Research article
J. Adv. Nutri. Sci. Technol. 6(1-2): 20-27, 2026 **DOI:10.15228/ANST.2026.v06.i01-2.p04**

Prevalence of Periodontal Bacteria in Smokers and Non-Smokers: A Comparative Study with Reference to Nutritional Considerations in Haripur

Umm-e habiba*, Muhammad Ali Syed, & Shiza Tariq,
The University of Haripur, Department of Microbiology
*Corresponding Author Email: cauzn233@gmail.com

Abstract

Periodontal disease develops when the oral microbial community becomes unbalanced. Smoking and nutrition both influence this balance. This study compared the prevalence of three bacterial species, including *Klebsiella pneumoniae*, *Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis*, among smokers and non-smokers in Haripur. This study also considered how nutrition might relate to these findings; for this purpose, 140 men were recruited, including 80 smokers and 60 non-smokers, for the collection of their dental plaque. The dental plaque samples were tested using PCR to detect the three bacterial species. The analysis showed that chi-square tests were used, and $p \leq 0.05$ was considered significant. In smokers compared to non-smokers, *K. pneumoniae* was observed in 45% versus 81% ($p = 0.02$); *A. actinomycetemcomitans* in 41% versus 39% ($p = 0.79$); and *P. gingivalis* in 61% versus 58% ($p = 0.67$). Only the difference for *K. pneumoniae* was statistically significant. Classic periodontal pathogens were common in both groups, but no clear evidence that smoking increased their prevalence. The higher prevalence of *K. pneumoniae* in non-smokers suggests that other factors, including diet, nutrition, and oral hygiene, may shape the oral microbiome as significantly as smoking. Consequently, oral health education should integrate nutritional guidance alongside smoking cessation advice.

Keywords: Periodontal disease, Smoking, Bacterial agents, Oral health, Nutrition, Micronutrients

Highlights

- *Porphyromonas gingivalis* was present in 61% of smokers and 58% of non-smokers.
- *Klebsiella pneumoniae* was more common in non-smokers (81%) than smokers (45%)
- Statistical tests showed no clear link between smoking and classical periodontal bacteria.
- Nutritional factors may help explain the bacterial patterns we observed
- Results suggest oral health programs should include dietary advice alongside smoking cessation

1. Introduction

The human mouth contains more than 700 bacterial species. Most of these coexist without causing harm and form what we call the normal oral flora (Kozak & Pawlik, 2023). Periodontal disease develops when this balance shifts. The condition inflames and damages the tissues that support the teeth, affecting roughly 538 million people worldwide (Chávez et al., 2022). Left untreated, it can lead to tooth loss and affect people's confidence and quality of life.The bacteria most often linked to periodontal disease are gram-negative anaerobes. These include *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, and *Treponema denticola* (Balan et al., 2024). These species form biofilms on tooth surfaces, structured communities that resist removal and trigger host inflammatory responses (Arias et al., 2024). The inflammation, intended to fight infection, eventually damages the surrounding tissues.

Smoking increases the risk of periodontal disease. Cigarette smoke contains nicotine and other chemicals that change the mouth's environment. These compounds reduce oxygen levels, alter the immune response, and shift which bacteria can thrive (Ye & Rahman, 2023). Smokers tend to have fewer beneficial bacteria and more pathogenic ones (Zięba et al., 2022). Long-term smokers also show lower blood levels of vitamins A and C, both of which are important for tissue repair and immune function.

1.1. Nutritional Links to Oral Disease

Diet shapes oral health in several ways (Bradshaw & Lynch, 2013). When people eat fermentable carbohydrates, such as sugars and refined starches, oral bacteria metabolize them and produce acids. These acids lower the pH in dental plaque. If the pH remains below 5.5 for extended periods, minerals such as calcium and phosphate can leach from tooth enamel. This demineralization, if it happens repeatedly without enough remineralization, leads to cavities. Several nutrients help protect teeth. Calcium and phosphorus support enamel strength. Fluoride promotes remineralization. Vitamins A, C, and D support the immune system and help maintain healthy oral tissues (Bradshaw & Lynch, 2013).

