A study on the characteristics of normal human oral flora in individuals who consume tobacco

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Abstract

Human mouth is natural incubator which harbor ample of microorganisms and this microflora are changing due to physical, hormonal and behavioral factors. Aim of the study is to compare the bacteria present in the tobacco users and non-users based on their morphology and gram reactions. Samples taken from the adults of slum area of Rajkot analyzed for presence of types of bacteria based on colony characterization and Gram's reaction. Results of the analysis showed that adults using tobacco has Gram Negative bacteria compared to non-users who harbor Gram positive bacteria with their particular colony characteristics. These finding suggest that use of tobacco change the microflora of oral cavity which may be responsible for many diseases.

1. Introduction

The human mouth, which contains all the necessary nutrients and proper temperature, is the best place where microbes can grow; around 700 bacterial species are found in the human oral cavity (Dewhirst et al., 2010). These microbes are normal flora of the human buccal cavity, which help in fighting with pathogens. These organisms can be replaced with unwanted bacteria due to changes in lifestyle, like consuming tobacco. Use of tobacco in any form leads to change in normal flora and thereby increases the pathogenic organisms that lead to many ailments of the oral cavity, including cancer (Tomar & Asma, 2000; Bagaitkar et al., 2011).

An investigation into the impact of tobacco consumption on the oral microbiome is crucial for the early identification and prevention of tobacco-related illnesses. Much research has studied the effect of tobacco on diseases of the oral cavity, with very little focus on the impact of tobacco on changes in the microbiome with respect to their morphology and diversity in people living in slum areas where use of tobacco is very common (Wu et al., 2016; Shchipkova et al., 2010).

Thus, a study was conducted to compare the microbial diversity of oral microflora of tobacco users and non-user adults, including males and females. Mainly colony morphology and gram reaction are studied in tobacco users and non-users to reveal any changes due to tobacco consumption.

2. Materials and Methods

2.1 Inclusion criteria

People aged 18 years and above who had been consumed tobacco product regularly for at least 10 years were selected as a test subject while those age 18 years and above who had never consumed tobacco were test as a control. Female were also included in the study who were consuming tobacco products for at least 4 years.

2.2 Study area

The sample were collected from the local slum area of Rajkot, Gujarat. An inclusion criterion for the study was any random people are selected accept children below the age of 18 years or people suffering from the multiple infection like dental plaque and periodontal etc.

2.3 The Collection of samples

During the investigation, two adult subjects were selected for sample collection. Their samples were stored in sterile phosphate buffer at 4°C.

Processing of samples

The collected sample was inoculated with sterile nutrient broth that contained peptone, yeast extract, sodium chloride, and beef extract and was incubated overnight at 37°C.

2.4 Culture

A loopful of culture from nutrient broth was taken with a sterile wire loop and streaked on a nutrient agar plate, which was kept at 37°C for 24 hours. After incubation, the isolated bacterial colonies were streaked onto a new plate.

2.5 Colony characterization of isolates

Isolates were examined for colony characters like color, size, consistency, shape, odor, margin and texture etc. Isolate were examined microscopically by Gram's Staining technique.

2.6 Maintenance of isolates

Isolated organisms were preserved using nutrient agar slant and stored at 2°C to 4°C temperatures in refrigerator. Stored culture was used for pre-culturing in nutrient broth.

3. Results and Discussion

Table 1: Growth characteristics of normal mouth flora of non-tobacco user adult male (control)

No.	Isolate	Gram's staining	Morphology	Colony characteristics						
				Size	Shape	Margine	Texture	Elevation	Opacity	Color
1	M1	+ve	Cocci arrange in chain	Small	Circular	Entire	Smooth	Convex	Opaque	Pale yellow
2	M2	+ve	Cocci arrange in group	Punctiform	Circular	Entire	Smooth	Convex	Opaque	White
3	M3	+ve	Cocci arrange in group	Small	Circular	Entire	Smooth	Convex	Translucent	Pale yellow
4	M4	+ve	Cocci arrange in group	Moderate	Irregular	Undulate	Smooth	Raised	Opaque	White
5	M5	+ve	Cocci arrange in group	Punctiform	Circular	Entire	Smooth	Flat	Opaque	Lemon yellow
6	M6	+ve	Cocci arrange in group	Punctiform	Circular	Entire	Smooth	Convex	Opaque	Pale yellow
7	M7	+ve	Large rod arrange in chain	Moderate	Circular	Entire	Smooth	Raised	Opaque	White

