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Comparative Analysis of Respiratory Disorders and Oral Microbial Flora in Smokers and Nonsmokers from District Swabi, Pakistan

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Article Details

ABSTRACT

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Tobacco smoking is a pervasive public health concern known to negatively affect both oral and systemic health. This study aimed to investigate the impact of cigarette smoking on the composition of oral microbial flora and associated respiratory symptoms among male residents of District Swabi, Pakistan. A total of 44 participants—22 smokers and 22 non-smokers—were included in the research. Oral samples were collected using sterile cotton swabs and subjected to microbiological analysis, including culturing on nutrient agar, Gram staining, and biochemical tests such as catalase and oxidase assays. Eight predominant bacterial species were identified in the oral cavities of smokers: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Acinetobacter baumannii*, *Haemophilus influenzae*, *Clostridium difficile*, and *Streptococcus pneumoniae*. These bacteria included several opportunistic and pathogenic strains, indicating a significant shift in the oral microbiome due to tobacco exposure. Most bacterial isolates from smokers tested positive for catalase, suggesting increased oxidative stress adaptation. Demographic and behavioral data revealed that smoking was most prevalent among individuals aged 18–30 years, and low to moderate smoking intensity was common. Additionally, a high prevalence of respiratory symptoms—such as morning cough, phlegm production, wheezing, and dyspnea—was reported among smokers, despite the absence of family history for respiratory illness. These findings highlight the detrimental effects of smoking on both microbial ecology and respiratory health, emphasizing the need for targeted tobacco cessation programs and early public health interventions.

INTRODUCTION

Tobacco smoking, characterized by the inhalation of smoke from burning plant materials, predominantly tobacco, remains one of the most detrimental habits to public health. While substances such as marijuana and hashish have also been used for smoking, tobacco remains the most extensively consumed, largely in the form of cigarettes, cigars, and pipes. The psychoactive component of tobacco, nicotine, has both stimulating and calming properties, contributing to its addictive potential. It is particularly widespread in regions including Pakistan, the United States, Europe, and across Mesoamerica and Eurasia (Helal et al., 2014). Each year, more than eight million deaths are attributed to the tobacco epidemic, including over 1.2 million from second-hand smoke exposure, ranking it among the most severe public health crises globally (Washington et al., 2021). Historically, tobacco use can be traced back to Mesoamerican cultures around 5000–3000 BC. It was introduced to Eurasia by European colonists in the 17th century. The World Health Organization (WHO, 2017) anticipates that tobacco will be responsible for over one billion deaths in the 21st century. In Pakistan, the use of tobacco is more common among men, with usage beginning to rise after age 18. Smoking has been directly linked to cardiovascular diseases, chronic bronchitis, emphysema, and cancers affecting the lips, larynx, esophagus, and lungs (Bharati et al., 2013).

A comprehensive survey conducted in Pakistan in 2017–18 found that 23% of men and 5% of women were tobacco users (WHO et al., 2017). More than 25 million individuals in Pakistan currently use tobacco in various forms, including cigarettes, shisha (water pipes), gutka, naswar, and bidis (Khan et al., 2012). In low-income countries like Pakistan, socioeconomic factors contribute significantly to high tobacco consumption, with 31.8% of males and 5.8% of females reported to use tobacco in some form as per WHO data from 2015. Approximately 19.1% of adult deaths in Pakistan have been attributed to smoking (Washington et al., 2019). Pakistan remains one of the largest consumers of tobacco globally. Alarming, the prevalence is increasing among youth, with 17.5% of boys and 9.6% of girls aged 13–15 reported to use tobacco in Karachi (John et al., 2013). The provinces of Khyber Pakhtunkhwa, Punjab, and Baluchistan are significant contributors to national tobacco production (Basit et al., 2020). The widespread availability and cultural acceptance of tobacco have perpetuated its use across socio-demographic groups in the country.

The human oral cavity presents a unique ecological niche, housing over 600 species of bacteria. This includes diverse surfaces such as the lips, tongue, gums, and hard and soft palates. Saliva, produced by major and minor salivary glands, plays a vital role in maintaining oral health by flushing away food particles, neutralizing acids, and providing antimicrobial activity through components like secretory IgA and various enzymes (Koliarakis et al., 2019; Van et al., 2016). When saliva flow is reduced or altered, the risk of oral infections such as dental caries, gingivitis, and periodontitis rises significantly (Van T et al., 2014). The oral microbiome is comprised primarily of bacterial phyla including Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria (Dewhirst et al., 2016). *Streptococcus*, *Haemophilus*, *Leptotrichia*, *Porphyromonas*, and *Prevotella* are among the most prevalent genera (Chattopadhyay et al., 2019). The mouth constantly encounters external microbes through food, drink, and breathing, making it difficult to distinguish resident flora from transient organisms. Pathogenic shifts in this microbiota, especially due to smoking, are of major concern. Smoking alters the oral microflora by reducing beneficial bacterial populations and encouraging the growth of harmful species (Charlson et al., 2010). These changes create a pathogenic environment that predisposes smokers to dental diseases, particularly periodontal disorders and oral cancers.