Vitamin C is essential for making collagen, which is basically the scaffolding that keeps your gums strong. Studies have shown that people with higher vitamin C intake tend to have a lower risk of periodontal disease (Tada & Miura, 2019). Vitamin D regulates bone metabolism and immune function, both of which are relevant to periodontal health. Recent

research confirms that low vitamin D levels are associated with more severe periodontal disease (Jagelavičienė et al., 2023). Iron also matters for oral health, though the relationship is complex. The body needs iron for hemoglobin synthesis and for immune cells to function properly. Iron deficiency weakens neutrophil responses and may reduce resistance to oral pathogens (Camaschella, 2015). At the same time, many bacteria require iron to grow. Some pathogens, including *Klebsiella pneumoniae*, have developed efficient systems to acquire iron even when it is scarce (Choby et al., 2020).

Klebsiella pneumoniae is not typically classified as a periodontal pathogen. It is an opportunistic bacterium found widely in the environment and can colonize various body sites. In people with weakened immunity or altered oral microbiota, it may contribute to oral inflammation (Perfileva et al., 2024). We included it in this study because emerging evidence suggests opportunistic species may play a larger role in periodontal disease than previously recognized.

This study had two aims. First, to compare the prevalence of *P. gingivalis*, *A. actinomycetemcomitans*, and *K. pneumoniae* in smokers and non-smokers in Haripur. Second, to examine how nutritional factors might help explain any observed patterns.

2. Materials and Methods

2.1. Ethical Approval

The Institutional Review Committee at The University of Haripur approved this study. All participants gave written informed consent after receiving information about the study's purpose, procedures, and their right to withdraw at any time. The study followed ethical guidelines for human research as outlined in the Declaration of Helsinki (World Medical Association, 2013).

2.2. Study Design and Participants

This comparative cross-sectional study conducted between March and August 2025 in Haripur, Pakistan. A total of 140 men were recruited from the local population, including 80 cigarette smokers and 60 non-smokers. Smokers were defined as individuals who had smoked at least 4–5 cigarettes daily for one year or more. Non-smokers were individuals who had never used any tobacco products. Sample collection and processing methods were adapted from standard microbiological techniques described by Balan et al. (2024) and Arias et al. (2024).

2.3. Inclusion and Exclusion Criteria

The study included men aged 18 to 75 living in Haripur district who met the smoking or non-smoking definitions above, followed by those excluded, including i) women and children, ii) people with diagnosed heart disease, diabetes, or autoimmune disorders, iii) anyone who had taken antibiotics in the previous three months, iv) people with fewer than 20 teeth and v) anyone who had received periodontal treatment in the previous six months.

2.4. Questionnaire

Participants completed a structured questionnaire covering age, smoking duration, number of cigarettes smoked per day, preferred cigarette brand, presence of dental cavities, frequency of tooth brushing, use of dental floss, and any observed changes in oral health. Each questionnaire was checked for completeness upon return.

2.5. Sample Collection

The dental plaque samples were collected using a standard approach adapted from Balan et al. (2024). Participants refrained from eating, drinking, or brushing their teeth for at least 1 hour before sampling. Supragingival plaque was collected from multiple teeth using sterile toothpicks and immediately placed into sterile 15 mL Falcon tubes containing 2 mL of normal saline (0.9% NaCl). The samples were kept on ice and transported to the Microbiology Laboratory at The University of Haripur within one hour.

Figure 1.1: Sample inoculation

Figure 1.2: Enriched media

2.6. Sample Processing

In the laboratory, each tube was vortexed to disperse the plaque. A 100 µL aliquot was transferred into 5 mL of peptone water and incubated at 37 °C for 24–48 hours to allow bacterial growth. After incubation, the cultures were examined for turbidity and used for DNA extraction. This enrichment step follows protocols described by Balan et al. (2024) for optimizing bacterial recovery from plaque samples (Fig. 1.1 & 1.2).

2.7. DNA Extraction

The DNA extracted using the phenol-chloroform method as described by Choby et al. (2020) with minor modifications followed by the centrifugation of 1 mL of enriched culture at 10,000 rpm for 5 minutes, discarded the supernatant, and resuspended the pellet in 500 µL of lysis buffer (10 mM Tris-HCl, 1 mM EDTA, 0.5% SDS, 20 µg/mL proteinase K). After incubating at 56°C for 1 hour, we added an equal volume of phenol:chloroform: isoamyl alcohol (25:24:1), mixed gently, and centrifuged at 12,000 rpm for 10 minutes. We transferred the aqueous layer to a fresh tube, added an equal volume of chloroform: isoamyl alcohol (24:1), mixed, and centrifuged again. The DNA was precipitated by adding 0.1 volume of 3 M sodium acetate and 2 volumes of cold absolute ethanol, then incubating at -20°C overnight. Then, the DNA pellet was washed with 70% ethanol, air-dried, resuspended in 50 µL of TE buffer, and stored the extracted DNA at -20°C until PCR analysis.

