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An Evaluation of Smoking Effect on the Normal Micro Flora of Oral Cavity

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Abstract:

Context: The microbial etiology of oral infections has for several decades been regarded as well established, reasonably consistent, and of limited interest**. Aim:** To study Evaluation of smoking effect on the normal micro flora on the oral cavity, Collection of information and data from the smoker consuming and non-smoker consuming form the different background people. Sample collection and isolation of Microflora from the oral cavity. Identification of bacteria, preparing the antibiotic susceptibility profile of oral pathogen of smoker users and non-smoker users**. Result:** A total of 100 throat swab sample were collected from different localities of Meerut city (28.9845°N, 77.7064°E and altitude 251 m (Latitude and Longitude of Meerut, 2020). The sample was collected from the oral cavity of the 50 smokers and 50 non-smokers with the standard protocols. A consent form will be filled by the sample donors which includes all the essential information regarding the research. Before sample collection first I mentioned the patient name, age, sex, address. Distribution of swab samples according to age group, smoker and non-smoker consuming. A total number of 100 swab sample out of which isolates of *staphylococcus aureus* were 51(47%), *micrococci spp.* 24 (24%), *bacillu spp* 13 (13%), *corny bacterium spp.* 16 (16 %). All isolates were screened for Antibiotic screening profile. The total number of 100 swab sample out of which 100 oralpathogen were screened for antibiotic screening profile (n=100). Distribution of isolated oral pathogens shown out of 100 patient samples, in which *staphylococcus aureus* were 47%, followed by *micrococci spp.*24%, *bacillu spp* 13%. & Corynebacterium *spp.* 16 %. All these isolates were identified by various bacteriological methods. Looking at the distribution of isolates oral pathogen of smoker and non- smoker, it was found that majority were isolated from smoker 63/100 (62.85%) and non- smoker 38/100(37.80%). Distribution of oral pathogen in smoker and non- smoker. 100 maximum isolates was seen in *Staphylococcus aureus* were 51 (47%), *micrococci spp.* 24 (24%), *bacillus spp.* 13 (13%), Corynebacterium *spp.* 16 (16 %).. distribution of isolated oral pathogen according to their sensitive and resistance pattern (n=100). This study showed that isolates in non- smoker group tetracycline was found most effective with the zone of

inhibition (ZOI) of 27±1.45mm against *S. aureus*, however, the most effective antibiotic against *Corynebacterium* was Ciprofloxacin with the ZOI of 25.00±0.15mm. In the smoker group, the zone of inhibition was ranged between 0.73±0.57-15.17±0.53mm, however, the same range for non-smokers are 13.73±30.80mm. For S. aureus Tetracycline was found most effective antibiotic with ZOI of 14.67±1.45mm. *Corynebacterium* was found resistant against cefotaxime and levofloxacin, however, ciprofloxacin was found most effective with ZOI of12.01±0.35mm however, ciprofloxacin was found most effective with ZOI of 12.01±0.35mm.**Conclusion**-Thepresent study indicated that despite the normal appearance of Gingival in smokers, cigarette smoke could change the structure of Human gingival mucosa in a dose-dependent manner. These changes may alter the virulence of bacteria and host-pathogen interactions and finally, contribute to the development of the oral disease. Although early studies based on traditional targeted molecular methods yield conflicting findings concerning the effects of smoking on sub gingival microflora associated with oral disease.

INTRODUCTION

Effect of smoking on the health of human health has become the topic of interest for the research workers throughout the world (Gupta & Kumar, 2018; Jiang et al., 2020; Millar & Locker, 2007;Saari et al., 2014; Sehgal & Tahir, 2016). At first, it was the relationship between smoking and lung diseases that excited interest, and particularly mouth cancer and chronic bronchitis, but more recently there has been concern about the possibility that smoking is in some measure responsible for the great increase in mortality from oral diseases noted in the last two to three decades (Manuscript, 2014).

Smoking is one of the most important public health problems in the world. Tobacco usage (Cigarette, cigar, pipe, hookah etc.) results in a very strong addiction syndrome. This syndrome reflects all basic features of addiction. Although the smoking rate is decreasing in developed countries, sadly, smoking is a common practice in developing countries (Sehgal & Tahir, 2016).The most important reasons for this situation are marketing strategies of international tobacco companies and lack of education in developing countries. It is a known fact that cigarette smoking causes adverse effects on the whole body (Pusterla et al., 2010). While struggling against smoking primary care physicians often neglect the effect of tobacco on oral health. This review aims to help primary care physicians to gain knowledge and improve their perspective on this topic.