Smoking has been identified as one of the principal risk factors for periodontitis, an inflammatory disease affecting the tissues surrounding the teeth. Unlike gingivitis, which is reversible, periodontitis leads to irreversible attachment loss and bone destruction (Razi et al., 2019). The buildup of dental plaque in the cervical region of teeth is exacerbated by smoking, which not only promotes bacterial adhesion but also impairs the host immune response, making smokers more vulnerable to disease progression (Fiorillo et al., 2019; Gloria et al., 2018). Studies show that smokers have more severe forms of periodontal disease, with increased bone loss, gingival recession, and deeper periodontal pockets (Lauritano et al., 2016; Razi et al., 2018). The risk is two to seven times higher in smokers compared to non-smokers. Periodontal disease remains the most common chronic inflammatory condition in the oral cavity, leading to tooth loss globally (Tonetti et al., 2020).

Tobacco use is also a well-documented risk factor for oral squamous cell carcinoma (OSCC). Tobacco smoke contains numerous carcinogens that can cause DNA damage, epigenetic alterations, and mutations in epithelial cells of the oral mucosa (Akbara et al., 2021). The risk increases with the frequency and duration of tobacco use. Individuals consuming even a single cigarette per day have a 48–74% increased risk of coronary heart disease (Hackshaw et al., 2018). Smoking is also linked to various other cancers, including those of the bladder, pancreas, kidney, and lungs (Cumberbatch et al., 2016; O'Keeffe et al., 2018; Santucci et al., 2019). The respiratory system is significantly affected by tobacco use. Cigarette smoking reduces lung volume and capacity, contributes to upper and lower respiratory infections, and is a major cause of chronic obstructive pulmonary disease (COPD) and asthma (Haddad et al., 2016; Soriano et al., 2017). Peak expiratory flow rate, FEV₁, and forced vital capacity are all reduced in smokers (Nawafleh et al., 2012). COPD is one of the most prevalent chronic diseases in Pakistan, affecting over 4% of the population, though many cases remain undiagnosed (Amir Khan M et al., 2019; Khan MA et al., 2020).

Tobacco use also impairs skeletal health. It reduces bone mineral density, increases the risk of fractures, and is associated with osteoporosis (Al-Bashaireh et al., 2018; Tarantino et al., 2021). Smokers are more likely to experience complications from orthopedic surgeries and dental implants (Moraschini et al., 2016). It has also been linked to delayed recovery from surgeries such as anterior cruciate ligament reconstruction and rotator cuff repair (Novikov et al., 2016; Santiago-Torres et al., 2015). Furthermore, smoking has mutagenic effects on male fertility. Nicotine and its metabolites can penetrate the blood-testis barrier, causing structural and functional damage to spermatozoa (Du Plessis et al., 2014; Pereira et al., 2014). These alterations include reduced sperm motility, abnormal morphology, and DNA fragmentation. Smoking is also associated with reduced sperm concentration and epigenetic changes that may affect fertility outcomes (Sharma et al., 2016; Pizzol et al., 2021). Chronic kidney disease is another systemic condition exacerbated by tobacco use. In Pakistan, where male tobacco use exceeds 72%, smoking has been implicated in the progression of renal disease. The nephrotoxic effects of tobacco compounds increase the risk of kidney failure and accelerate the deterioration of renal function in patients with pre-existing conditions (WHO et al., 2017).

Keeping in view the above facts this study aimed to compare the oral microbial flora between cigarette smokers and non-smokers in District Swabi, Pakistan. It focused on identifying the predominant bacterial species present in the oral cavities of smokers using microbiological methods such as culturing, Gram staining, and biochemical tests. Additionally, the research sought to determine the impact of smoking on oral microbiota composition, particularly examining the shift from beneficial to pathogenic bacteria.

MATERIALS AND METHODS

The materials utilized in this study included sterile cotton swabs, Eppendorf tubes, Petri dishes, inoculating wire loops, a spirit lamp, laminar airflow hood, an incubator maintained at 37 °C, a compound light microscope, alcohol, distilled water, test tubes, an autoclave, nutrient broth medium, nutrient agar, hydrogen peroxide (H₂O₂), N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) oxidizing reagent, and Gram's stain.

DATA COLLECTION

Data were collected from cigarette smokers residing in District Swabi through a structured, self-administered questionnaire and personal interviews. The questionnaire comprised two sections. The first section addressed socio-demographic information and tobacco use behavior, including age, gender, age at smoking initiation, type of tobacco products used (e.g., cigarettes or cigars), and the average number of cigarettes consumed daily. The second section focused on medical history, particularly respiratory conditions such as asthma, allergies, chronic cough, dyspnea, phlegm production, wheezing, and age-related health issues within the family.

SAMPLE COLLECTION

Oral samples were collected from smokers using sterile cotton swabs, which were rubbed inside the oral cavity. The swabs were then transported to the Center of Biotechnology and Microbiology Laboratory in Peshawar for analysis under aseptic conditions.

INCLUSION CRITERIA

Participants included in the study were habitual smokers aged between 18 and 50 years who had been using tobacco products regularly for at least one year prior to sampling.

CULTURE MEDIA PREPARATION

To cultivate bacterial isolates from the oral samples, both nutrient broth and nutrient agar were prepared. Nutrient broth was prepared by dissolving 13 g of nutrient broth powder in 1 L of distilled water and sterilizing the solution at 121 °C for 15 minutes using an autoclave. A drop of the sterile broth was then transferred into Eppendorf tubes and incubated at 37 °C for 24 hours. Nutrient agar was prepared by dissolving 28 g of nutrient agar powder in 1 L of distilled water, followed by autoclaving at 121 °C for 15 minutes. The sterilized agar was poured into Petri dishes within a laminar airflow hood and allowed to solidify (Figure-1).



