

Identification of dominant aerobic bacteria in saliva of the patient with fixed orthodontic appliance



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Abstract

Objective: To understand the kind of dominant aerobic bacteria in patient's saliva with an orthodontic appliance.

Material and Methods: This is a descriptive observational study with cross-sectional design. Saliva samples from as many as 16 patients with fixed orthodontic appliances were collected. Saliva was taken by a swab method using wood a cotton swab, then put into tube filled with stuart medium. The samples was then isolated to blood agar, put into incubator for 24 hours and then assessed for the catalase test (H_2O_2 , 3%) and gram colour test to obtain aerobic bacteria results.

Results: Identified bacteria from patient's saliva with fixed orthodontic appliance were as many as 44 aerobic gram positive bacteria, such as streptococcus mitis, streptococcus pneumoniae, staphylococcus aureus and enterococcus faecalis. Gram-negative aerobic bacteria found 2 kinds of bacteria, pseudomonas aeruginosa and klebsiella pneumoniae.

Conclusion: The most dominant bacteria is the gram positive aerobic bacteria staphylococcus aureus.

Keywords: Saliva, Fixed orthodontic appliance, Aerobic bacteria

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Introduction

The healthiness of teeth and the oral cavity are important factors that must be maintained. Dental and oral health problems include malocclusion that requires orthodontic treatment to improve.¹ Orthodontic treatment may be performed with either removable or fixed orthodontic appliances.²

The human oral cavity consists of many bacteria both normal and pathogenic flora. A drop of saliva contains 50.000 pathogenic bacteria and can easily spread into the orthodontic component appliances.³ Bacterial concentrations increase in the patient's oral cavity with orthodontic treatment.⁴ The bacteria will multiply if the patient lacks oral hygiene resulting in damage of the tooth hard tissue or caries and soft tissue injury.⁵ It is also reported that the number of bacteria before using a fixed orthodontic appliance is 8.2% and after using an orthodontic appliance for 3 months bacteria increased to 13.3%. This suggests that orthodontic treatment induces changes in the oral flora with increased bacterial concentration, salivary pH and salivary flow rate.⁴ Buffer capacity and salivary pH affect its ability to inhibit acid production locally in the oral cavity.⁶ There are changes in the amount of bacteria in saliva over time caused by the installation of fixed orthodontic appliances.⁷ However, significant improvements were only detected during the first 6 weeks after the installation of fixed orthodontic

appliances, and the highest number was recorded after a 12 weeks period of treatment.⁶

Fixed orthodontic appliances have a design that is harder to clean compared to removable orthodontic appliances which can be easily cleaned because it can be removed by patient, therefore the number of bacteria in a removable orthodontic appliance is less than a fixed orthodontic appliance.⁸

The most common bacteria found in the fixed orthodontic appliances is aerobic bacteria, which require oxygen for respiration, growth, survival, and reproduction.⁹ Previous studies stated that aerobic bacteria commonly found in fixed orthodontic appliances are streptococcus mutans, streptococcus salivarius and lactobacillus.¹⁰ These bacteria are suspected to be associated with the incidence of caries occurrence. However, it does not mention which bacteria specifically cause caries from the above three aerobic bacteria and no studies have shown that.¹⁰ High caries prevalence has been reported in about 96% of patients undergoing orthodontic treatment, due to difficulties in oral hygiene maintenance. The frequency increases with the patient's age and length of treatment.¹¹

Based on the above description, the researcher wanted to know the dominant type of aerobic bacteria that may cause caries in saliva of patient using a fixed orthodontic appliance.

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Material and Methods

The type of research is descriptive observational study with cross sectional design. This research was conducted in the Faculty of Dentistry Hasanuddin University and Microbiology Laboratory of Hasanuddin University Hospital. The samples used in this research are saliva samples from patients using orthodontic appliances using a purposive sampling method.

Sampling of saliva

Saliva samples from 16 patients using fixed orthodontics were collected. Saliva samples were taken by a swab method using a wooden cotton swab, before insertion it into an incubator. Saliva samples were isolated into blood agar by way of scratching.

Catalase and H₂O₂ 3% test

A small drop of saliva was placed on a clean dry glass slide and then mixed with H₂O₂ by scratching. Rapid oxygen formation (bubbling) means catalase (+), if it does not form a bubble means catalase (-). The samples were then heated over a bunsen flame until dry and Gram staining was performed.

Gram staining stage

Liquid crystal violet was poured on the glass slides and let stand for 1 minute before rinsing. Lugol liquid was then added and let stand for 40 seconds before rinsing. Next, alcohol was poured onto the slide and let stand for 10 seconds before rinsing. Finally, fuchsin was poured then let stand for 20 seconds before rinsing.

Observation of Colonies

Colonies were observed under a microscope to see whether the samples were coccus or bacil. The mixed colonies were purified again, the basil colonies were purified in MC while the coccus colonies were purified in MSA.

Results

Research data were grouped based on several things, namely type of aerobic bacteria from all samples, the dominant type of gram positive aerobic bacteria and dominant type of gram negative aerobic bacteria.