The oral cavity is the initial portion of the digestive tract and it is surrounded by the lips, cheeks, palate, tongue and the mouth floor. The section between teeth, gums, lips and cheeks is called “vestibule ores”. “Caritas ores propriety” is the inner section surrounded by teeth and gums includes the tongue. The oral cavity is an important structure that hosts both soft and rigid surfaces washed by saliva and open to the external environment (Antolin et al., 2006). Smoking causes cancers, mucosal lesions and periodontal diseases in all regions of the oral cavity. It increase coronal and root caries. Smokers are notorious for large carries and missing teeth as well as bad breath (Gometz, 2011).

A Cigarette smoker, in addition to having a high spread is position to develop cancer of the respiratory and gastrointestinal epithelium, have tendencies to develop gingival infections, bronchitis, and pneumonia. These infections occur as are salt of the complex interaction of the host, bacteria, and the short- and long-term effects of cigarette smoke (Spell et al., 2014). There is a possibility that cigarette smoke may directly affect the normal flora of the mouth and thus permit a reselection of colonizing flora of species more resistant to smoke. The direct effects of smoke on the growth of many bacterial species isolated from humans are mostly unknown, although the effect of smoke on dental flora has been examined extensively (Dagli&Baroudi, 2016).It is important to determine these effects of cigarette smoke on bacterial species and to determine the contribution of this reselection of the particular species in the pathogenesis of bacterial infections in smokers (Ozturketal., 2017).

However, recent research has suggested that in the long term, it depresses the ability of the brain to experience a pleasure. So, smokers and chewers need greater amounts of the drug to achieve the same levels of satisfaction. Oral changes due to tobacco are 1) irritations of oral mucosa by toxins and carcinogens found naturally in tobacco 2) mucosal drying effects, 3) high intraoral temperature, 4) change in intraoral pH, 5) local alteration of membrane barriers 6) alteration in the immune response 7) altered resistance to fungal and viral (Sehgal & Tahir, 2016)

The primary object of the present investigation was to examine the association between smoking and oral disease by the co-twin control method. Since the connection between smoking and oral respiratory function is most probably causal, this association was examined chiefly to verify the effect of smoking on the series. Another object was to evaluate the significance of genetic factors in oral disease by applying the conventional twin method (Karuniawati et al., 2011).

Scope of the study

This study helps us to have a better understanding of the role of normal micro flora of the oral cavity in maintaining health. Smoking and other food habits directly encounter to the oral microflora of the individuals. This microflora creates competition with the pathogens and does not allow them to invade in our body.

Collection of Sample and Bacterial Isolates

A total of 100 isolates of bacterial culture were collected from different location of the nearby area of Chhatra Pati Shivaji Shubharti Hospital, Meerut. 50 smokers and 50 non-smoker individuals were selected for the study. The sample was collected according to their age, sex, food habits and profession (Table 7 and Table 8). According to the data, the maximum numbers of smokers belongto the age of 30-40 years age group (Figure 6) and in the profession; wise distribution data showedthe student's group carry the maximum strength of the smokers with the value of 27% (Figure 7). Followed that the health care workers have a remarkable number of smokers with the value of 17% (Figure 7).

Table7. Distribution of smokers and non-smokers among different sexes and age groups

Age	No of non smoker		No of smoker		No of alcoholic smoker	
	Male	Female	Male	Female	Male	Female
20-30	6	1	7	3	5	1
30-40	5	3	9	2	10	1
40-50	2	1	6	1	7	2
50-60	0	2	2	3	5	1
60-70	1	0	3	0	4	2
70-80	2	0	0	0	3	0