FIGURE-1: POURED NUTRIENT AGAR IN EPPENDORF TUBE AND ON PETRI DISH

STREAKING TECHNIQUE

Once the nutrient agar plates were solidified, bacterial inoculation was performed using the streak plate method. A sterile wire loop was used to transfer approximately 0.002 mL of the nutrient broth containing bacterial culture onto the agar surface. The streaking was performed in a zigzag pattern to allow discrete colony formation. Plates were incubated at 37 °C for 24

hours to facilitate bacterial growth.

CULTURE OBSERVATIONS

Post-incubation, bacterial colonies were observed for variations in color, shape, and size to differentiate microbial species. Morphological features were used as preliminary indicators of bacterial identity (Figure-2).



FIGURE-2: Streaking method of bacterial sample in petri dishes of nutrient agar plates on sterilized wire loop

IDENTIFICATION VIA COLONY MORPHOLOGY AND GRAM STAINING

Colony morphology was assessed based on characteristics such as colony shape, margin, elevation, surface appearance, color, and size. These traits served as preliminary identifiers prior to microscopic and biochemical confirmation.

For Gram staining, a specific bacterial colony was selected and emulsified in 100 mL of distilled water on a clean slide. After air drying, the slide was heat-fixed by passing it briefly through a flame. The fixed smear was then stained using the Gram staining technique to classify bacteria as either Gram-positive or Gram-negative based on cell wall properties.

BIOCHEMICAL TESTS

CATALASE TEST

The catalase test was employed to determine the presence of the catalase enzyme, which catalyzes the breakdown of hydrogen peroxide into water and oxygen. A drop of 3% hydrogen peroxide was placed on a clean glass slide, and a loopful of the 24-hour culture was mixed into it using a sterilized wire loop. The formation of oxygen bubbles indicated a positive catalase reaction, confirming the presence of catalase-producing bacteria.

OXIDASE TEST

The oxidase test was performed using tetramethyl-p-phenylenediamine (TMPD) as an electron donor reagent. A solution was prepared by dissolving 10 mg of TMPD in 10 mL of distilled water and allowed to react for 15 minutes, developing a dark purple color. Filter paper impregnated with the reagent was streaked with bacteria from a 24-hour culture using a sterilized wire loop. A rapid color change to purple indicated a positive oxidase reaction, suggesting the presence of cytochrome c oxidase in the bacterial isolate.

RESULTS AND DISCUSSION

This study analyzed 44 oral samples collected from male participants in District Swabi, comprising 22 smokers and 22 non-smokers as a control group. The microbial assessment demonstrated a marked difference in the diversity and composition of oral flora between the two groups. Eight distinct bacterial species were isolated from the oral cavities of smokers, including *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Additionally, smokers were found to be more frequently colonized by *Clostridium difficile* and *Streptococcus pneumoniae*, which are known opportunistic pathogens. These findings suggest that smoking may promote colonization by more pathogenic and potentially harmful bacteria in the oral cavity.

A comparison of bacterial prevalence in relation to smoking intensity revealed that *Staphylococcus* and *Streptococcus* species were consistently present in individuals who smoked between one to ten cigarettes per day. However, in individuals who smoked more than ten cigarettes daily, a greater diversity of bacterial species was observed, indicating a more pronounced alteration in the oral microbiome. Furthermore, the long-term effects of smoking were evident in participants with more than five years of tobacco use, where a higher prevalence of *Staphylococcus* and other pathogenic bacteria was noted. This suggests a cumulative effect of smoking on oral microbial dysbiosis over time.

The analysis also incorporated participants' socio-demographic profiles and smoking-related variables, including age, duration of smoking, type of tobacco products used, and average daily consumption. In addition, clinical histories were recorded, covering a range of respiratory symptoms such as chronic cough, phlegm production, dyspnea, wheezing, and allergy-related conditions. These symptoms were more frequently reported by smokers than non-smokers, supporting a correlation between tobacco use and the deterioration of oral and respiratory health.

CHARACTERIZATION OF ISOLATED BACTERIA THROUGH BIOCHEMICAL TESTS

To confirm the identity of the bacterial isolates obtained from smokers, standard biochemical assays were performed, specifically the catalase and oxidase tests. These tests validated the presence of eight bacterial species, including *Staphylococcus aureus*, *Streptococcus* species, *Haemophilus influenzae*, *Acinetobacter baumannii*, *Clostridium* species, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. These organisms are commonly associated with respiratory tract infections and opportunistic conditions, particularly in individuals with compromised immune defenses.

CATALASE TEST

The catalase test was employed to detect the presence of the catalase enzyme, which plays a vital role in protecting bacteria from oxidative damage. Catalase catalyzes the breakdown of hydrogen peroxide (H₂O₂) into water and oxygen, neutralizing a reactive compound that is frequently used by immune cells such as macrophages and neutrophils during the host defense response. All bacterial isolates in this study tested positive for catalase activity, suggesting their capacity to survive oxidative stress within the oral environment. The universal catalase positivity among isolates from smokers indicates that smoking may selectively favor the survival and growth of catalase-producing bacteria, contributing to a pathogenic shift in the oral microbiota (Figure-3).