2.8. PCR Amplification

The species-specific primers based on published sequences were used (Table 1). Primer sequences for *P. gingivalis* and *A. actinomycetemcomitans* were obtained from Balan et al. (2024), while *K. pneumoniae* primers were based on Choby et al. (2020). A commercial supplier synthesized the primers, and we reconstituted them according to the manufacturer's instructions (Fig. 2 & 3).

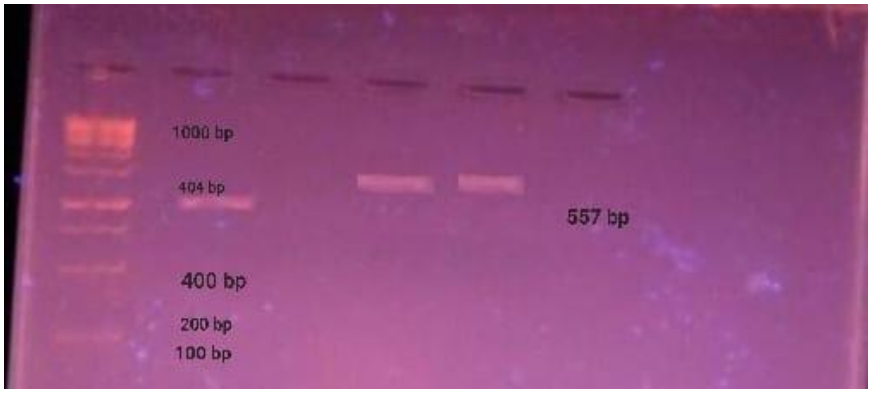

Figure 2: Electrophoresis results of PCR amplification that was obtained by using specific primers against the targeted organisms. Lane 4 *Porphyromonas gingivalis,* Lane 5 *Actinobacillus actinomycetemcomitans.*

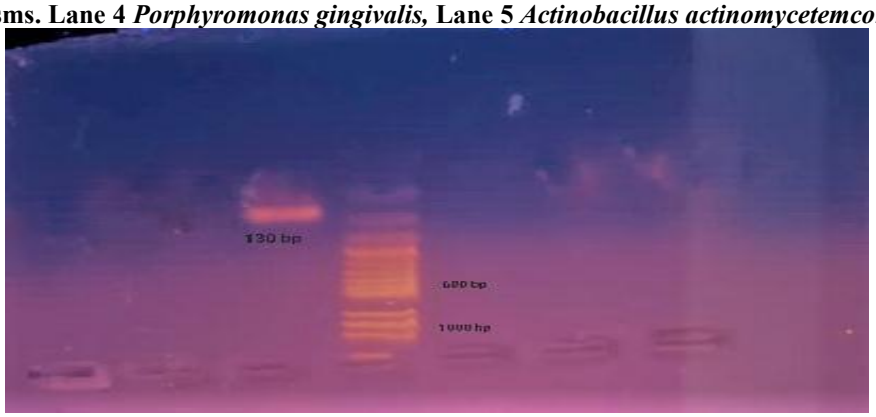

Figure 3: shows the results of *Klebsiella pneumoniae* in Lane 1. The DNA ladder was 1 Kb.

Table 1: Primers used in this study

Target Species	Primer Sequence (5'→3')	Product Size (bp)	Annealing Temp (°C)	Source
P. gingivitis	Forward: AGGCA GCTTG CCATA CTGCG Reverse: ACTGT TAGCA AGTAC CGATG T	404	68	Balan et al., 2024
A. actinomycetemcomitans	Forward: GCTAA TACCG CGTAG AGTCG G Reverse: ATTTC ACACC TCACT TAAAG GTCCG	557	68	Balan et al., 2024
K. pneumoniae	Forward: ATTTG AAGAG GTTGC AAACG AT Reverse: TTCAC TCTGA AGTTT TCTTG TGTTC	130	55	Choby et al., 2020

A 25 µL mixture for each PCR reaction was prepared, and 5 µL of 5X PCR Master Mix (Solis BioDyne) was used, which contains buffer, dNTPs, and Taq polymerase. To this, 2 µL of template DNA, 1.5 µL each of forward and reverse primers

(10 pmol/µL), and 15 µL of nuclease-free water were added to reach the final volume. The samples were placed in a thermocycler with the following conditions: initial denaturation at 94°C for 5 minutes; then 35 cycles of denaturation at 94°C for 30 seconds, annealing for 30 seconds, and extension at 72°C for 45 seconds; followed by a final extension at 72°C for 10 minutes. The annealing temperature varied by species: 55°C for *K. pneumoniae* and 68°C for both *P. gingivalis* and *A. actinomycetemcomitans*.