Table 2: Growth characteristics of normal mouth flora of non-tobacco consuming adult female (control)

No.	Iso-late	Gram's staining	Morphology	Colony characteristics						
				Size	Shape	Margine	Texture	Elevation	Opacity	Color
1	F1	+ve	Short rod arrange in chain	Small	Rhizoid	Rhizoid	Rough	Convex	Opaque	White
2	F2	+ve	Cocci arrange in group	Small	Irregular	Entire	Smooth	Flat	Translucent	Pale yellow
3	F3	+ve	Cocci arrange in chain	Punctiform	Circular	Entire	Smooth	Raised	Opaque	White
4	F4	+ve	Large rod arrange in single	Small	Irregular	Curled	Rough	Raised	Opaque	White
5	F5	+ve	Spores	Small	Irregular	Undulate	Smooth	Raised	Opaque	Off White
6	F6	+ve	Bacillus coccus	Large	Irregular	Undulate	Smooth	Raised	Opaque	Pale yellow
7	F7	+ve	Large rod arrange in single	Large	Irregular	Undulate	Smooth	Raised	Opaque	White
8	F8	+ve	Cocci arrange in group	Punctiform	Circular	Entire	Smooth	Convex	Translucent	White
9	F9	+ve	Spores	Large	Irregular	Undulate	Smooth	Raised	Opaque	Milky white