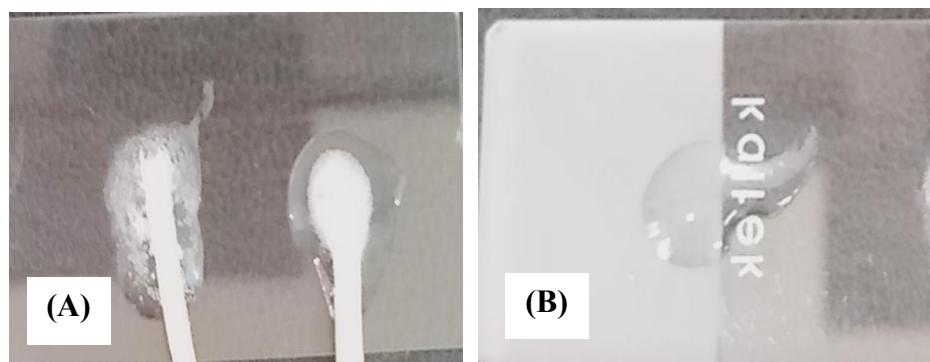


FIGURE-3: CATALASE TEST (A) SHOWING CATALASE POSITIVE AND CATALASE (B) SHOWING CATALASE NEGATIVE

OXIDASE TEST

The test detected in presence of indophenol oxidase or cytochrome c oxidase in several bacterial species. Due to the presence of the cytochrome-C oxidase enzyme, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* displayed oxidase positive results, resulting in a dark purple color formed as shown in figure 4.

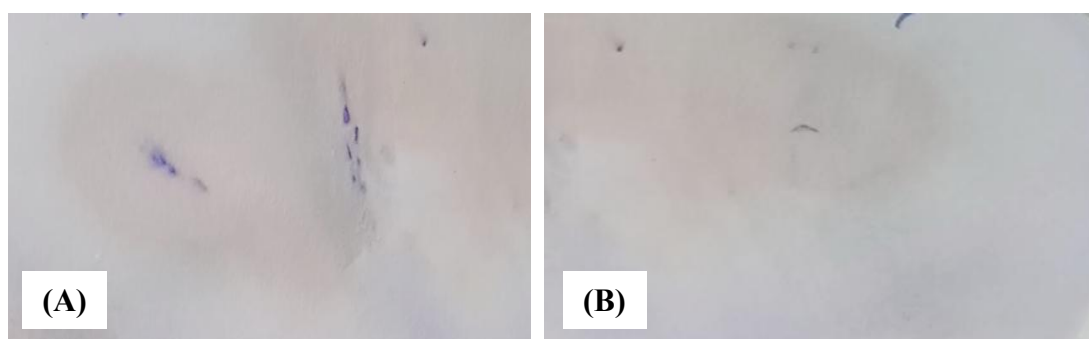


FIGURE-4: OXIDASE TEST (A) SHOWING OXIDASE POSITIVE AND (B) SHOWING OXIDASE NEGATIVE

TABLE-1: BIOCHEMICAL CHARACTERIZATION OF BACTERIAL ISOLATES FROM SMOKERS' ORAL MICROFLORA

Bacterial Species	Gram Stain / Morphology	Oxidase Test	Catalase Test
<i>Acinetobacter baumannii</i>	Gram-negative bacilli	–	+
<i>Haemophilus influenzae</i>	Gram-negative bacilli	+	–
<i>Serratia marcescens</i>	Gram-negative bacilli	–	+
<i>Klebsiella pneumoniae</i>	Gram-negative bacilli	–	+
<i>Pseudomonas aeruginosa</i>	Gram-negative bacilli	+	–
<i>Staphylococcus aureus</i>	Gram-positive cocci	+	–
<i>Streptococcus pneumoniae</i>	Gram-positive cocci	–	+
<i>Clostridium difficile</i>	Gram-positive bacilli	–	–

The table presents the biochemical profiling of bacterial species isolated from the oral cavities of smokers, based on Gram staining, oxidase activity, and catalase production. Among Gram-negative bacilli, *Acinetobacter baumannii*, *Serratia marcescens*, and *Klebsiella pneumoniae* tested positive for catalase, while *Haemophilus influenzae* and *Pseudomonas aeruginosa* were oxidase-positive, reflecting their differing metabolic capabilities. Among the Gram-positive cocci,

Staphylococcus aureus demonstrated oxidase activity, whereas *Streptococcus pneumoniae* exhibited catalase positivity, suggesting variable enzymatic defenses against host immune responses. *Clostridium difficile*, a Gram-positive bacillus, was negative for both oxidase and catalase, consistent with its anaerobic nature. These biochemical traits aid in the differentiation and identification of oral pathogens, highlighting shifts in microbial composition potentially induced by chronic tobacco exposure.

IDENTIFICATION OF ORAL BACTERIA USING COLONY MORPHOLOGY AND GRAM STAINING

Colony morphology and Gram staining served as essential preliminary techniques for the identification and classification of bacteria isolated from the oral cavities of smokers. After inoculation on nutrient agar and incubation at 37 °C for 24–48 hours, colonies were differentiated based on observable characteristics such as shape, size, color, elevation, texture, and margin. Gram staining further enabled the classification of isolates into Gram-positive and Gram-negative groups based on structural differences in their cell walls, providing critical information about their taxonomy and pathogenic potential.

BACTERIAL ISOLATES IDENTIFIED IN SMOKERS' ORAL MICROFLORA

The results revealed eight predominant bacterial species in the oral microbiota of smokers. These isolates differed in their morphological features and Gram reaction, reflecting microbial shifts potentially induced by tobacco exposure.

