two grams of BHIB powder were weighed, and dissolved. The media were homogenized using a magnetic stirrer and sterilized by an autoclave at 121°C. After being sterile and cold, the transport media were aseptically poured into the microtubule by working next to an alcohol burner.[16]

Tooth extraction pliers are one of dentistry's most critical instruments. Critical instruments are those that come into direct contact with structures or tissues covered by skin or mucosa. They must be sterilized using an autoclave, used immediately after sterilization, or packaged after sterilization and left in the packaging until use.[17]

Following the tooth extraction, the operators used gloves and sterile masks for the sampling procedure, and the tools were in sterile condition. The operators took a swab from the active part of the tooth extraction pliers, also known as beak, which they used to extract the teeth from the patients. All samples were

put into a microtube and placed in an ice box. They were then examined at the Universitas Syiah Kuala's Faculty of Dentistry Laboratory.[18,19]

This study used four agar media: three general media and one selective medium. The production processes of general media included *Nutrient Agar* (NA), *Mueller Hinton Agar* (MHA), and *Triptic Soy Agar* (TSA). These four agar media were chosen since these are universal media that is commonly used for the growth of most bacteria and has been clinically tested to be good for bacterial growth. To begin, 62.5 ml of distilled water was poured into each beaker glass for each agar medium. The agar was initially weighed to ensure the correct amount was used. The nutrient agar media was weighed at 1.4 grams, MHA at 2.37 grams, and TSA at 2.5 grams. Next, 62.5 ml of distilled water was added. A magnetic stirrer was then inserted to dissolve the media. The agar was heated in the microwave, and after it came to a boil, the beaker glass was removed and covered with aluminum foil, plastic wrap, newspaper, and corn thread. This setup was then sterilized using an autoclave at a temperature of 121°C for 15 minutes. After the materials had cooled slightly, they were then transferred to petri dishes using an alcohol burner.

For *Trypticase Soy-Yeast 20 Percent Sucrose with Bacitracin* (TYS20B) media, the following ingredients were used: 40 gr of sucrose, 2 gr of yeast extract, 8 gr of trypticase soy agar, and 1 gr of bacto agar. Once the ingredients were accurately weighed, they were combined in an Erlenmayer flask, and 200 ml of sterile distilled water were added. The mixture was then homogenized and heated in the microwave until it came to a boil, and covered with aluminum foil, plastic wrap, and newspaper. Then, they were sterilized in an autoclave at a temperature of 121°C for 15 minutes. After the sterilization process was complete, the materials were allowed to cool slightly before being transferred to petri dishes immediately by using the spiritus-lamp.[20,21]

The method used for bacterial culture on tooth extraction pliers was the streak method. The first step was to homogenize the sample within the transport media using a vortex. Then, it was inoculated on the agar media using the loop needle, which had been priorly sterilized by heating the tip of the loop needle over the spiritus. Furthermore, using the zigzag method, the bacteria were spread across the surface of the agar medium. At a temperature of 37°C, all the cultured-agar media were incubated for 24 hours for NA, MHA, and TSA under aerobic condition, and for 48 hours under anaerobic condition for TYS20B.

The gram staining was used to observe the nature and morphology of bacteria. The tested bacteria were collected with the loop needle and smeared on the microscope slide. First, a crystal violet stain was applied to the microscope slide and allowed to sit for 1-2 minutes. Following this, the slide was rinsed with running water. Next, it was treated with lugol solution and left for 30 seconds. After rinsing again under running water, the pigment on the microscope slide faded with 96% alcohol. Afterward, the stain safranin was applied to the microscope slide for 2 minutes, followed by rinsing under running water, and left to dry at room temperature. Finally, the sample was observed under a microscope with a magnification of 100x.[22]

RESULTS

The samples obtained from the Teeth and Mouth Dentistry Hospital were cultured on NA (nutrient agar) media, which is a common medium for bacterial growth. Bacterial colonies growing on NA media could be observed after being incubated for 24 hours at a temperature of 37^{0}C.

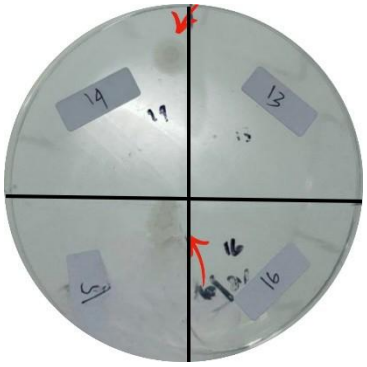

Figure 1. Bacterial Colonies Growing on NA Media on media number 14 and 15

The results of incubation on NA revealed that 8 of the 16 cultured samples were not covered by bacteria, whereas the other 8 were. The dominant bacteria that grow on this medium have the following characteristics: soft, yellowish-white, and slimy.