Gram's reaction

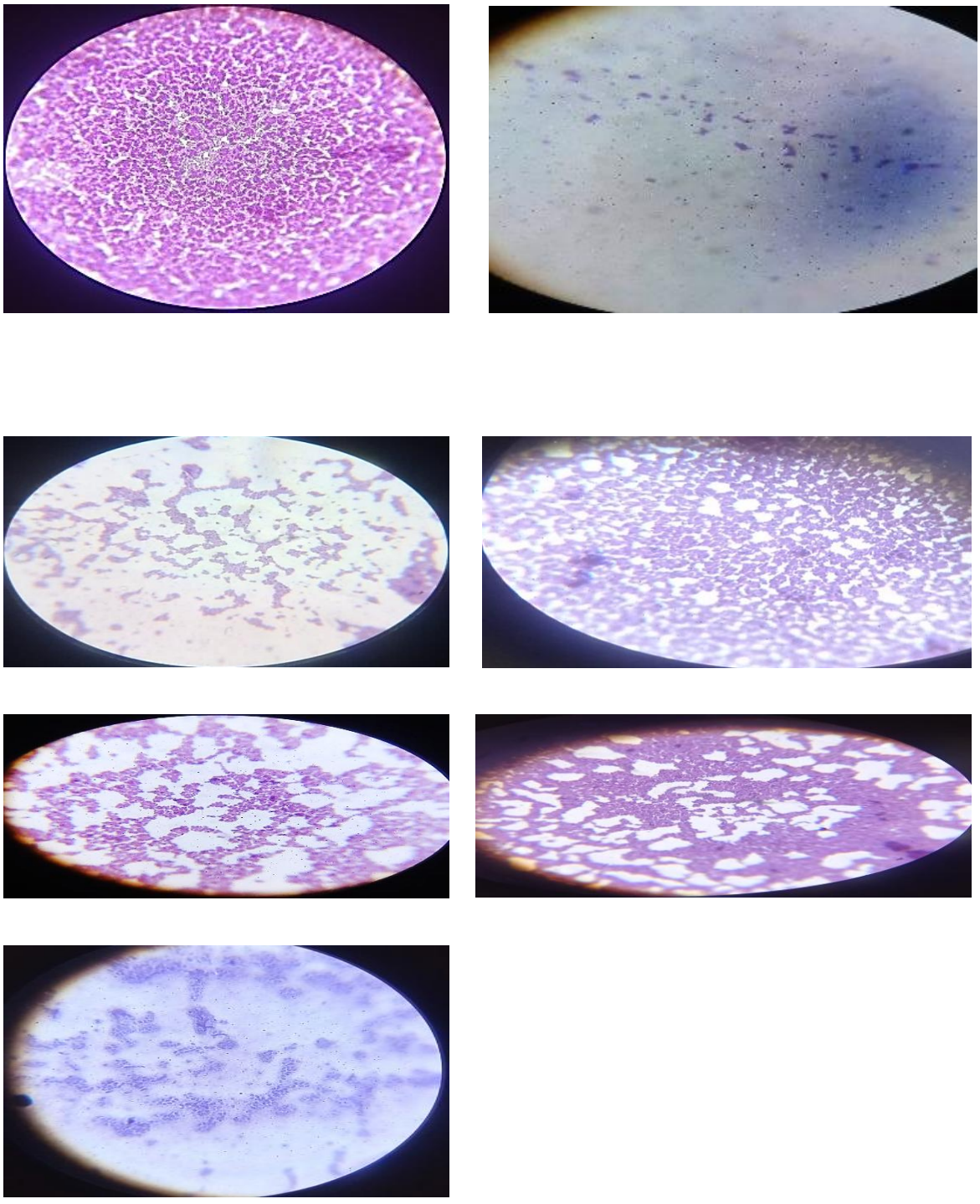


Fig. 1. Gram's reactions of normal mouth flora of non-tobacco user adult male (control)