2.9. Gel Electrophoresis

PCR products were analyzed on 2% agarose gels prepared in 0.5× TBE buffer. Gels were stained with ethidium bromide (0.5 µg/mL), and a 1 kb DNA ladder was used as a size marker. Electrophoresis was performed at 100 V for 45 minutes. Bands were visualized under UV light and recorded. A sample was considered positive if it showed a band at the expected size, following the interpretation criteria described by Balan et al. (2024).

2.10. Statistical Analysis

Data were entered into SPSS version 25. Descriptive statistics were calculated for demographic variables and bacterial prevalence. The chi-square test was used to compare bacterial prevalence between smokers and non-smokers. A p-value ≤ 0.05 was considered statistically significant. Similar statistical approaches have been used in periodontal microbiology studies (Balan et al., 2024; Perfileva et al., 2024). Chi-square distribution plot showing the statistical significance of differences in bacterial prevalence between smokers and non-smokers. The p-value for *K. pneumoniae* (0.02) was below the significance threshold of 0.05, while p-values for *P. gingivalis* (0.67) and *A. actinomycetemcomitans* (0.79) were not significant (Fig. 4).

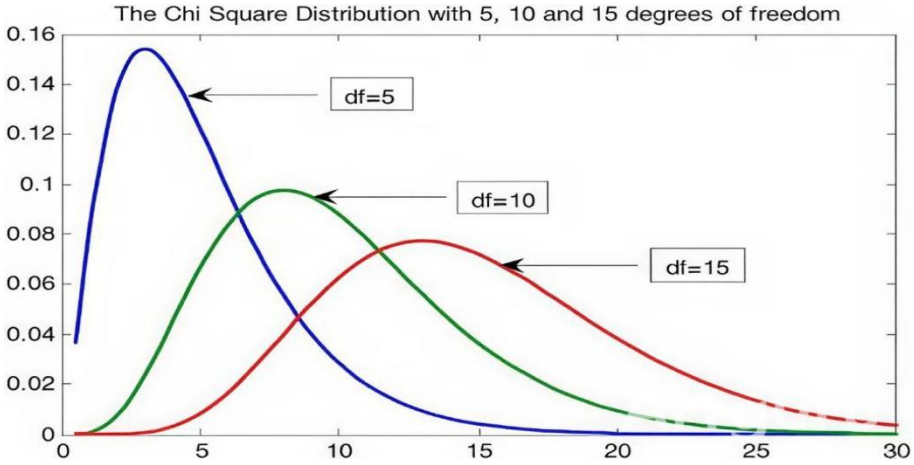

Figure 4: Chi-square distribution plot showing the statistical significance

3. Results and Discussion

3.1. Participant Characteristics

The age distribution of the 140 participants is shown in Table 2. Most smokers (44%) were in the 26-35 age range. Non-smokers were more evenly distributed across age groups (Fig. 5).

Table 2: Age distribution of participants

Age Group (years)	Smokers (n=80)	Non-Smokers (n=60)
15-25	15 (19%)	16 (27%)
26-35	35 (44%)	19 (32%)
36-45	10 (12%)	14 (23%)
46-55	13 (16%)	9 (15%)
56-65	4 (5%)	2 (3%)
66-75	3 (4%)	3 (5%)

A total of 80 smoker and 60 non-smoker samples were collected from the local population of Haripur. Of the total 80 samples of smokers, 36 samples were *Klebsiella* positive, 33 were *Actinobacillus acetinomycetemcomitans*, and 49 were *Porphyromonas gingivalis* positive. However, there were almost 60 non-smoker samples, of which 23 were *Actinobacillus actinomycetemcomitans*-positive, 48 were Klebsiella pneumoniae-positive, and 34 were Porphyromonas gingivalis-positive (Fig. 5).