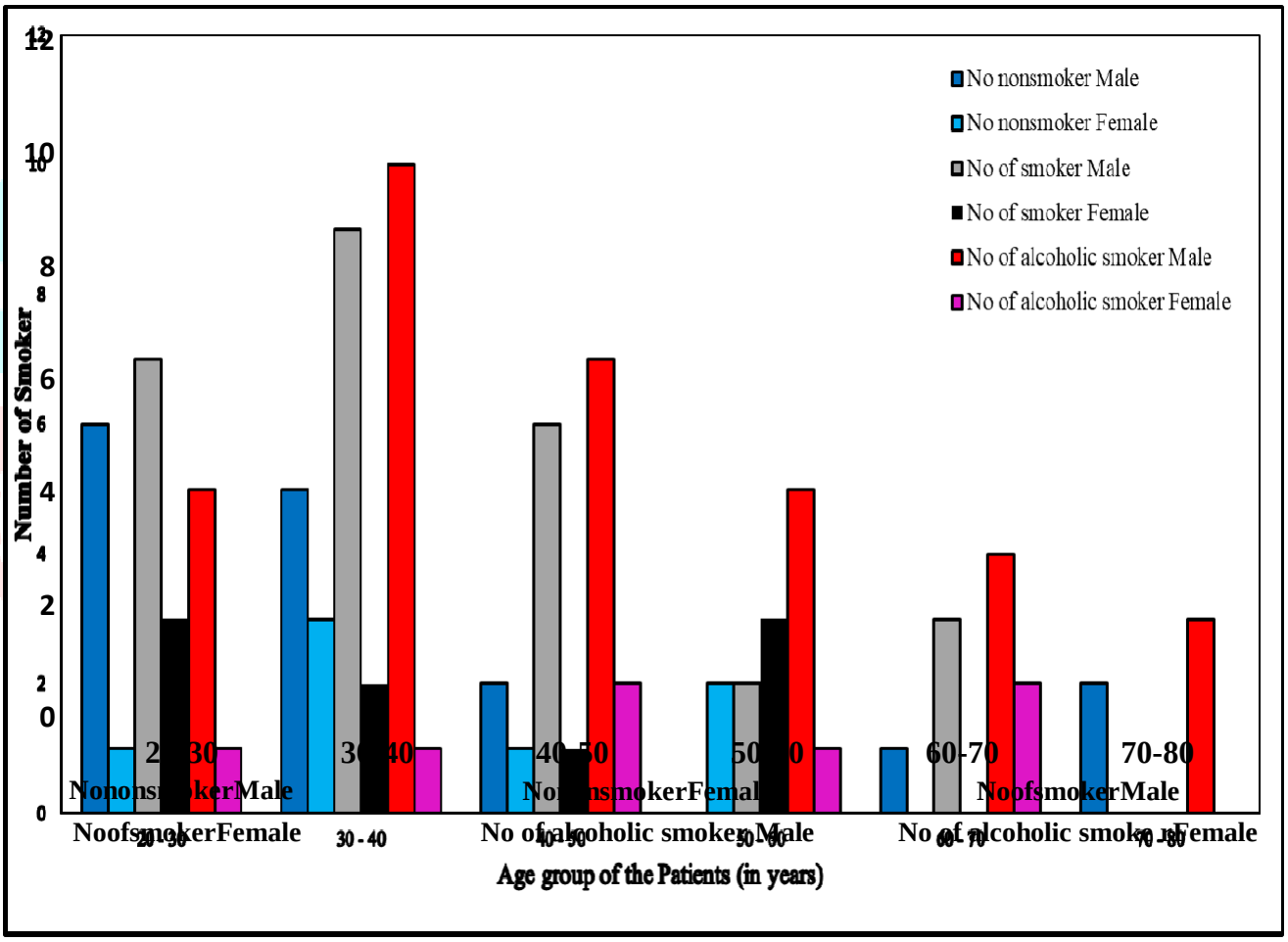

Figure6. Distribution of Smokers and non-smokers in different age groups.

Table8. Distribution of smokers and non smoker according to the professions

S. No	Category	No. of smoker	No. of non-smoker	Total no. of sample
1	Teaching staff	6	4	10
2	Student	16	14	30
3	HCWs	10	5	15
4	Hospital workers	6	4	10
5	Sweeper	7	5	12
6	Guards	11	5	16
7	Shopkeepers	3	4	7