ACINETOBACTER BAUMANNII

Acinetobacter baumannii appeared as Gram-negative coccobacilli, often in pairs or short chains. Colonies were smooth, pale yellow to grayish-white, and typically measured 1–2 mm in diameter. This organism is a known nosocomial pathogen and was found colonizing the oral cavities of smokers, aligning with reports that smoking promotes its prevalence in respiratory secretions. It has been associated with pneumonia, urinary tract infections, and wound infections, particularly in immunocompromised individuals (Figure-5).



FIGURE-5: SMOOTH PALE YELLOW TO GRAYISH WHITE COLONIES OF *ACINETOBACTER BAUMANNII* ON NUTRIENT AGAR MEDIA

PSEUDOMONAS AERUGINOSA

This Gram-negative, rod-shaped bacterium formed large, flat, opaque colonies with irregular margins. Known for its distinct fruity odor, *P. aeruginosa* is an opportunistic pathogen capable of causing severe respiratory and systemic infections. In smokers, it has been linked to increased biofilm production and airway colonization, especially under conditions of chronic inflammation (Figure-6).

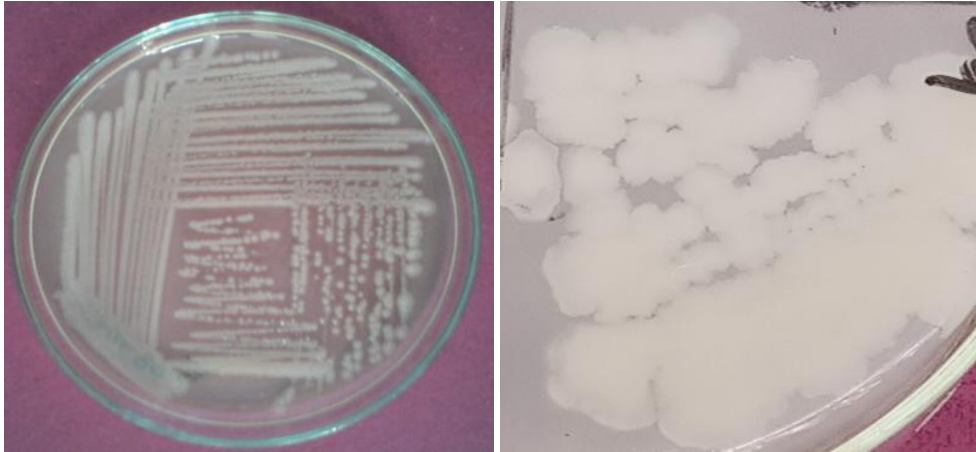


FIGURE-6: LARGE FORM OPAQUE FLAT COLONIES WITH IRREGULAR MARGINS AND FRUITY ODOR COLONIES OF PSEUDOMONAS AERUGINOSA ON NUTRIENT AGAR MEDIA

HAEMOPHILUS INFLUENZAE

Identified as small, pleomorphic Gram-negative coccobacilli, *H. influenzae* produced smooth, convex, colorless to grey colonies. It is implicated in both upper and lower respiratory tract infections, particularly among individuals with chronic obstructive pulmonary disease (COPD). Cigarette smoke is known to compromise mucosal defenses, facilitating colonization by *H. influenzae* in smokers (Figure-7).

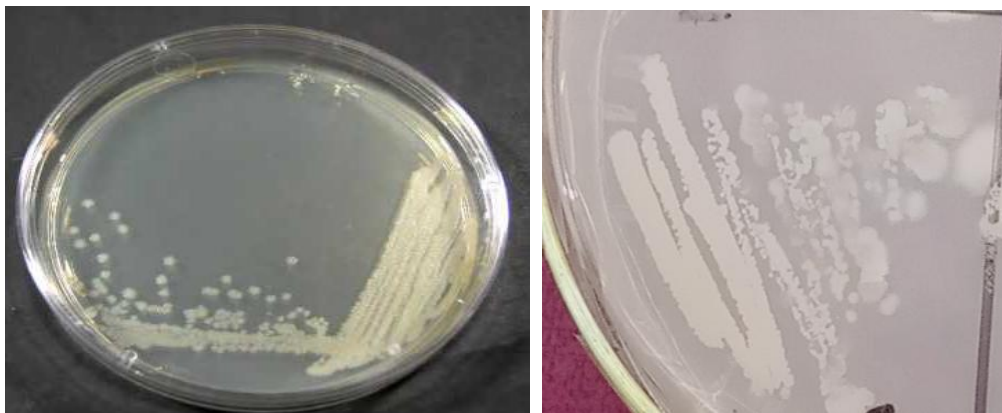


FIGURE-7: APPEAR LARGE SMOOTH AND CONVEX COLORLESS TO GREY OPAQUE COLONIES OF HAEMOPHILUS INFLUENZAE ON NUTRIENT AGAR MEDIA

SERRATIA MARCESCENS

This facultative anaerobic, rod-shaped Gram-negative bacterium formed pigmented colonies

and was present in a notable portion of smokers. As a member of the Yersiniaceae family, *S. marcescens* is an opportunistic pathogen and was found at a prevalence rate consistent with other studies that have associated it with tobacco-induced changes in oral ecology (Figure-8).



FIGURE-8: ROD SHAPED YELLOW COLONIES OF SERRATIA MARCESCENS ON NUTRIENT AGAR MEDIA

KLEBSIELLA PNEUMONIAE

K. pneumoniae appeared as large, mucoid, white colonies and was identified as Gram-negative bacilli. It is a common cause of nosocomial infections such as pneumonia, bloodstream infections, and urinary tract infections. In smokers, *K. pneumoniae* colonization may begin as early as 30 to 60 days after initiation of smoking and is associated with disruption of the oral microbial community (Figure-9).

