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Comparative study on bacterial flora of oral cavity of smokers and non-smokers

Abstract

The oral cavity is a complex heterogeneous microbial habitat. The presence of nutrients, saliva and epithelial debris make the mouth favorable habitat for great variety of bacteria. Cigarette smoking is a public health problem. It decreases the commensal population of oral cavity resulting in an increase of pathogenic microbes. The study was designed to compare the bacterial flora of oral cavity of smokers and non-smokers and their antibiotic sensitivity pattern.

Twelve individuals comprising of 6 smokers and 6 nonsmokers were enrolled for the study. Oral swabs were collected from the oral cavity using sterile cotton swab stick under standard aseptic methods. The specimens were subjected to microscopy and culture. Organisms were identified using standard microbiological techniques. Only Gram positive bacteria were isolated from oral cavity of both smokers and non-smokers. Higher rates of bacteria were isolated from oral cavity of non-smokers (55%) than smokers (45%). *Streptococcus* species were found to be prevalent in both smokers and non-smokers. *Streptococcus mutans* (28.57%) and *Streptococcus salivarius* (28.57%) was found to be the most dominant bacteria in smokers. While *Staphylococcus epidermidis* (33.33%) was found to be most prevalent bacteria among non-smokers. Our study suggests that smoking may have altered bacterial acquisition. The study also showed the prevalence of potential pathogens and lesser number of commensals in oral cavity of smokers. The presence of potential pathogens may lead to greater susceptibility to oral as well as respiratory infections. Antibiotic susceptibility test showed higher resistivity of bacteria isolated from oral cavity of smokers towards commonly used antibiotics in comparison to non-smokers. This study suggests that campaign against smoking should be intensified and more people should be aware about the effect of smoking on oral microbiome and related diseases.

Keywords: oral cavity, oral microbiome, cigarette smoking, antibiotic susceptibility test

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Introduction

Oral cavity is the part of the mouth behind the gums and teeth that is bounded above by the hard and soft palates and below by the tongue and by the mucous membrane connecting it with the inner part of the mandible.[1] The oral cavity is made up of the lips and its inner lining, buccal mucosa, teeth, gums, the front two-thirds of the tongue, and the hard palate.[2] Oral health reflects the wellbeing of an individual, thus maintaining oral hygiene is important. The oral cavity is a complex heterogeneous microbial habitat. The environment present in the human mouth allows the growth of characteristic microorganisms found there. It provides a source of water and nutrients, as well as a moderate temperature. The presence of nutrients, saliva and epithelial debris make the mouth favorable habitat for great variety of bacteria.[3] At birth, the oral cavity is sterile but rapidly becomes colonized particularly from the mother in the first feeding with *Streptococcus salivarius* which contain about 98% of the oral flora until the appearance of the teeth. The eruption of the teeth during the first year leads to colonization by *S. mutans* and *S. sanguis*. They will persist as long as teeth remain.[4]

Some of the commonly found bacteria in oral cavity are:

Staphylococcus epidermidis, Streptococcus oralis, Streptococcus sanguis, Streptococcus mitis, Streptococcus salivarius Actinomyces sps, *Arachnia* sps, *Bacteroides* sps, *Eubacterium* sps, *Peptococcus* sps, *Peptostreptococcus* sps and *Veillonella* sps.[5]

Genera of fungi that are frequently found in the mouth include:

Candida sps, *Cladosporium* sps, *Aspergillus* sps, *Penicillium* sps and *Crptococcus* sps.[6]

Smoking is a practice in which a substance is burned and the resulting smoke is breathed in to be tasted and absorbed into the bloodstream. Most commonly, the substance used is the dried leaves of the tobacco plant, which have been rolled into a small rectangle of rolling paper to create a small, round cylinder called a "cigarette". Cigarette smoking could enhance microbial colonization by biofilm formation on oral epithelial cells. This may impair host immune responses against pathogens and also disrupt effective nasal mucociliary clearance.[7] Despite the warning, the number of smokers are increasing day by day. Almost all people are aware of the fact that smoking causes cancer but few are aware about the microbial status of oral cavity. This study will shade more light on possible health implications of smoking related to oral health.