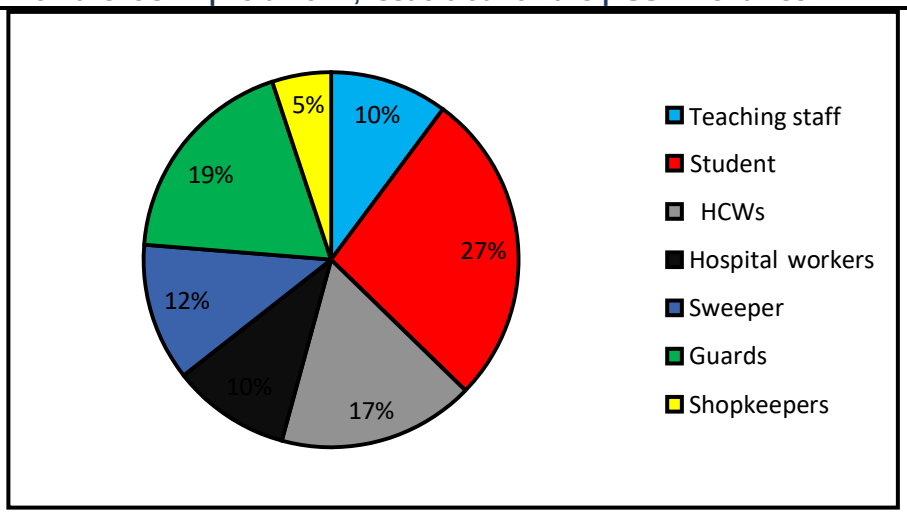

Figure7. Distribution of smokers and nonsmoker in different professions

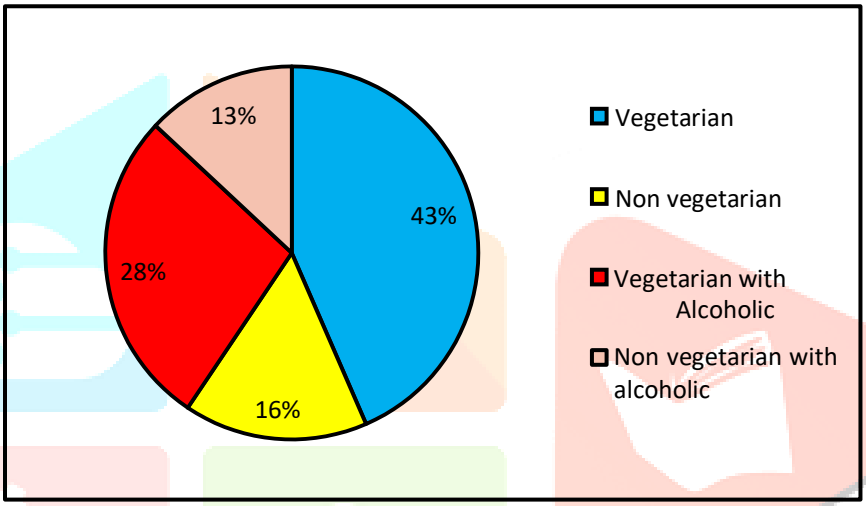

Figure.8 Distribution of Smokers in different food habits of people

Table.9. Distribution of smokers and non smokers among different food habits of Peoples

Category	Smokers	No. of non smoker	Total
Vegetarian	30	18	48
Non vegetarian	11	4	15
Vegetarian With alcoholic	19	6	25
Non vegetarian with alcoholic	9	3	12