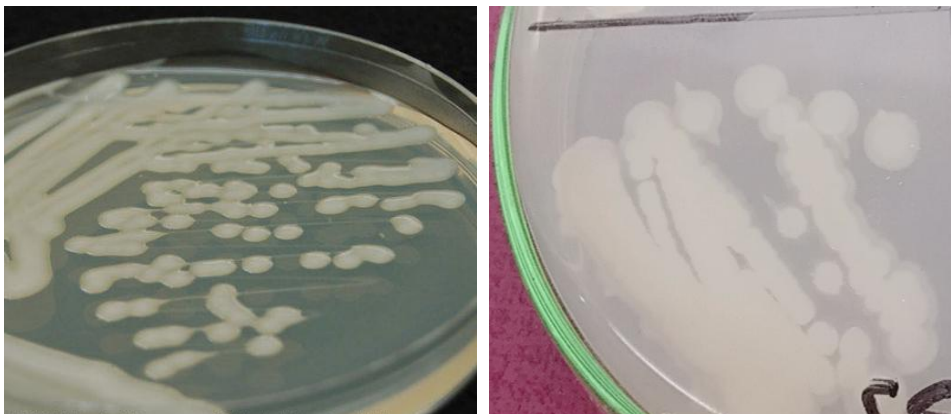


FIGURE-9: LARGE MUCOID WHITE COLONIES OF KLEBSIELLA PNEUMONIAE IN NUTRIENT AGAR MEDIA

STREPTOCOCCUS PNEUMONIAE

This Gram-positive, catalase-negative coccus occurs in pairs or chains and typically forms smooth, mucoid colonies. *S. pneumoniae* is part of the normal flora but can become pathogenic, causing pneumonia, sinusitis, and meningitis. The organism was detected with high prevalence in smokers, supporting existing evidence of its involvement in smoking-related respiratory infections (Figure-10).



FIGURE-10: MUROID OR SMOOTH GRAY TO WHITISH /GRAY COLONIES OF STREPTOCOCCUS SPECIES IN NUTRIENT AGAR MEDIA STAPHYLOCOCCUS AUREUS

Identified as Gram-positive cocci arranged in grape-like clusters, *S. aureus* produced round, light golden colonies with a smooth, convex morphology. It is a major pathogen responsible for skin infections, pneumonia, and bloodstream infections. In this study, its prevalence among smokers was significantly higher compared to non-smokers, indicating a strong association with tobacco use (Figure-11).

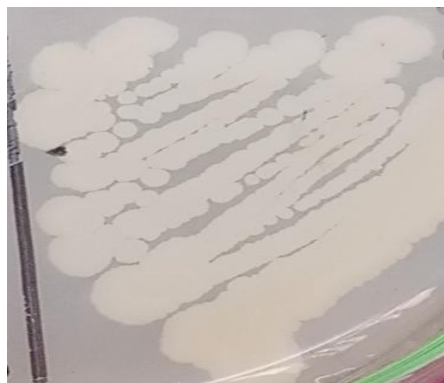


FIGURE-11: LIGHT GOLDEN COLOR ROUND COLONIES OF STAPHYLOCOCCUS AUREUS IN NUTRIENT AGAR MEDIA

CLOSTRIDIUM DIFFICILE

C. difficile appeared as Gram-positive, rod-shaped, spore-forming bacilli and was found in both current and former smokers. This anaerobic bacterium is known for causing gastrointestinal and respiratory tract infections. The increased prevalence among smokers suggests that smoking may favor conditions conducive to its growth, such as reduced oxygen tension and altered mucosal immunity (Figure-12).

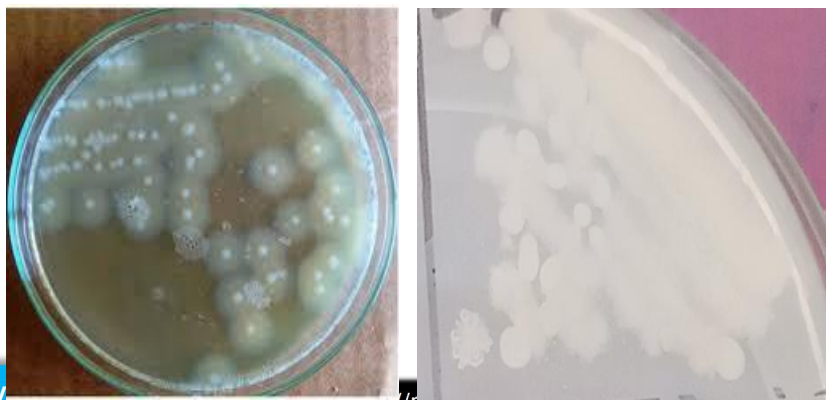


FIGURE-12: YELLOW GREEN OVAL OR SPHERICAL COLONIES OF *CLOSTRIDIUM* IN NUTRIENT AGAR MEDIA.**DEMOGRAPHIC DISTRIBUTION OF SMOKERS AND NON-SMOKERS BY AGE**

Understanding the age distribution of participants is critical for evaluating the prevalence and behavioral patterns associated with cigarette smoking. In this study, demographic data were collected from a total of 44 male individuals—22 smokers and 22 non-smokers—ranging in age from 18 to above 50 years. The analysis revealed that smoking was more prevalent among younger adults, particularly those between the ages of 18 and 30, with decreasing prevalence observed in older age groups. Conversely, non-smoking behavior was more commonly reported among participants aged 50 years and above. These patterns may reflect social, behavioral, and health awareness differences across age cohorts.