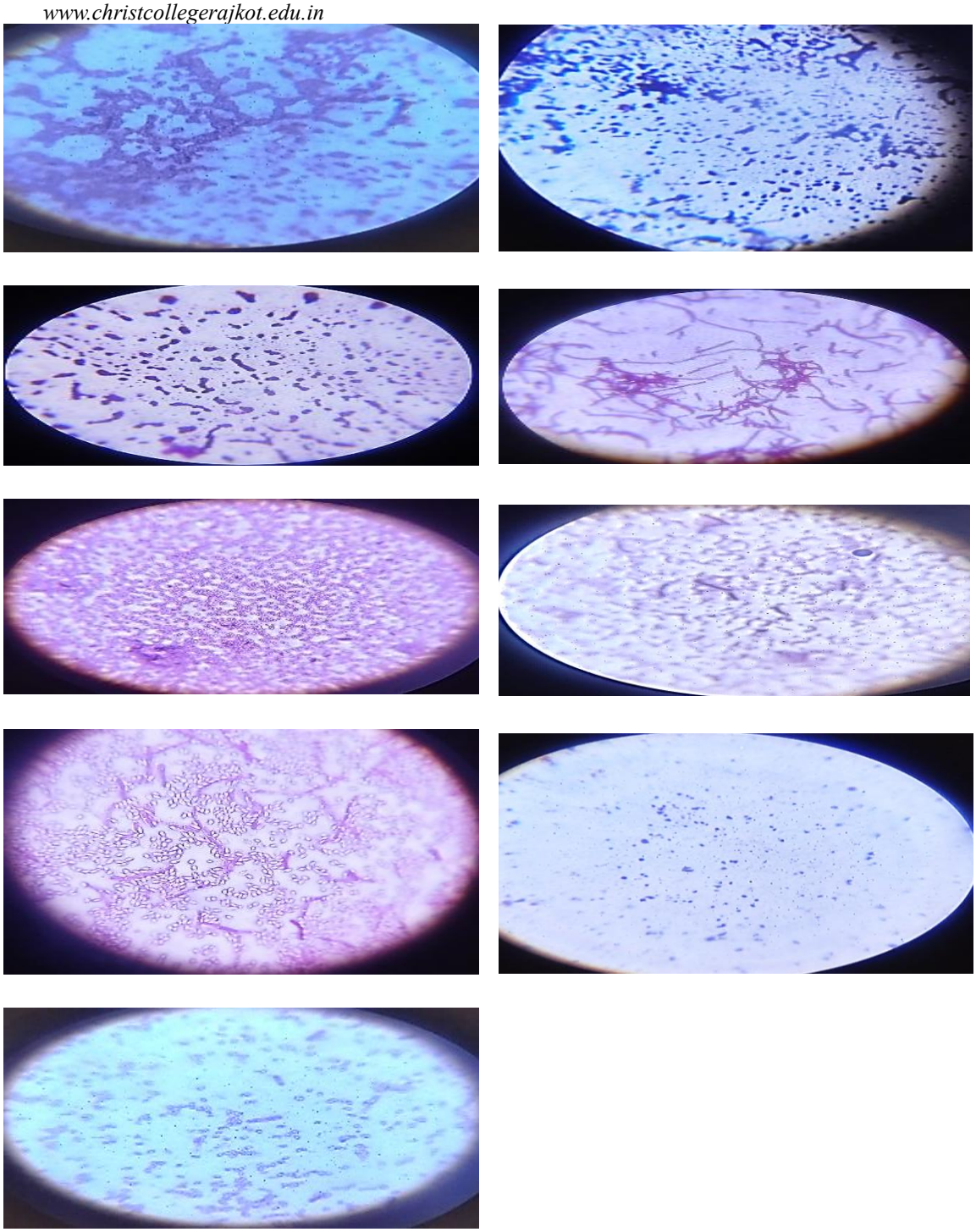


Fig. 2. Gram's reactions of normal mouth flora of non-tobacco user adult female (control)

As observed in table 1, 2, and Fig. 1 and 2 all isolates of males not using tobacco were Gram-positive cocci or rods, predominantly arranged in chains or groups. Colonies were mostly pale yellow, lemon yellow, or white in color, with smooth textures, entire margins, convex or raised elevations, and opaque or translucent appearances. In Females, similar to males, the isolates were primarily Gram-positive cocci and rods. Colony morphology showed more variation, with

the presence of rhizoid and irregular shapes. Spore-forming bacteria were also observed, that indicate that diverse group of organisms are present in the oral cavity of tobacco non users but most of them are Gram-positive bacteria.

Table 3: Growth characteristics of mouth flora of tobacco consuming adult male

No.	Isolate	Gram's staining	Morphology	Colony characteristics						
				Size	Shape	Margin	Texture	Elevation	Opacity	Color
1	M1	-ve	Short rods with single arrangement	Moderate	Circular	Entire	Smooth	Convex	Opaque	White
2	M2	-ve	Short rods arrange in group	Small	Circular	Entire	Smooth	Raised	Opaque	Pale yellow
3	M3	-ve	Coccus arrange in chain	Punctiform	Circular	Entire	Smooth	Raised	Opaque	Pale yellow
4	M4	-ve	Short rods with single arrangement	Small	Irregular	Undulate	Rough	Flat	Opaque	Yellow
5	M5	+ve	Cocci arranges in group	Punctiform	Circular	Entire	Smooth	Raised	Opaque	Yellow
6	M6	-ve	Cocci arranges in group	Small	Circular	Entire	Smooth	Convex	Translucent	White
7	M7	-ve	Cocci arranges in group	Moderate	Irregular	Undulate	Smooth	Convex	Opaque	Pale yellow
8	M8	+ve	Large rods arrange in group	Moderate	Irregular	Undulate	Smooth	Flat	Opaque	white

Table 4: Growth characteristics of mouth flora of tobacco consumption by adult female

No.	Iso-late	Gram's staining	Morphology	Colony characteristics						
				Size	Shape	Margin	Texture	Elevation	Opacity	Color
1	F1	+ve	Cocci with single arrangement	Small	Irregular	Curled	Smooth	Flat	Opaque	White
2	F2	+ve	Cocci arrange in group	Punctiform	Circular	Entire	Smooth	Raised	Opaque	White
3	F3	-ve	Cocci arrange in group	Small	Circular	Entire	Smooth	Raised	Opaque	Pale yellow
4	F4	-ve	Short rods with single arrangement	Moderate	Irregular	Undulate	Smooth	Raised	Translucent	Pale yellow
5	F5	-ve	Rods arrange in group	Punctiform	Circular	Entire	Smooth	Convex	Opaque	Cream white
6	F6	+ve	Cocci arrange in group	Punctiform	Circular	Entire	Smooth	Convex	Opaque	Off white
7	F7	-ve	Cocci arrange in chain	Punctiform	Circular	Entire	Smooth	Raised	Opaque	Pale yellow
8	F8	-ve	Short rods arrange in group	Moderate	Irregular	Undulate	Smooth	Raised	Opaque	White
9	F9	-ve	Cocci arrange in group	Small	Circular	Entire	Smooth	Raised	Translucent	White