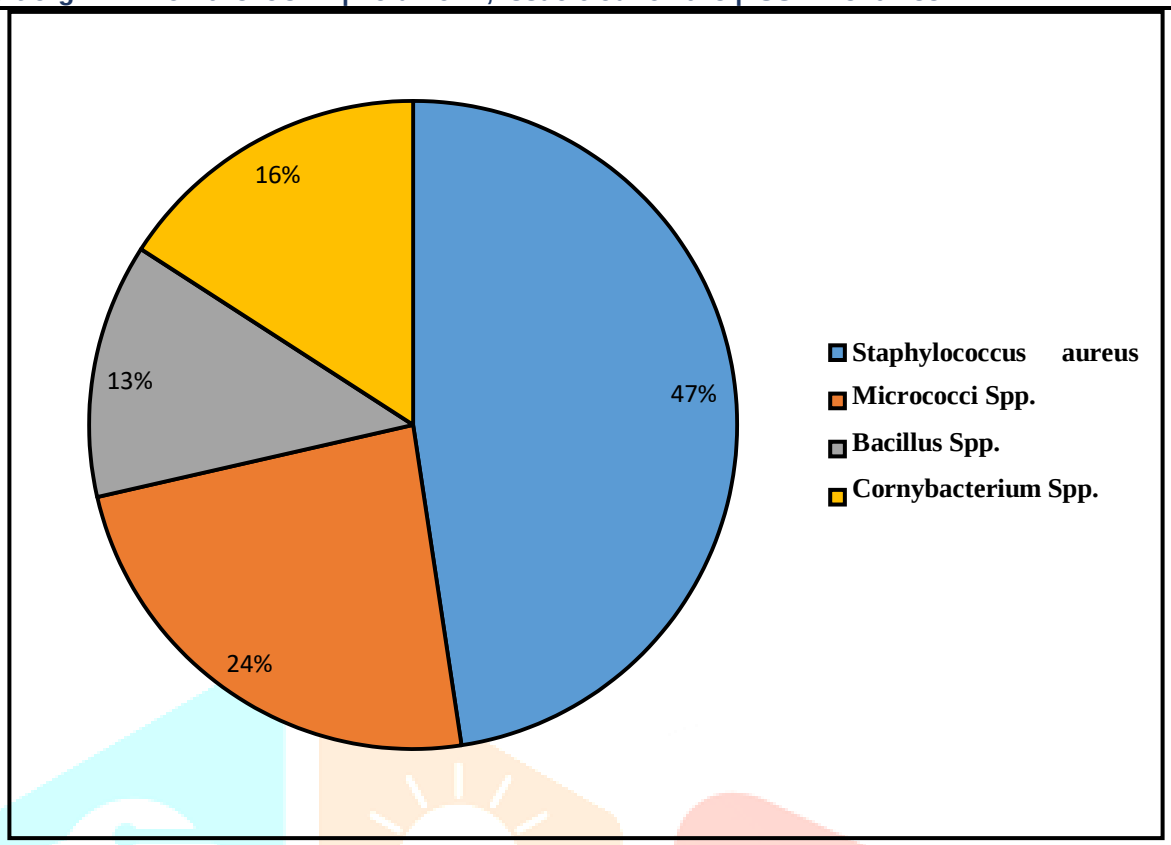

Figure.13. Distribution of different microorganisms among the oral microflora of smokers and non-smokers

Table.10. Distribution of different microorganism among the oral microflora of smoker and nonsmoker

Name of the organism	Smoker	Non-smoker
Staphylococcus aureus	30	21
Micrococci spp.	15	08
Bacillus spp.	08	05
Cornybacterium spp.	10	04

MORPHOLOGY:

Based on colony morphology, color and shape different isolates were selected from the mother culture late. The pure culture was obtained by the streak plate method. The pure colonies appeared small circular in shape, elevation flat, slightly raised or markedly raised sometimes the colonies were pigmented and appear a pale yellow or golden yellow. The size of the 24 hours mature colonies was ~0.5-1cm (Figure 9 (A) and (B).