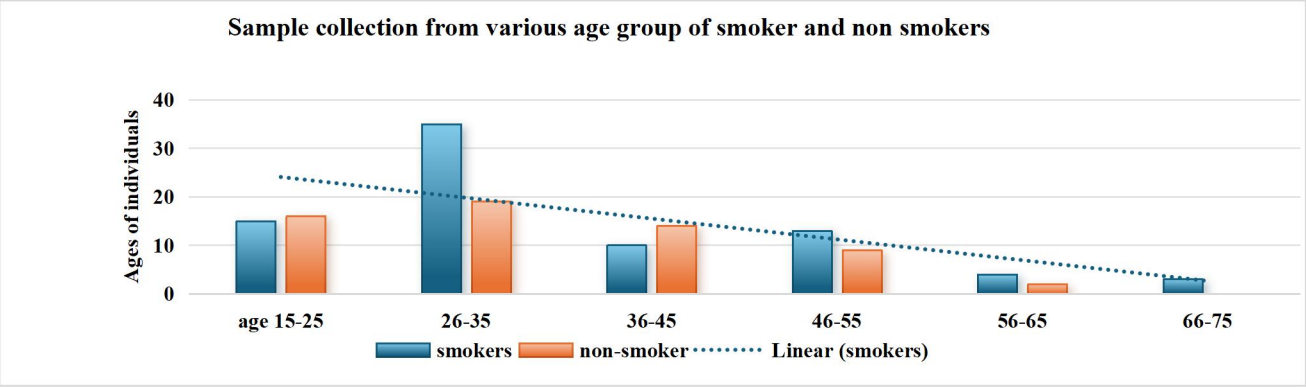

Figure 5: This graph represents the prevalence of sample collection from the various age groups among smokers and non-smokers.

3.2.Bacterial prevalence

PCR produced bands at the expected sizes for all three species. Table 3 summarizes the results.

Table 3: Bacterial prevalence in smokers and non-smokers

Bacterial Species	Smokers (n=80)	Non-Smokers (n=60)	χ^2	p-value	Significance
P. gingivalis	49 (61%)	34 (58%)	0.18	0.67	ns
A. actinomycetemcomitans	33 (41%)	23 (39%)	0.07	0.79	**ns**
K. pneumoniae	36 (45%)	48 (81%)	5.41	0.02	*

- ns = not significant ($p > 0.05$); * = significant at $p < 0.05$*
- *P. gingivalis* was the most common species in both groups. It appeared in 61% of smokers and 58% of non-smokers. This difference was not statistically significant ($p = 0.67$).
- *A. actinomycetemcomitans* showed similar rates: 41% in smokers and 39% in non-smokers. The difference was not significant ($p = 0.79$).
- *K. pneumoniae* followed a different pattern. It was significantly more common in non-smokers (81%) than in smokers (45%) ($p = 0.02$) (Fig. 6).

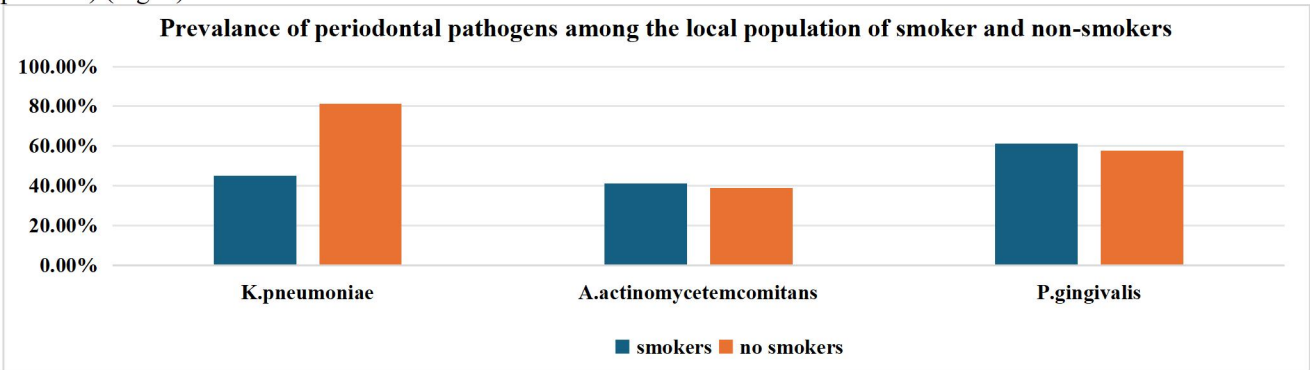

Figure 6. The figure shows the prevalence of periodontal pathogens that are responsible for causing periodontitis among smokers and non-smokers