Gram's Reaction

Fig. 3. Gram's reactions of normal mouth flora of tobacco user adult male (test)

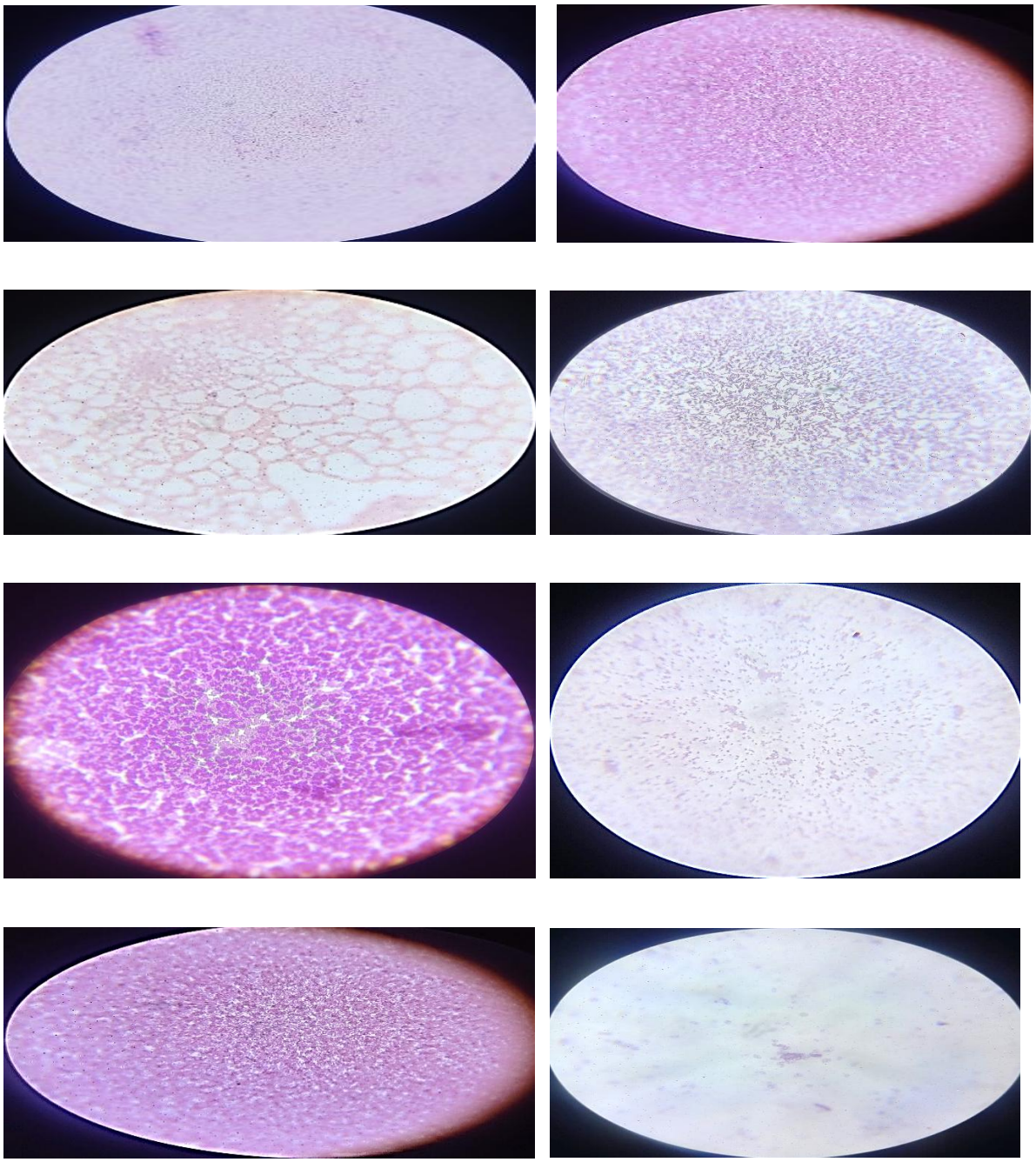
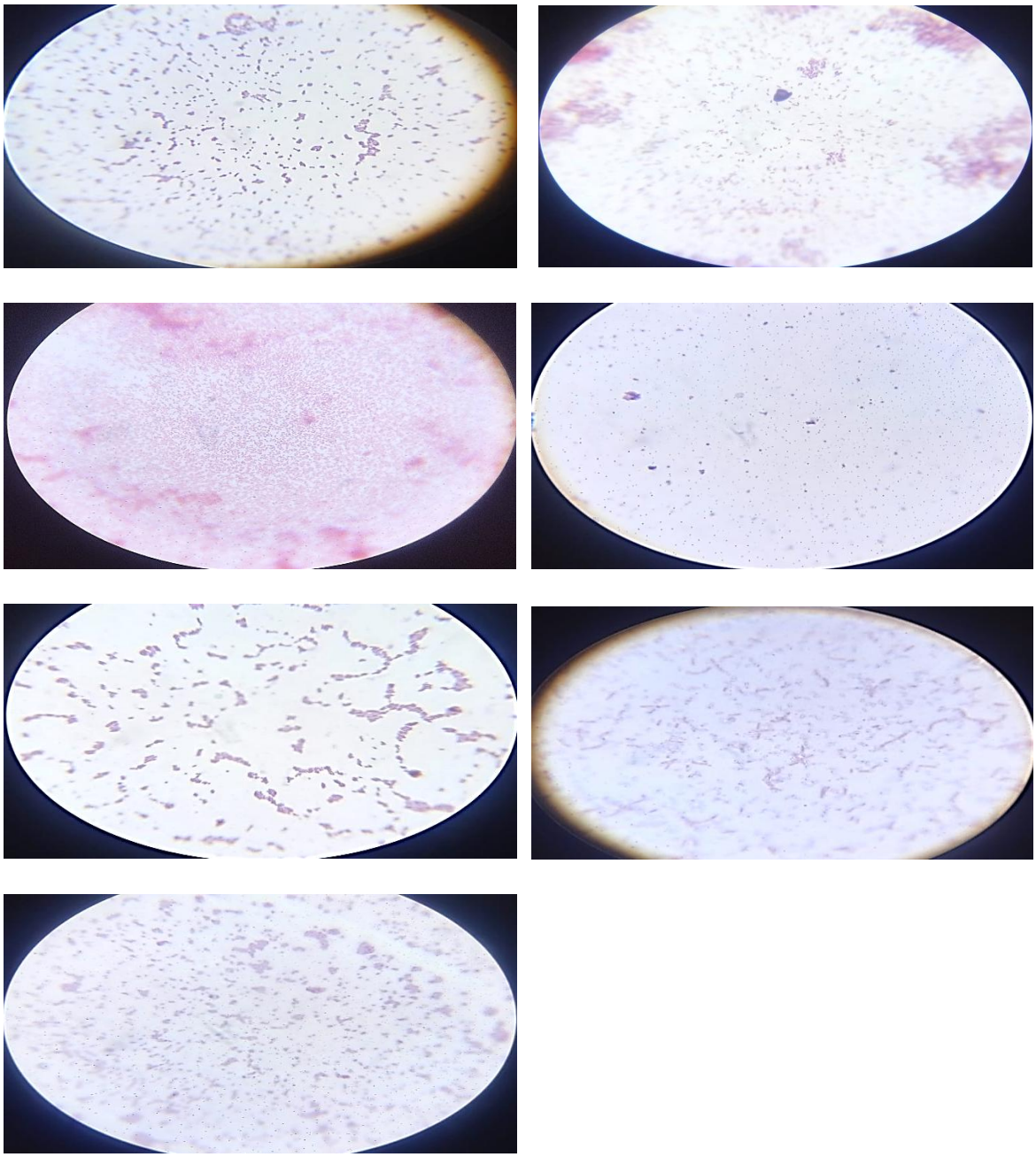