Materials and method

Study design

The study design of this research is cross sectional type.

Study site

The study was conducted in the microbiological laboratory, Department of Microbiology, DAV College.

Study duration

The study was conducted from 16th January-12th March 2020.

Sample size

Twelve individuals comprising 6 smokers and 6 non-smokers were enrolled for the study.

Inclusion criteria

Individuals aged 18 years and above were enrolled for the study but for smokers, people who have been smoking tobacco products regularly for at least 1 year were enrolled for the study.

Collection of sample

For examination of oral flora, oral swab was collected. Before collecting the swab, the inside of the mouth was examined for any inflammation or the presence of any membrane exudates or pus. Using a sterile cotton swab, the sample was taken by swirling it around the cheeks and gum for about 30 seconds. The contamination of swab with saliva was avoided. The swab stick was transported within 1 hour of collection to the laboratory for analysis.

Sample processing

Stock preparation

The swab sticks were aseptically placed in peptone water broth which was incubated at 37° C for 24 hours and was used as stock.

Culture

A loopful of stock was streaked on various media (Blood agar, Eosin Methylene Blue agar, Mannitol Salt agar and MacConkey agar) and incubated at 37° C for 24 hours. Isolates were identified macroscopically for colony morphology. It was then sub cultured on Nutrient agar and was observed microscopically (Gram staining) and biochemically (coagulase, catalase, oxidase, urease and TSIA).[8]

Antibiotic susceptibility test (AST)

AST by Kirby-Bauer's disc diffusion method was performed for the isolate using commercially available antibiotic discs on Muller Hinton agar (MHA). Standard suspension of the isolates was adjusted to standard 0.5 McFarland solutions. The antibiotics were selected as per CLSI Guidelines and interpretive criteria of CLSI.[9] After incubation the zone diameter was measured for each antibiotic and was compared with standard provided by CLSI guideline.[10] The antibiotics discs used were Chloramphenicol (30µg), tetracycline (30µg), ofloxacin (5µg), Ciprofloxacin (5µg), Vancomycin (5 µg), Erythromycin (10µg) and penicillin (10 µg). Thereby the zone of inhibition is interpreted as sensitivity(S) or resistant(R). (Himedia Laboratories Pvt. Ltd., Mumbai, India)

Result

Out of twelve individuals comprising six smokers and six non-smokers, higher rates of bacteria (55%) were isolated from oral cavity of non-smokers than smokers (45%).

Total isolates among smokers and non-smokers (Figure 1)

Streptococcus salivarius (28.57%) and *Streptococcus mutans* (28.57%) was found to be most prevalent among the smokers followed by *Streptococcus pneumoniae* (21.42%). Five microbial isolates were identified from the oral cavity of smokers.

Isolates in smokers (Figure 2)

Staphylococcus epidermidis (33.33%) was found to be the most prevalent among non-smokers followed by *Streptococcus salivarius* (22.22%). Seven microbial isolates were identified among non-smokers. Only two potential pathogens *Staphylococcus aureus* (11.11%) and *Bacillus subtilis* (5.56%) were isolated from oral cavity of non-smokers.

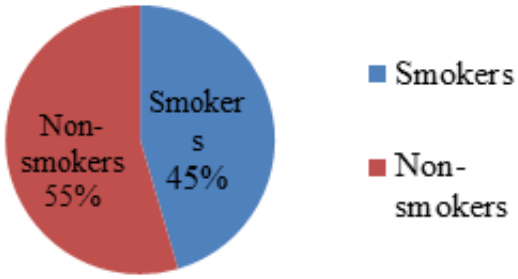

Figure 1 Pie chart showing total isolates.

