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COMPARATIVE STUDY OF ORAL NORMAL FLORA OF TOBACCO USERS AND NON-TOBACCO USERS

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Abstract

Tobacco smoking is an important environmental factor that has a negative impact on the oral micro flora. Tobacco users have a higher number of pathogenic microorganisms than non-tobacco users. Cigarette smoking, in fact, exposes the oral cavity to a wide range of toxins thus, disrupting the microbial ecology. Tobacco smoke has direct contact with the microbial populations of the oral fluid, which may have an impact on these communities. The imbalance of these microbial flora lead to oral and systemic diseases, including tooth decay, oral cancer, periodontitis and gingivitis. The purpose of this study was to analyze the micro flora in the oral cavity of tobacco users and compare it to the normal flora of non-tobacco users. A total of 30 samples were collected from tobacco users and non-tobacco users in Arbab Yaseen Town, Peshawar. Among them, 15 samples were from tobacco users and 15 were collected from non-tobacco users. The samples collected from tobacco users and non-tobacco users were evaluated for the presence of normal flora. Various morphological and biochemical assays were performed to identify the bacterial isolates. The 5 samples collected from tobacco users were culture negative, while the remaining 10 samples were culture positive. Eleven of the 15 samples collected from non-tobacco users was culture negative and the remaining 4 were culture positive. Staphylococci (5 species) was the most frequently isolated bacteria in both groups, however, tobacco users had a larger proportion than non-tobacco users. Neisseria species, methicillin resistant Staphylococcus aureus (MRSA) and Streptococcus species were the least frequently isolated

Introduction

Tobacco use is prevalent in more than 70 nations, particularly in South East Asia regions, which account for over 89% of global smokers (Abakar et al., 2020). Tobacco has been linked to malignancies of the nasal cavity, oral cavity, liver, trachea, esophagus, lungs and pancreas, with more than 30 carcinogens detected in various cigarette brands. Tobacco smoking is a public health concern and the leading cause of periodontitis, oral cancer, tooth discoloration, and halitosis (Ilankizhai & Leelavathi, 2018). It causes significant reduction in commensal population of normal flora in the oral cavity, allowing harmful bacteria to flourish. There are more than 1 billion adult smokers worldwide (Yousuf et al., 2020), with tobacco responsible for almost 20% of all fatalities in the United States annually. The mouth provides an ecological niche for various microbial communities with significant consequences for human health (Sedghi et al., 2021). Tobacco products are used by over 2 billion people globally, causing at least 4 million deaths every year. The dramatic rise in diseases linked to tobacco smoking, such as cardiovascular disorders, pulmonary disorders, and Crohn's disease, suggests smoking plays a negative role in human disease development. Environmental variables may contribute to creating human-associated microbial ecosystems and immunological responses. Smoking, whether active or passive, is linked to colonization of potentially harmful microorganisms (Huang et al., 2019). In an era where microorganisms are rapidly recognized as etiologic agents for chronic diseases such as malignancies and neurologic disorders, understanding the impact of smoking on the microbiome is critical (Huang & Shi, 2019).

The oral microbiota is the second largest and most diversified microbiome, important for homeostasis as it protects the mouth from pathogenic colonization, reduces nitrate species, and aids food digestion (Robert & Kreth, 2017). It contains adherent bacteria that cling to gums and teeth surfaces, while non-adherent microorganisms can be found until mechanical flushing removes them. The most prevalent bacteria in healthy individuals include Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria. Non-bacterial microorganisms such as fungi, protozoa, and viruses have also been reported (Sampaio-Maia et al., 2014). Tobacco cigarettes are the most common causes of preventable diseases worldwide. Several studies demonstrate that exposure to cigarette smoking removes indigenous bacteria such as Prevotella and Peptostreptococcus and permits pathogenic bacteria including Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pneumoniae in the upper airways (Brooks et al., 2018). It also increases antimicrobial resistance profiles in Staphylococcus aureus (Lacoma et al., 2018). Cigarette smoking is linked to oral cancer, regulation of natural flora, periodontal diseases, oral infections, and interference with taste. Investigations have found substantial variations in subgingival bacteria between smokers and non-smokers (Bartlett et al., 2020; Sherwin et al., 2013). The prevalence of oral pathogens such as Prevotella nigrescens, Prevotella intermedia, Porphyromonas gingivalis, and Tannerella is considerably greater in smokers. Various bacterial species identified in the human oral cavity include anaerobic periodontal pathogens associated with periodontal infections such as P. gingivalis, Tannerella forsythia, P. intermedia, Eikenella corrodens, Campylobacter rectus, Aggregatibacter actinomycetemcomitans, Treponema denticola, and Fusobacterium nucleatum (Shakhatreh et al., 2018).