The age-stratified frequency and percentage distribution of smokers and non-smokers are presented in the following table-2:

TABLE-2: AGE GROUP DISTRIBUTION OF SMOKERS AND NON-SMOKERS

Age Group (Years)	Number of Smokers (n = 22)	% of Smokers	Number of Non-Smokers (n = 22)	% of Non-Smokers
18–30	8	36.36%	4	18.18%
30–40	5	22.72%	4	18.18%
40–50	6	27.27%	5	22.72%
Above 50	3	13.63%	9	40.90%

The data indicate that smoking is most common in the youngest age group (18–30 years), accounting for over one-third (36.36%) of all smokers, while the highest proportion of non-smokers (40.90%) was observed in individuals aged above 50 years. This age-based variation in smoking behavior may be attributed to factors such as increased health consciousness in older adults and the influence of peer behavior or stress-related habits among younger populations. These findings emphasize the need for age-targeted tobacco cessation strategies, particularly focused on early adulthood, where smoking initiation appears to be most prevalent.

SMOKING INTENSITY AMONG SMOKERS

Smoking intensity, measured in pack-years, serves as a critical indicator of long-term exposure to tobacco and its potential health implications. In this study, the smoking intensity of 22 cigarette smokers from District Swabi was categorized into four groups based on the number of cigarette packs consumed per year: 0–14, 15–24, 30–40, and above 50. The data reveal that the highest proportion of smokers fell within the 0–14 pack-years category, representing 45.45% of the total smoking population. This suggests that low to moderate cigarette consumption is most common among smokers in this region. In contrast, individuals with a smoking intensity exceeding 50 pack-years constituted the smallest group (9.09%), indicating a lower prevalence of heavy chronic smokers.

These findings are crucial in understanding local smoking patterns and can inform the design of public health interventions. The frequency and percentage distribution of smoking intensity are presented below (Table-3).

TABLE-3: DISTRIBUTION OF SMOKING INTENSITY AMONG CIGARETTE SMOKERS IN DISTRICT SWABI

Smoking Intensity (Packs/Year)	Frequency (n = 22)	% of Smoking Intensity
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0–14	10	45.45%
15–24	6	27.27%
30–40	4	18.18%
Above 50	2	9.09%

The analysis shows that nearly half of the smokers in District Swabi have a relatively low smoking intensity (0–14 packs/year), which may suggest early-stage addiction or less frequent usage. The decreasing trend in frequency with increasing smoking intensity indicates that fewer individuals engage in heavy long-term smoking. Nonetheless, even low levels of tobacco exposure can have significant adverse effects on oral and systemic health. These insights underscore the importance of early intervention and targeted cessation efforts, particularly for individuals in the early phases of tobacco use.

FAMILY HISTORY OF RESPIRATORY DISORDERS AMONG SMOKERS

The assessment of familial predisposition to respiratory illnesses is essential to differentiate between genetically inherited conditions and those acquired due to environmental or behavioral factors, such as smoking. In the current study, most smokers reported no family history of respiratory disorders, including asthma or allergic diseases. This finding suggests that the respiratory issues observed among participants are more likely attributed to personal smoking behavior rather than hereditary causes. Among the smokers studied, respiratory symptoms or difficulties were reported by 45.45% of individuals, indicating a significant burden of smoking-related pulmonary complications. However, only 22.72% of these individuals had sought medical treatment for their breathing problems, highlighting a potential gap in healthcare access, awareness, or willingness to seek care. These findings underscore the importance of health education and early intervention in populations where smoking-related respiratory symptoms are present but often unmanaged or undiagnosed (Table-4).

TABLE-4: PREVALENCE OF RESPIRATORY SYMPTOMS AND FAMILY HISTORY IN SMOKERS

Category	Frequency (n = 22)	Percentage (%)
Smokers with respiratory difficulties	10	45.45%
Smokers who received treatment for symptoms	5	22.72%
Smokers with family history of respiratory disease	0	0%

RESPIRATORY PROBLEMS IN SMOKERS

Tobacco smoke is a major risk factor for the development of chronic respiratory symptoms. In the current study, a high prevalence of respiratory issues was observed among cigarette smokers compared to non-smokers. The most frequently reported symptoms included persistent cough, dyspnea (shortness of breath), phlegm production, and wheezing.

Among the smokers surveyed, 72.72% reported waking up with a morning cough, which is a classic symptom of smoking-related airway irritation. Additionally, 68.18% of participants experienced smoke-induced cough or increased sensitivity to environmental triggers such as weather changes. These symptoms typically began within the first few years of smoking, with 40.90% reporting onset within 1–2 years of starting smoking, while 59.09% experienced symptom onset after more than one year (Table-5).

These findings demonstrate the early and progressive respiratory effects of smoking and emphasize the urgent need for preventive strategies, early diagnosis, and smoking cessation support.