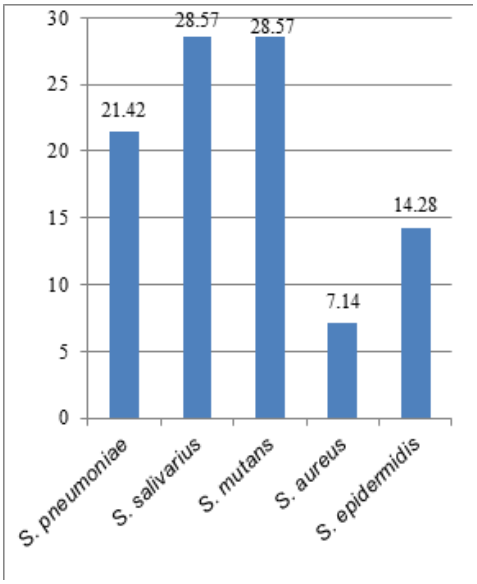

Figure 2 Isolates in smokers.

Isolates in non-smokers (Figure 3)

It was observed that most of the organisms showed resistance to Chloramphenicol (37%) followed by Tetracycline (11%), Erythromycin (11%) and Penicillin (25%). None of the organisms showed resistance to Ofloxacin and Ciprofloxacin.

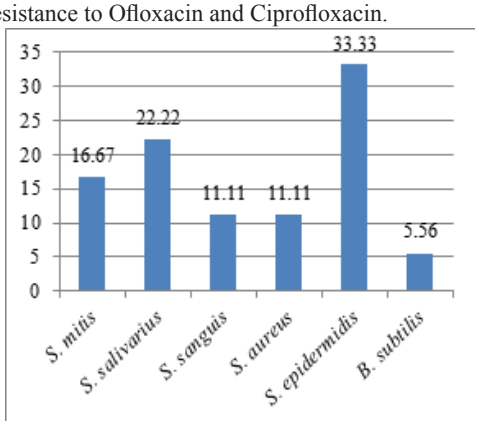

Figure 3 Isolates in non-smokers.

Antibiotic susceptibility test among smokers and non-smokers (Figure 4)

Among the bacterial isolates recovered from the oral cavity of smokers, most of the isolates showed resistance against antibiotics used. The potential pathogens isolated from showed the resistance to

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the antibiotics used. While the few commensals isolated did not show any resistance toward the used antibiotics.

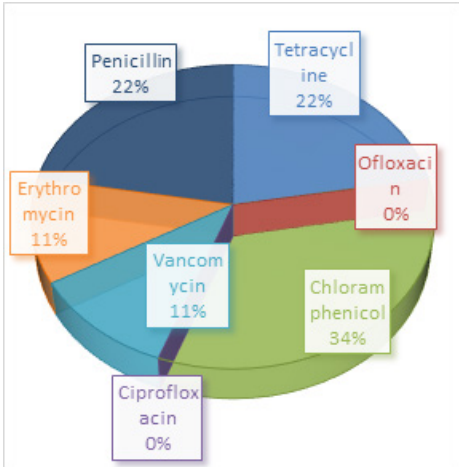

Figure 4 AST among smokers and non-smokers.

Antibiotic Susceptibility test (smokers) (Figure 5)

Among the isolates recovered from non-smokers, only *Staphylococcus aureus* was the resistance to Tetracycline. Similar to what was observed in samples collected from oral cavity of smokers, the commensals isolated from the oral cavity of non-smokers also did not show any resistance towards the antibiotics used.

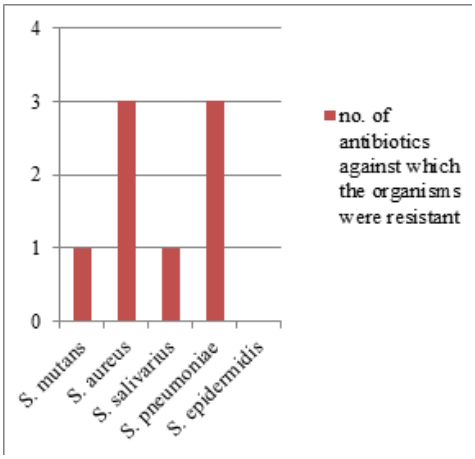

Figure 5 Antibiotic susceptibility test (smokers).