The data in Table 3 show distinct patterns for each bacterial species. *P. gingivalis* was the most common species in both groups. It appeared in 61% of smokers and 58% of non-smokers. This difference was not statistically significant ($p = 0.67$), indicating that smoking status did not influence colonization by this bacterium in our study population. Figure 6 shows the probability density function for the chi-square distribution with the given degrees of freedom. As the degree of freedom increases, the distribution shifts to the right and becomes more symmetrical. This is used to determine the critical value for the statistical analysis of bacterial prevalence. *A. actinomycetemcomitans* showed similar rates: 41% in smokers and 39% in non-smokers. The difference was not significant ($p = 0.79$). Like *P. gingivalis*, this classical periodontal pathogen occurred at comparable frequencies across smoking status. *K. pneumoniae* followed a different pattern. It was significantly more common in non-smokers (81%) than in smokers (45%) ($p = 0.02$). This unexpected finding suggests that non-smokers in our population were more likely to carry this opportunistic bacterium than smokers. This study examined how often three bacterial species appeared in dental plaque from smokers and non-smokers in Haripur. The results reveal distinct patterns and suggest that factors beyond smoking, notably nutrition, may significantly shape the oral microbiome.

3.3. Classical Periodontal Pathogens

P. gingivalis was the most frequently detected species in both groups. This matches what other researchers have found: *P. gingivalis* is widespread in adult populations regardless of smoking status (Balan et al., 2024; Łasica et al., 2024). The bacterium is considered a keystone pathogen because it can disrupt immune responses and create conditions that favor the growth of other harmful bacteria.

No significant difference in *P. gingivalis* prevalence between smokers and non-smokers was observed. This suggests that in this population, smoking may not directly increase colonization by this species. It may also suggest that other factors, including diet, oral hygiene, and genetic background, are equally or more important. *A. actinomycetemcomitans* showed similar prevalence in both groups. This species produces a leukotoxin that kills immune cells, which helps explain its association with aggressive periodontitis (Balan et al., 2024). The similar rates between smokers and non-smokers suggest that smoking cessation alone may not reduce carriage of this organism.

3.4. *Klebsiella pneumoniae* and the Role of Nutrition

The higher prevalence of *K. pneumoniae* in non-smokers was unexpected. Smoking typically promotes colonization by pathogens, so finding an opportunistic bacterium more often in non-smokers requires explanation. One possibility involves nutrition. *K. pneumoniae* has efficient systems for acquiring iron, which may give it an advantage in people with certain iron statuses (Choby et al., 2020). Iron deficiency is common in South Asian populations, and rates may differ between smokers and non-smokers due to dietary habits or other factors. Smokers often have different food preferences and nutrient absorption patterns, which could affect their oral microbial communities. Vitamin and mineral status influences oral health in several ways. Vitamin C deficiency impairs collagen synthesis and wound healing, potentially facilitating bacterial colonization (O'Leary & Samman, 2010; Tada & Miura, 2019). Vitamin D regulates immune responses, and low levels are linked to more severe periodontal disease (Jagelavičienė et al., 2023). Antioxidants help control oxidative damage associated with inflammation (Bradshaw & Lynch, 2013). Oxidative stress plays a central role in periodontal tissue destruction, which is why antioxidant status matters so much for gum health (Sczepanik et al., 2020).

Dietary patterns may also differ between smokers and non-smokers. Smoking affects taste and smell, which can change food choices. Non-smokers may consume more diverse diets, supporting a broader range of microorganisms, which includes opportunistic species such as *K. pneumoniae*. Research has shown that dietary intake directly influences the composition of oral bacteria, as diets rich in fiber and nutrients support microbial communities distinct from those fostered by processed foods (Kato et al., 2017). Another possibility is that smoking directly inhibits *K. pneumoniae*. Tobacco leaves contain compounds with antimicrobial activity against some bacteria. However, this is different from the effects of tobacco smoke. While tobacco leaves may inhibit certain bacteria, tobacco smoke damages immune cells and increases susceptibility to respiratory infections like pneumonia and tuberculosis. This apparent contradiction may explain our findings: smokers in our study had lower oral carriage of *K. pneumoniae*, possibly due to the direct antimicrobial effects of smoke components, but this does not mean they are protected from infection. The damage smoking causes to immune defenses means that if *K. pneumoniae* does colonize or infect smokers, they may be less able to clear it, leading to more severe outcomes.