Tobacco smoking is predicted to be prevalent in 36% of males and 9% of females in Pakistan, with

smoking particularly prevalent among young adults and university students (Aryaie et al., 2021). The heat produced by smoking irritates oral mucosa, resulting in alterations in vascularization and salivary secretions. Smoking disrupts body defense cells by lowering antibodies (IgA, IgG, and IgM), enzymes (lysozyme, lactoperoxidase, and lactoferrin), mucin and histatin in saliva, resulting in impaired immune function (Rahmi et al., 2019). The decrease in salivary components causes increased bacterial numbers, making the oral cavity susceptible to infection. Studies show that people with reduced salivary flow rates have increased Lactobacillus and Streptococcus mutans bacteria. Cigarette smoke contains around 5,000 chemicals including carbon monoxide and tars. Carbon monoxide binds to hemoglobin with 200-250 times greater affinity than oxygen, causing tissue hypoxia and elevated hemoglobin and red blood cell values (Aldosari et al., 2020). Harmful substances including cyanide and carcinogens such as polycyclic aromatic hydrocarbons, formaldehyde, cadmium, nickel, arsenic, tobacco-specific nitrosamines, and phenols contribute to smoking's adverse effects (Proctor, 2012).

Staphylococci, methicillin-resistant Staphylococcus aureus, and Neisseria species are common pathogenic bacteria found in smokers and non-smokers. Staphylococci are Gram-positive cocci causing pneumonia, bacteremia, and endocarditis. MRSA is resistant to oxacillin, methicillin, and all beta-lactam antibiotics. Smokers have higher MRSA colonization rates than non-smokers, increasing risk of serious infections (Belkaid & Hand, 2014). Cigarette smoking promotes bacterial virulence since colonizing microbiota inhabit human mucosal areas exposed to environmental stimuli. Smoking may expose S. aureus to survival pathways against antibacterial action. Smokers with acute and chronic sinusitis have higher prevalence of S. aureus and MRSA than non-smokers (Carr, 2011). The genus Neisseria has more than 20 species of Gram-negative bacteria colonizing mucosal surfaces and oral cavities (Weyand, 2017). These bacteria can become pathogens due to predisposing factors such as oral hygiene and bad habits (Petrusic et al., 2021; Brooks et al., 2018). Despite increasing smoker numbers and cancer warnings, little is known about microbial profiles of smokers' oral cavities in Pakistan, where poor oral hygiene practices are common among both smokers and non-smokers.

Methodology

Research Study Area

This research was conducted in the Microbiology Laboratory of Sarhad Institute of Allied Health Sciences, Sarhad University of Science & IT, Peshawar.

Duration of the Research

Samples from tobacco users and non-tobacco users were collected during the period of August 2023 to September 2023.

Sample Size

A total of 30 samples were collected. Of these 30 samples, 15 were collected from tobacco users and 15 were collected form non-tobacco users.

Inclusion and Exclusion Criteria

Smokers and non-smokers of aged 18 and above were included.Females were not included in this study, as this habit is not acceptable among women in our community. We thought it would be challenging to recruit females who admit they were

Sample Collection

The samples were collected from tobacco users and non-tobacco users of Arbab Yaseen Town, Peshawar. During sample collection, a history of tobacco user was taken, including the brand of cigarette, oral diseases, addiction, age and duration of tobacco use. The samples were obtained using sterile swabs and transported within 1 hour to the

Laboratory for further examination. The samples were collected in accordance with CLSI guidelines

Sample Processing

Nutrient agar and blood agar media was prepared according to the instructions given by manufacturer (Solis et al., 2016).

Identification of the Bacterial isolates

All the bacterial isolates were further identified by observing their colony morphology, Gram Staining and several biochemical tests, including Catalase, Coagulase and Hockey Puck test (Holt et al., 1994).

Results

Total number of Samples Processed

A total of 30 samples were processed in the Microbiology Lab of Institute of Biological Sciences, Sarhad University of Science & IT, Peshawar. Out of these samples, 15 were collected from tobacco users and 15 were collected from non-tobacco users as shown in table 1. Staphylococcus aureus was the most frequently isolated bacteria in both groups. Neisseria and Streptococcus species were the least frequently isolated

Table. 1. Total number of Samples Processed

Total samples	Tobacco users	Non-tobacco users
30	15	15

No. of culture negative and culture positive samples

Five of the 15 samples collected from tobacco users were culture negative, while the remaining 10 samples were culture positive. Eleven of the 15 samples collected from non-tobacco users was culture negative and the remaining 4 were culture positive as shown in table

Table. 2. No. of culture negative and culture positive samples

Group	No. of Samples	Culture Positive Samples	Culture Negative Samples
Tobacco Users	15	10	5
Non-tobacco Users	15	4	11

Identification of the Bacterial Isolates based on Morphology

The isolates of Staphylococcus aureus when streaked on nutrient agar media formed yellow or golden color colonies, while the same Staphylococcus aureus when streaked on blood agar plates formed golden colonies. Neisseria species formed non-hemolytic and flat grey colonies on blood agar. Streptococcus on blood agar formed grey pigmented

Gram Staining of the Isolated Bacteria

Staphylococcus aureus formed purple color cocci or round shaped colonies, while Neisseria species formed pink color round or cocci shaped colonies under the microscope. Streptococcus formed purple oval or round shaped colonies in chains. Results of the Gram staining of species isolated from tobacco users and non-tobacco users are given in the table 3 and table