Antibiotic Susceptibility test (non-smokers) (Figure 6)

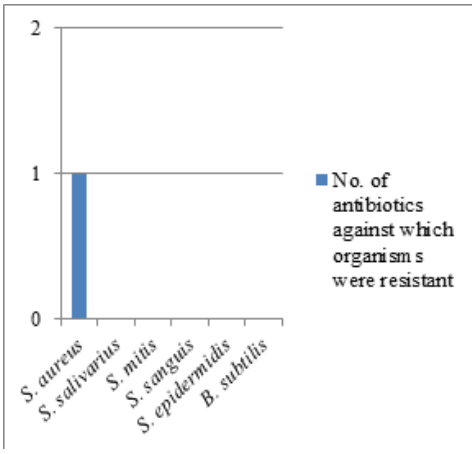

Figure 6 Antibiotic susceptibility test (non-smokers).

Discussion

In this study, higher rates of microbial isolates were recovered from oral cavity of non-smokers (55%) than smokers (45%). *Streptococcus salivarius* (28.57%) and *Streptococcus mutans* (28.57%) were found to be the most prevalent bacteria among smokers while *Staphylococcus epidermidis* (33.33%) was found to be prevalent bacteria among non-smokers which is different from Ogbate[11] whose major isolate was Staphylococcus (25%) then Klebsiella pneumonia (20%). The variation of microbial flora of both studies may be due to the variation in oral hygiene habits. The predominant isolate was found to be *Streptococcus* sps in both groups. Comparing the oral bacteria of smokers and non-smokers, only gram positive organisms were isolated from both smokers and non-smokers. Some of the commensals (*Staphylococus mitis, Staphylococcus sanguis* and *Bacillus subtilis)* found in non-smokers were not recovered from the smokers. The presence of commensals may play a role in the prevention of upper respiratory tract infections. The absence of these commensals may contribute to the increased risk of acquiring respiratory pathogens and the greater susceptibility to oral as well as respiratory infections. This may be due to the effect of exposure to cigarette smoking.

Most of the isolates were resistant to Chloramphenicol (34%), Tetracycline (22%) and Penicillin (22%). Some of the isolated were resistant to Vancomycin (11%) and Erythromycin (11%). However, the isolates were not resistant to Ofloxacin and Ciprofloxacin. Beside one isolate (*Streptococcus aureus*) remaining were sensitive to all most all antibiotic used. The potential pathogens isolated from oral cavity of smokers were resistant to the antibiotics used. Two of the isolates (*S. aureus* and *S. pneumoniae*) were resistant to three classes of antibiotics indicating multidrug resistance similar to the study by Fayaz[12]. The resistance showed by the isolated strains to commonly prescribed antibiotics is a matter of concern. The wide spread abuse of these antibiotics should be avoided and a drug policy must be designed to control the unnecessary use of the antibiotics. Some of the isolates were found to be resistant to multiple drugs. The resistance towards antibiotics shown by the isolates recovered from smokers is higher than that of non-smokers. So it can be concluded that besides the wide spread abuse of antibiotic, there is another factor making the organisms resistant towards antibiotics. The other factor in this case may be the cigarette smoke or the nicotine present in the cigarette. The combination of cigarette smoke and improper use of antibiotics may lead to more dangerous consequences, which is also supported by Fledman.[13] Further research should be conducted for identifying the factor making the bacteria isolated from oral cavity of smokers more resistant towards the antibiotics used.

Conclusion

This study concluded that the smokers had diverse bacterial colonization compared to non-smokers. This study also showed the more prevalence of potential pathogens in oral cavity of smokers. Smokers had less number of commensals than non-smokers. Our study suggests that smoking alters the bacterial acquisition and oral mucosal colonization in favor of potential pathogens. Our study also showed the greater resistivity of bacteria isolated from oral cavity of smokers towards commonly used antibiotics. Most of the bacteria were resistant to tetracycline and chloramphenicol which are the antibiotics most commonly prescribed in dental practice. So, the wide spread abuse of antibiotics must be prevented in order to decrease the multi-drug resistant property of bacteria.

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Conflicts of interest

The authors declare no conflict of interest.

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