3.5.Microbial Diversity and Environmental Factors

Microbial communities in the mouth vary across populations. Diet, water source, living conditions, and genetic background all shape which bacteria colonize and persist. Research has shown that dietary habits directly influence the composition of oral bacteria, as diets high in fiber and nutrients support distinct microbial communities compared with those high in processed foods (Kato et al., 2017). The higher *K. pneumoniae* rate in non-smokers may reflect the normal microbial diversity in this population rather than any protective effect of non-smoking. The bacteria detected by PCR may not represent an active infection. PCR amplifies DNA from both living and dead bacteria, so a positive result indicates the organism was present at some point but does not indicate whether it was causing disease. This distinction matters because some bacteria can colonize without causing harm.

3.6.Study Limitations

This study has several limitations. First, we only included men, so the results may not apply to women. Second, we did not directly measure dietary intake or nutritional status, so our discussion of nutrition remains speculative. Third, the sample size was modest, and we did not perform a formal sample size calculation. Fourth, the study's cross-sectional design precludes the determination of cause and effect. Consequently, while our findings highlight key associations, they do not confirm that smoking or nutrition habits directly drive these specific bacterial profiles. Fifth, we did not quantify bacterial loads, so we cannot say whether smokers and non-smokers differed in the number of bacteria they carried. Sixth, participants reported their oral hygiene habits, and people often overestimate how well they care for their teeth. Seventh, *K. pneumoniae* is not a standard periodontal pathogen, and its role in gum disease remains understudied.

3.7.Implications

Despite these limitations, the findings have practical implications. Periodontal pathogens were common in both smokers and non-smokers, which means everyone in this population faces some risk. The high rate of *K. pneumoniae* matters because this bacterium can cause infections elsewhere in the body, particularly in people with weakened immunity (Perfileva et al., 2024). The mouth may serve as a reservoir for such organisms. Public health efforts should help people understand how their daily choices affect their gums. Simple messages about reducing sugar and eating foods rich in vitamins and minerals can make a difference. Regular dental check-ups matter because they catch problems early. Some researchers have suggested that integrating oral health services into general medical settings could reach people who do not regularly see dentists (Harnagea et al., 2017). This kind of integrated approach could help more people get the care they need.

Advanced molecular methods, such as gene sequencing, are increasingly used to study oral diseases. These techniques can identify genetic mutations and microbial communities with high accuracy, which may help in developing personalized treatment plans (Abdul et al., 2024). While our study used conventional PCR, future research could benefit from next-generation sequencing approaches to better understand the complex microbial interactions in periodontal disease.

Conclusion

This study found high rates of periodontal bacteria in both smokers and non-smokers in Haripur. *P. gingivalis* was the most common species in both groups. *K. pneumoniae* was significantly more common in non-smokers, a finding that may reflect nutritional or dietary differences between the groups. It was concluded that there was no clear evidence that smoking increased the prevalence of classical periodontal pathogens in this population. This finding may seem unexpected given that smoking is known to alter the oral environment by reducing oxygen levels, impairing immune responses, and shifting bacterial populations toward more pathogenic species. However, similar rates of *P.gingivalis* and *A. actinomycetemcomitans* between smokers and non-smokers suggest that, in this population, other factors, such as diet, oral hygiene, and genetic background, may play equally important roles in shaping bacterial colonization.The results suggest that multiple factors shape the oral microbiome. Smoking matters, but so do nutrition, diet, and oral hygiene. Future studies should directly measure nutritional status, use quantitative methods to assess bacterial loads, and follow participants over time to establish causal relationships.

In this study, the key outcome was that *K. pneumoniae* was significantly more common in non-smokers (81%) than in smokers (45%), while classical periodontal pathogens showed no significant differences between groups. This indicates that non-smokers in this population carried this opportunistic bacterium more frequently, which may reflect dietary or nutritional differences rather than a protective effect of smoking. Prevention strategies should therefore address smoking, nutrition, and oral hygiene together. Future studies should directly measure nutritional status, employ quantitative methods to assess bacterial loads, and follow participants over time to establish causal relationships.

Acknowledgment

We thank the participants who volunteered for this study. We are grateful to our parents for their support and encouragement. The Department of Microbiology at The University of Haripur provided laboratory facilities and technical assistance.

Conflict of Interest

The authors declare no conflict of interest. No financial, personal, or professional relationships influenced the study design, data collection, analysis, or interpretation.