Table. 3. Results of the Gram staining of species isolated from tobacco users

Sample No	Gram staining
S-1	Gram +ve cocci
S-2	Gram +ve cocci
S-3	Gram +ve cocci
S-4	Gram +ve cocci
S-5	Gram +ve cocci
S-6	Gram +ve cocci
S-7	Gram +ve cocci
S-8	Gram +ve cocci
S-9	Gram -ve cocci
S-10	Gram -ve cocci

Table. 4. Results of the Gram staining of species isolated from Non-tobacco users

Sample No.	Gram Staining
S-1	Gram +ve cocci
S-2	Gram +ve cocci
S-3	Gram -ve cocci
S-4	Gram -ve cocci

Biochemical Tests for the Identification of Isolated Bacteria

Table 5 and table 6 summarizes the results of several biochemical assays performed to identify Staphylococcus aureus and Neisseria species isolated from tobacco

Table. 5. Results of several biochemical assays performed to identify species isolated from tobacco

Sample No.	Catalase test	Coagulase Test	Hockey Puck Test	Species
S-1	-ve	-ve	–	Streptococcus
S-2	-ve	-ve	–	Streptococcus
S-3	+ve	-ve	–	Coagulase negative Staphylococci
S-4	+ve	-ve	–	Coagulase negative Staphylococci
S-5	+ve	-ve	–	Coagulase negative Staphylococci
S-6	+ve	-ve	–	Coagulase negative Staphylococci
S-7	+ve	-ve	–	Coagulase negative Staphylococci
S-8	+ve	+ve	–	Staphylococcus aureus (MRSA)
S-9	+ve	–	+ve	Neisseria species
S-10	+ve	–	+ve	Neisseria species

Table. 6. Results of several biochemical assays performed to identify species isolated from Non-tobacco

Sample No.	Catalase test	Coagulase Test	Hockey Puck Test	Species
S-1	+ve	-ve	–	Coagulase negative Staphylococci
S-2	+ve	-ve	–	Coagulase negative Staphylococci
S-3	+ve	–	+ve	Neisseria species
S-4	+ve	–	+ve	Neisseria species

Discussion

Despite smoking's harmful health effects, the trend remains high globally, with many beginning as recreational activity that quickly becomes addiction (Dadipoor et al., 2019). This study compared normal flora between tobacco users and non-tobacco users, finding significant differences in microbial profiles with considerable variations in health-compatible species numbers.

According to Radaic and Kapila (2021), Staphylococcus aureus, Klebsiella, Streptococci, and Lactobacilli are among normal human flora.

The normal flora prevents pathogen colonization by competing for sites and nutrients, regarded as their most important beneficial effect. The imbalance of microbial flora contributes to oral and systemic diseases including gingivitis, oral cancer, periodontitis, and tooth decay (Lee et al., 2021). Tobacco smoking reduces commensal population of normal oral flora, resulting in increased harmful bacteria (Ogba et al., 2021).

In the current study, coagulase negative and coagulase positive Staphylococci, Neisseria, and Streptococcus species were isolated from tobacco users, while non-tobacco users yielded only coagulase negative Staphylococci and Neisseria species. Heavy growth was observed in swab cultures from tobacco users compared to non-tobacco users. These findings are in line with Grine et al. (2019), who reported Streptococcus and Staphylococci. Similarly, Al-Assiri et al. (2019) also isolated Staphylococci from saliva of tobacco users.

When comparing normal oral flora between smokers and non-smokers, a divergence was observed from Gram negative bacteria to Gram positive bacteria. Similarly, Nagarajan and Geetha (2020) also observed this shift from Gram negative to Gram positive bacteria. These findings are supported by Alaidarous et al. (2019), who reported Gram positive bacteria from tobacco users. These results were further strengthened by Moussa et al. (2020).

In the current study, Staphylococci were the most frequently isolated bacteria in both groups; however, tobacco users had a larger proportion than non-tobacco users. These results are similar to the study conducted by Gargin et al. (2021). In contrast, Altindis et al. (2020) also reported Staphylococci predominance among tobacco users.

In the current study, Neisseria and Streptococcus species were the least frequently isolated bacteria. Similarly, Bugova et al. (2018) reported Neisseria species. These findings were also strengthened by Luo et al. (2021), who reported Neisseria species. In the current study, methicillin-resistant Staphylococcus aureus was also reported, which is similar to the findings of Al-Zoubi et al. (2020). Osman et al. (2020) also reported MRSA in tobacco users.

Conclusion

Our results showed an increase in number of Gram positive bacteria in tobacco users as compared to non-tobacco users. Oral microflora was significantly higher in tobacco users than non-tobacco users. Tobacco use alters the oral normal flora, resulting in the emergence of pathogenic bacteria. These alterations may have an impact on immunity and health of tobacco users, as well as cause major oral and systemic health problems.

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