Table 1 shows that gram positive aerobic bacteria contain 4 types of bacteria, namely streptococcus mitis, streptococcus pneumoniae, staphylococcus aureus and enterococcus faecalis. The gram-negative aerobic bacteria found was pseudomonas aeruginosa and Klebsiella pneumoniae.

Table 2 shows the most common type of gram-positive aerobic bacteria identified in saliva of patients using fixed orthodontic appliances staphylococcus aureus bacteria occupy the highest percentage of 38% of the total 15 specimens.

Table 3 shows the most common types of gram negative aerobic bacteria found in saliva of patients using fixed orthodontic appliances. Pseudomonas aeruginosa bacteria occupy the highest percentage of 10% with 4 specimens.

Table 4 shows the dominant bacteria based on growth medium in saliva of patients using fixed orthodontic appliances, is 85% gram positive bacteria with 33 colonies. While the gram negative bacteria contained only 15% with the number of 15 colonies. The total amount of colonies in the oral cavity of patients using fixed orthodontic appliances was 39 colonies.

Discussion

The results of this study identified the most gram positive aerobic bacteria, namely the genus staphylococcus and streptococcus. Which stated that most aerobic bacteria found in the fixed orthodontic appliance is streptococcus that can potentially cause caries.¹⁰ Increased caries risk during treatment is due to several factors, such as the difficult localization of the lesion, low pH rest, increased dental plaque volume and rapid changes in bacterial flora.¹²

From the gram staining test conducted in this study, the most common bacteria were gram-positive aerobic bacteria with as much as 85% and 33 colonies, whereas the gram negative aerobic bacteria was only found in 15% and 6 colonies.

Table 1 Identification of aerobic bacteria in saliva of patients using a fixed orthodontic appliances

| Identification of aerobic bacteria | |
|------------------------------------|------------------------|
| Gram positive | Gram negative |
| Streptococcus mitis | Pseudomonas aeruginosa |
| Streptococcus pneumoniae | Klebsiella pneumonia |
| Staphylococcus aureus | |
| Enterococcus faecalis | |

Table 2 Identification of dominant gram positive aerobic bacteria

| Type of bacteria | Number of Specimens | Percentage (%) |
|--------------------------|---------------------|----------------|
| Streptococcus mitis | 10 | 24 |
| Streptococcus pneumoniae | 5 | 15 |
| Staphylococcus aureus | 15 | 38 |
| Enterococcus faecalis | 3 | 8 |
| Total | 33 | 85 |

Table 3 Identification of dominant gram negative aerobic bacteria

| Type of Bacteria | Number of Specimens | Percentage (%) |
|-------------------------------|---------------------|----------------|
| <i>Pseudomonas aeruginosa</i> | 4 | 10 |
| <i>Klebsiella pneumoniae</i> | 2 | 5 |
| Total | 6 | 15 |

Table 4 Classification of aerobic bacteria based on growth medium in saliva of patients using fixed orthodontic appliances

| Type of Bacteria | Number of Colonies | Percentage (%) |
|--------------------------------|--------------------|----------------|
| Gram positive aerobic bacteria | 33 | 85 |
| Gram negative aerobic bacteria | 6 | 15 |
| Total | 39 | 100 |

However in this study, this type of bacteria is not commonly found. This may occur because patients with fixed orthodontic appliances have an increased salivary flow rate that causes the salivary pH to become basic and inhibits bacteria to thrive therefore, enabling the growth of bacterial species to be selective. There is an increase in salivary flow rate one month after the use of a fixed orthodontic appliance.¹³ This is supported by findings that the salivary flow rate is stimulated and the saliva pH shows improvement after 12 and 18 weeks of using fixed orthodontic appliances, although its buffer capacity was virtually unchanged.⁷

An increase in stimulated salivary flow rate, pH, buffer capacity and aerobic bacterial levels after three months of active use has been proven.^{14,15} A person may experience changes in salivary function over time and these clinical findings may be significant in the long term.¹⁶ While there are many studies of salivary function using different methods for measuring proper salivary function and bacterial interactions, the relationship between salivary function and outcomes related to the number of bacteria in saliva is unclear, so further research is needed.

From the results of this study, bacteria that have been identified from saliva of patients using fixed orthodontic appliances. As many as 4 types of gram positive aerobic bacteria, namely *Streptococcus myitis*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis* and as many as 3 types of gram negative bacteria, namely *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, have been found.

The most common gram positive aerobic bacteria found was *Staphylococcus aureus* by 38% with a total of 15 specimens and the most common gram-negative aerobic bacteria found was *Pseudomonas aeruginosa* in 10% or 4 specimens. *Streptococcus mutans* and *Lactobacillus* are the most common

bacteria found in patients using fixed orthodontic appliances.⁷ This proves the type of bacteria commonly found in patients using fixed orthodontic appliances is different from previous studies. Due to the limitations of this study, a further study into the causes of different types of bacteria is needed.

Conclusion

Bacteria from saliva of patients wearing fixed orthodontic appliances has been identified, namely *Streptococcus mitis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The most dominant type of bacteria is a gram-positive aerobic bacteria, namely *Staphylococcus aureus*.

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Conflict of interest

The authors report no conflict of interest.

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