Funding Resource

This research received no external funding. The Department of Microbiology at The University of Haripur provided institutional support.

References

Abdul, N. S., Shenoy, M., Reddy, N. R., Sangappa, S. B., Shivakumar, G. C., Di Blasio, M., … Minervini, G. (2024). Gene sequencing applications to combat oral-cavity related disorders: A systematic review with meta-analysis. *BMC Oral Health*, 24(1), 103.

Arias, L. S., da Silva, P. D. A., da Silva, G. P., de Souza, A. H., & dos Santos, A. A. D. M. (2024). Biofilms in the context of oral diseases. In *Microbial Biofilm Dynamics* (pp. 31-43). CRC Press.

Balan, D. K., John, J., Ahamed, S., Syam, N., Sudhakaran, G., Lakshmy, M., & Ali, H. (2024). An anaerobic culture study to assess the prevalence of *Porphyromonas gingivalis* in periodontal disease incidences among adults. *Cureus*, 16(7).

Bradshaw, D. J., & Lynch, R. J. (2013). Diet and the microbial aetiology of dental caries: New paradigms. *International Dental Journal*, 63, 64-72.

Camaschella, C. (2015). Iron-deficiency anemia. *The New England Journal of Medicine*, 372(19), 1832-1843.

Chávez, E. M., Kossioni, A., & Fukai, K. (2022). Policies supporting oral health in ageing populations are needed worldwide. *International Dental Journal*, 72(4), S27-S38.

Choby, J. E., Howard-Anderson, J., & Weiss, D. S. (2020). Hypervirulent *Klebsiella pneumoniae*—clinical and molecular perspectives. *Journal of Internal Medicine*, 287(3), 283-300.

Ganz, T., & Nemeth, E. (2012). Hepcidin and iron homeostasis. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1823(9), 1434-1443.

Harnagea, H., Lamothe, L., Couturier, Y., Esfandiari, S., Voyer, R., Charbonneau, A., & Emami, E. (2017). From theoretical concepts to policies and applied programs: The landscape of integration of oral health in primary care. *BMC Oral Health*, 17(1), 1-17.

Jagelavičienė, E., Vaitkevičienė, I., Šilingaitė, D., Šinkūnaitė, E., & Daugėlaitė, G. (2023). The relationship between vitamin D and periodontal pathology. *Medicina*, 59(3), 572.

Kato, I., Vasquez, A., Moyerbrailean, G., Land, S., Djuric, Z., Sun, J., & Ram, J. L. (2017). Nutritional correlates of human oral microbiome. *Journal of the American College of Nutrition*, 36(2), 88-98.

Kozak, M., & Pawlik, A. (2023). The role of the oral microbiome in the development of diseases. *International Journal of Molecular Sciences*, 24(6), 5231.

Łasica, A., Golec, P., Laskus, A., Zalewska, M., Gędaj, M., & Popowska, M. (2024). Periodontitis: Etiology, conventional treatments, and emerging bacteriophage and predatory bacteria therapies. *Frontiers in Microbiology*, 15, 1469414.

O'Leary, F., & Samman, S. (2010). Vitamin B12 in health and disease. *Nutrients*, 2(3), 299-316.

Perfileva, D. Y., Miroshnichenko, A. G., Kulikov, E. S., Perfilev, V. Y., Boykov, V. A., & Nesterovich, S. V. (2024). Antibiotic resistance of infectious agents is associated with prior hospitalization. *Research Results in Pharmacology*, 10(1), 15-21.

Sczepanik, F. S. C., Grossi, M. L., Casati, M., Goldberg, M., Glogauer, M., Fine, N., & Tenenbaum, H. C. (2020). Periodontitis is an inflammatory disease of oxidative stress: We should treat it that way. *Periodontology 2000*, 84(1), 45-68.

Tada, A., & Miura, H. (2019). The relationship between vitamin C and periodontal diseases: A systematic review. *International Journal of Environmental Research and Public Health*, 16(14), 2472.

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA*, 310(20), 2191-2194.

Ye, D., & Rahman, I. (2023). Emerging oral nicotine products and periodontal diseases. *International Journal of Dentistry*, 2023(1), 9437475.

Zięba, S., Maciejczyk, M., & Zalewska, A. (2022). Ethanol-and cigarette smoke-related alterations in oral redox homeostasis. *Frontiers in Physiology*, 12, 793028.

Received: February 28th, 2025 **Accepted: March 31st, 2026**