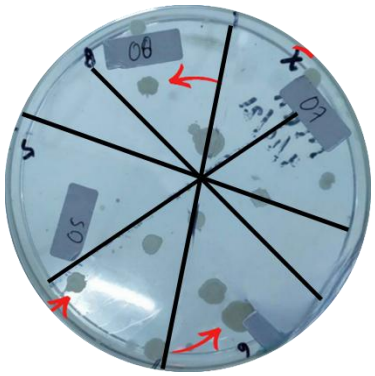

Figure 2. Bacterial Colonies Growing on MHA Media

The 24-hour incubation results revealed that all the samples cultured were overgrown with bacteria. It was found that 8 samples of MHA media were overgrown with bacteria with the following characteristics: solid, yellowish white, and not slimy, as seen at samples 6 and 7, and 8 other samples contained bacteria with the following characteristics: soft, yellowish white, and slimy, as seen at samples 5 and 8.

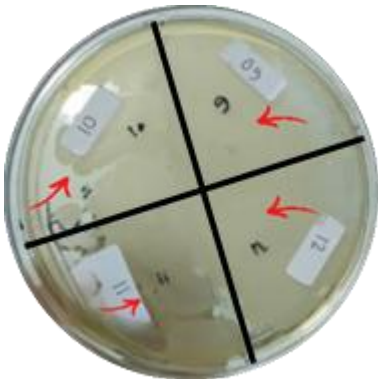

Figure 3. Bacterial Colonies Growing on TYS20B Media

After a 48-hour incubation period, it showed that 9 samples of TYS20B media were overgrown with bacteria that exhibiting the following characteristics: solid, yellowish-white, and not slimy. In contrast, the other 7 samples did not show bacterial growth.

Figure 4. Bacterial Colonies Growing on TSA Media

Upon the incubation on TSA, it was observed that 5 samples were not overgrown with bacteria. However, 9 samples were overgrown with bacteria exhibiting these following characteristics: solid, yellowish-white, and not slimy. Additionally, 2 other samples contained bacteria that appeared soft, yellowish-white, and slimy.

The samples collected from the Teeth and Mouth Dentistry Hospital were then used for gram staining. The results revealed a variety of shapes. The bacterial growth data, as described in Table 1, further shows the shape of the bacteria.

Table 1. The number of bacterial colonies and results of the identification of bacteria types growth on four agar media.

		n=16							
		NA		MHA		TSA		TYS20B	
		n	%	n	%	n	%	n	%
The Shape of The Bacteria	*Monobacillus*	2	12,5	7	43,8	9	56,3	0	0
	Diplobacillus	0	0	3	18,8	0	0	1	6,3
	Streptobacillus	6	37,5	6	37,5	2	12,5	5	31,3
	Monobacillus + Streptococcus	0	0	0	0	0	0	3	18,8
	Streptobacillus + Streptococcus	0	0	0	0	0	0	1	6,3
	Total	**8**	**50**	**16**	**100**	**11**	**65,7**	**10**	**62,5**
The Types of Bacteria	*Bacillus sp.*	8	50	14	87	11	68,7	6	37,5
	Diphtheroid basil sp.	0	0	2	12,5	0	0	0	0
	Streptococcus mutans	0	0	0	0	0	0	4	25
	Total	**8**	**50**	**16**	**100**	**11**	**68,7**	**10**	**62,5**

*Note: NA (Nutrient Agar), MHA (Mueller Hinton Agar), TSA (Triptic Soy Agar), TYS20B (Trypticase Soy-Yeast 20 Per Cent Sukrose with Bacitracin)

Bacteria growing on the agar media showed distinct characteristics based on their shape, consistency, and color. Among these, the *monobacillus* form was the most dominant form found in four agar media. *Monobacillus* is a single-rod bacteria species that is often found within the *Bacillus sp*. family. The agar medium showed a soft and solid surface consistency. The results of this study indicated that solid consistency was the predominant characteristic. The identified bacteria in the tooth extraction pliers sample are *Bacillus sp., Diphtheroid basil sp*., and *Streptococcus mutans*. *Diphtheroid basil sp*. is a gram-positive bacteria commensal to mucosa and skin that is associated with nosocomial infections.

In this study, bacterial culturing and gram staining for bacterial identification were carried out. In order to determine the species of bacteria, it is necessary to conduct a bacterial culture on a specific agar medium for these bacteria, such as mannitol salt agar (MSA) for *Staphylococcus aureus* bacteria, bismuth sulfite agar (BSA) for *Salmonella bacteria* among others. The types of agar media used in this study, included three general media (NA, MHA and TSA) and one selective media

(TYS20B), were certainly insufficient in accurately identifying bacterial species. To accurately identify bacterial diversity, it is important to use more selective media, providing that not all bacteria can grow on general media.

This research is consistent with Dhani et al.'s (2020) research, which states that MHA media is very good and sensitive to bacterial and antimicrobial activity.[23] MHA contains complex carbohydrates derived from amylum which are needed in the metabolic process of bacteria. Amylum is a polysaccharide that is mostly produced from plants and consists of two kinds of polysaccharides: amylose and amylopectin. Amylum can be completely hydrolyzed by acids to produce glucose, so this environment is suitable for oral conditions.[24]

The results of this study are also in line with the research of Evangelista et al.,[25] which states that bacteria commonly present on instruments after surgical procedures include *Staphylococcus, Streptococcus sp., Actinomyces spp., Pseudomonas, Candida sp.*, and *Diphtheroid basil*. Tooth extraction pliers may serve as potential vectors for transmitting infection. However, their use is essential in the field of oral surgery in hospitals.