TABLE-5: PREVALENCE AND ONSET OF RESPIRATORY SYMPTOMS IN SMOKERS

Respiratory Problem	Frequency (n = 22)	Percentage (%)
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Morning cough (wakes up with cough)	16	72.72%
Cough due to smoking and weather sensitivity	15	68.18%
Symptom onset within 1–2 years of smoking	9	40.90%
Symptom onset after more than 1 year of smoking	13	59.09%

DYSPNEA IN SMOKERS

Dyspnea, or difficulty in breathing, was a commonly reported symptom among smokers in this study. Approximately 45.45% of smokers experienced some form of breathing disability, yet only 27.27% had received treatment for it. A significant proportion (68.18%) reported worsened breathing when exposed to cigarette smoke, while 77.27% had ongoing respiratory issues. In 40.90% of cases, the onset of these symptoms had occurred within the past year, indicating a recent progression in respiratory decline among smokers (Table-6).

TABLE-6: PREVALENCE OF DYSPNEA AND BREATHING-RELATED ISSUES AMONG SMOKERS

Indicator	Frequency (n = 22)	Percentage (%)
Breathing disability	10	45.45%
Received treatment for breathing issues	6	27.27%
Breathing worsens with smoke exposure	15	68.18%
General breathing problems reported	17	77.27%
Onset of breathing issues within past year	9	40.90%

PHLEGM AND ASSOCIATED RESPIRATORY SYMPTOMS IN SMOKERS

Phlegm production and related respiratory symptoms were notably common among smokers in the study. A total of 63.63% of smokers reported phlegm production without associated cold symptoms, particularly in the mornings and during winter months. Wheezing was also reported in 50% of smokers, suggesting lower respiratory tract involvement. Additionally, 63.63% of smokers experienced nasal problems, and 81.81% reported feeling fatigued easily, which may be attributed to chronic smoke exposure—especially in occupational settings (Table-7).

TABLE-7: PREVALENCE OF PHLEGM, WHEEZE, AND RELATED SYMPTOMS AMONG SMOKERS

Symptom	Frequency (n = 22)	Percentage (%)
Phlegm without cold (morning/winter)	14	63.63%
Wheezing	11	50.00%
Nasal problems	14	63.63%
Easily fatigued (tiredness)	18	81.81%

PREVALENCE OF RESPIRATORY SYMPTOMS AMONG SMOKERS

The study revealed a high prevalence of respiratory symptoms among smokers, indicating significant compromise of respiratory health due to tobacco use. Cough (both morning and weather-induced), persistent phlegm without cold, wheezing, nasal issues, and increased fatigue were commonly reported. A majority of smokers experienced these symptoms within the first year of smoking initiation, while others developed chronic conditions over time (Table-8).

TABLE-8: PREVALENCE OF RESPIRATORY SYMPTOMS IN SMOKERS (N = 22)

Symptom Type	Specific Item	Response	Frequency	Percentage (%)
Cough	Wake with cough	Yes	15	68.18%
	Cough affected by weather	Yes	16	72.72%
Phlegm	Without cold	Yes	14	63.63%
	In morning and winter	Yes	14	63.63%
	Duration: One year	Yes	14	63.63%

	Duration: More than one year	Yes	8	36.36%
Wheeze	Wake with wheezing	Yes	11	50.00%
Other Issues	Nasal problems	Yes	14	63.63%
	Increased fatigue/tiredness	Yes	18	81.81%

CONCLUSION AND RECOMMENDATIONS

The results of this study clearly demonstrate that cigarette smoking induces significant alterations in the composition of oral microflora. In the population of District Swabi, smoking emerges as a major public health concern, notably influencing the microbial environment of the oral cavity. These changes result in an increased presence of pathogenic bacterial species, which not only disrupt the normal oral microbiota but also contribute to a higher prevalence of respiratory complications among smokers compared to non-smokers. Bacterial species such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Acinetobacter baumannii*, *Clostridium difficile*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were significantly more prevalent in the oral cavities of smokers. These pathogens are associated with a wide range of clinical complications, including respiratory infections, systemic illnesses, and increased antimicrobial resistance.

Based on these findings, several recommendations can be made to address the health risks posed by smoking-related microbial alterations. Infections caused by *Staphylococcus aureus*, especially in cases involving pneumonia, bloodstream infections, or osteomyelitis, require hospitalization and should be treated with targeted antibiotic therapy. Empirical treatment should begin within 2 to 4 days, and transesophageal echocardiography (TEE) is recommended for suspected endocarditis, followed by a 5–7 day course of antibiotics depending on the severity of infection.

For *Clostridium difficile*, which is known to cause ulcerative colitis and persistent diarrhea, management should include both medical and lifestyle approaches. Smokers experiencing frequent bowel disturbances may benefit from establishing regular bowel habits, engaging in pelvic floor exercises, and maintaining a dietary journal to identify and eliminate trigger foods. These measures, in conjunction with antibiotic therapy when necessary, can help control symptoms and reduce recurrence.

In the case of *Serratia marcescens*, a bacterium commonly associated with nosocomial infections, treatment can be challenging due to antibiotic resistance. A combination of an aminoglycoside and an antipseudomonal β -lactam is generally effective, although resistance to agents such as gentamicin and tobramycin has been observed. In resistant or biofilm-associated infections, a supratherapeutic dose of chloramphenicol may be required, administered under close clinical supervision.

Overall, there is a pressing need for public health intervention in District Swabi. Routine oral microbial screening for smokers should be implemented to monitor colonization by pathogenic bacteria and to prevent the progression of infections. Educational campaigns on the risks of smoking—particularly regarding its impact on oral and respiratory health—should be strengthened. Additionally, smoking cessation programs should be made widely available, and healthcare facilities must prioritize preventive care strategies to mitigate the microbial and respiratory health burden caused by tobacco use.

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