Fig. 4. Gram's reactions of normal mouth flora of tobacco user adult female (test)



As illustrated in Tables 3 and 4, and Figures 3 and 4, the majority of bacteria present in the oral cavities of both male and female tobacco users were Gram-negative rods and cocci. Colonies observed also showed changes in shape, margin texture and pigmentation when compared with colonies of tobacco non users. These changes in colony characteristics and Gram's reaction suggest there is shift of organisms towards pathogenic bacteria. While in adult females, Gram-negative rods and cocci predominated in the samples, and colony morphology indicated increased

undulation, roughness, and translucent characteristics. Notably, a rise in the number of Gram-negative isolates was seen compared to non-users.

The comparison across groups suggests that tobacco consumption leads to a notable shift from Gram-positive to Gram-negative organisms. Gram-negative bacteria such as Prevotella, Fusobacterium, and Porphyromonas are known for their association with periodontal disease and inflammatory conditions (Paster et al., 2006; Socransky et al., 1998).

These findings are similar to previous studies that shows that there is imbalance in the oral microbiome of tobacco users. The changes in microbial types of oral cavity may increase the risk of oral diseases, lower immune defense, and pave the way for opportunistic infections (Shchipkova et al., 2010; Wu et al., 2016).

Conclusion

The present study reveals that the oral microbial flora of tobacco users was significantly different from the non-users. Tobacco nonusers contain more Gram-positive bacteria, while tobacco users contain more Gram-negative bacteria with variation in their colony morphology, like texture, margin, and pigmentation. This change in microbes in the oral cavity due to tobacco consumption shows a change in the oral environment.

Such change in microbial load can make tobacco user prone to many oral diseases including periodontal diseases, dental caries and mucosal infections. These finding suggests that importance is to be given to monitor oral microbial load of tobacco users of slum areas where to use is very common. Early detection of disease, awareness program and regular checkup of oral microflora can solve the problems of disease related to oral health.

References

Bagaitkar, J., Demuth, D. R., & Scott, D. A. (2011). *Tobacco use increases susceptibility to bacterial infection*. Tobacco Induced Diseases, 9(1), 1–10. https://doi.org/10.1186/1617-9625-9-12

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C. R., Yu, W. H., ... & Wade, W. G. (2010). *The human oral microbiome*. Journal of Bacteriology, 192(19), 5002–5017. https://doi.org/10.1128/JB.00542-10

Paster, B. J., Boches, S. K., Galvin, J. L., Ericson, R. E., Lau, C. N., Levanos, V. A., & Dewhirst, F. E. (2006). *Bacterial diversity in human subgingival plaque*. Journal of Bacteriology, 183(12), 3770–

3783. https://doi.org/10.1128/JB.183.12.3770-3783.2001

Shchipkova, A. Y., Nagaraja, H. N., & Kumar, P. S. (2010). *Subgingival microbial profiles of smokers with periodontitis*. Journal of Dental Research, 89(11), 1247–1253. https://doi.org/10.1177/0022034510377203

Shchipkova, A. Y., Nagaraja, H. N., & Kumar, P. S. (2010). *Subgingival microbial profiles of smokers with periodontitis*. Journal of Dental Research, 89(11), 1247–1253. https://doi.org/10.1177/0022034510377203

Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., & Kent Jr, R. L. (1998). *Microbial complexes in subgingival plaque*. Journal of Clinical Periodontology, 25(2), 134–144. https://doi.org/10.1111/j.1600-051X.1998.tb02419.x

Tomar, S. L., & Asma, S. (2000). *Smoking-attributable periodontitis in the United States: findings from NHANES III*. Journal of Periodontology, 71(5), 743–751. https://doi.org/10.1902/jop.2000.71.5.743

Wu, J., Peters, B. A., Dominianni, C., Zhang, Y., Pei, Z., Yang, L., ... & Ahn, J. (2016). *Cigarette smoking and the oral microbiome in a large study of American adults*. The ISME Journal, 10(10), 2435–2446. https://doi.org/10.1038/ismej.2016.37

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