Based on Table 1, the bacterium found in all agar media was *Bacillus sp*. This bacterium is characterized as a positive-gram bacterium, exhibiting a rod-like shape, and belonging to a family of bacteria that is commonly found in humans, animals, water, and the surrounding environment. This bacterium produces endospores so that it can survive in any condition. Besides this bacterium, other bacteria found in this study were the *Diphtheroid basil sp*. and *Streptococcus mutans*, both of which are responsible for causing cavities.

DISCUSSION

The results of planting samples on agar media showed there was bacterial growth in each medium. In the nutrient agar (NA) media, it was found that 7 samples showed growth on agar surfaces with a solid consistency, yellowish white color, and were not slimy. However, 1 sample grew on agar surfaces with a softer consistency, yellowish color, and was slimy. In this study, the NA media was incubated aerobically for 24 hours, at a temperature of 370° C. Furthermore, Mueller Hinton Agar (MHA) was used as the second medium. In this medium, all the samples were inoculated with bacteria, each exhibited distinct characteristics. They were white in color, and had a solid yet soft consistency on agar surface. The final common medium used in this study was Tryptic Soy Agar (TSA), and it was observed that 11 samples implanted in this medium experienced bacterial overgrowth.

This research is consistent with a study by Shayeghi.,[10] who cultured bacteria on agar media (nutrient agar, blood agar and chocolate agar) and then incubated for 24-48 hours and found that bacteria were growing on these media.

This study shows that among the three general media used, Mueller Hinton Agar (MHA) is the best growth medium for bacterial growth because it provides an environment similar to the oral cavity. This study is in line with the research by Dhani et al.,[23] which states that MHA media is excellent and sensitive to bacterial and antimicrobial activity. MHA contains complex carbohydrates derived from starch, which is needed in the bacterial metabolism process. Starch is a polysaccharide that is mostly produced from plants and consists of two kinds of polysaccharides, namely amylase and amylopectin. The starch can be completely hydrolyzed by acid to produce glucose, so this environment is suitable for oral cavity conditions.[24].

The study's findings revealed the presence of *Streptococcus mutans*, *Bacillus sp.*, and *Diphtheroid basil* bacterial colonies on the tooth extraction pliers used. These results align with the research conducted by Evangelista et al. (2020), which identified common bacteria found on instruments after surgical procedures, including *Staphylococcus*, *Streptococcus sp.*, *Actinomyces spp.*, *Pseudomonas* and *Candida sp.*, *Diphtheroid basil*. Tooth extraction pliers can be wrong, which can

transmit infection, but the use of tooth extraction pliers is required in the field of oral surgery in hospitals.[25] [The use of tooth extraction pliers is crucial in the oral surgery department in hospitals, given their potential to transmit infections.]

HAIs (Healthcare Associated Infections) are infections that occur when a patient visits a hospital or other healthcare facility. This infection can be transmitted directly through contaminated medical equipment or acquired by patients while in public places through contact with other patients' care. All over the world, HAIs are the most common side effects in health care delivery, affecting hundreds of millions of patients. They lead to significant illness (morbidity) and death (mortality), impacting both developing and developed countries.[26]

Based on the characteristics of the bacteria found in this study, it is imperative to give special attention to the sterilization of surgical instruments used to prevent cross-infection between patients and dentists. The tooth extraction pliers, in particular, must undergo sterilization through an autoclave.[27]

The types of agar media used in this study are limited to three general media (NA, MHA and TSA) and one selective medium (TYS20B). There are relatively few media types, which can lead to a lack of variation in identifying the types of bacteria. More selective media may be required to identify bacterial diversity because not all bacteria can grow on general media.

CONCLUSION

Three species of gram-positive bacteria, including *Bacillus sp., Diphtheroid basil sp.*, and *Streptococcus mutans,* were found on the agar media of the tooth extraction pliers used in tooth extraction procedures. In addition, MHA media is identified as the most effective general growth medium for bacteria. This is due to its content of essential nutrients such as complex carbohydrates, which are necessary for the bacteria to grow and create an environment similar to that of the human oral cavity. On TYS20B media, *Streptococcus mutans* bacteria were detected in four samples. In terms of sensitivity to this specific bacteria, TYS20B media is the best *Streptococcus mutans* grows on nutrient-rich complex media and TYS20B provides complex nutrients and contains bacitracin, which is selective for these bacteria.

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Institutional Review Board Statement: All research procedures were approved by the Ethical Committee of Faculty of Dentistry Universitas Syiah Kuala with ethical approval number: 297/KE/FKG/2021.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The result of research data would be provided by request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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