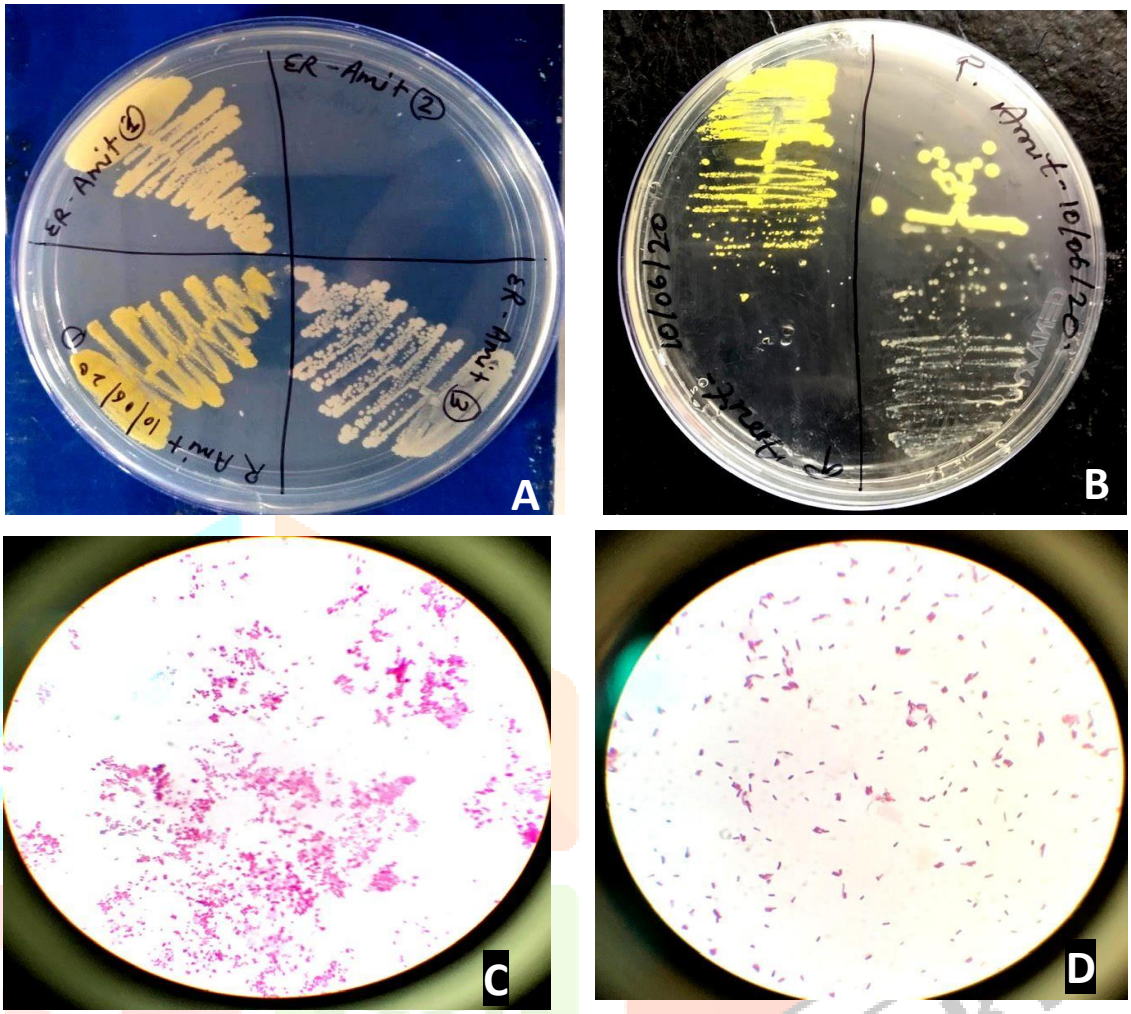

Figure .9. Colony morphology and microscopic morphology of the bacterial isolates: A and B showing the different colour and shape of the bacterial colonies on the nutrient agar plates, C and D are the gram's stain bacterial cells under the compound microscope at100X magnification.

Bio-Chemical Characterization

Bacteria identification was carried out by Gram stain, morphology and biochemical reaction to specific media to obtain pure isolates. Biochemical tests were carried out using the motility test, catalase production, Coagulase test, Voges Proskauer **(**V.P), indole production, citrate utilization, and Sugar test (Table11).

Gram's staining: All the isolates were found grams positive. All the isolates showed the coccoid shape with a bunch like an arrangement or free cells except ER15 and ER65. These two isolates were large rod-shaped cells with endospores. Isolate ER28 showed Chinese letter like or V-

shaped road like structure, therefore tentatively identified as *Corynebacterium* sp. (Figure 9 (C) and (D))

Catalase Test:

Catalase Test is a basic test to differentiate between *Staphylococci* and *Streptococci*. *Staphylococcus* sp., *Corynebacterium* sp. and *Bacillus* sp. All showed positive results for the catalase tests (Figure 10).

Coagulase Test:

Isolates ER5, ER8, ER10, ER36, and ER45 all were tentatively identified as *S. aureus and* supportingto the result, it was found negative for coagulase test. In the same way, the isolates ER9 and ER55 were also coagulase-negative, hence designated as *Enterococcus* sp. *Bacillus* isolates ER15 and ER65, as well as *Corynebacterium*, isolates ER28 also showed the coagulase-negative results Figure 11).

Voges ProskauerTest: All the isolates showed positive results for the V.P. test.

Sugar Fermentation test: All the isolates showed positive results for Maltose and Dextrose fermentation. Isolates of *Micrococcus* and *Corynebacterium ER9*, ER55 and ER 28 were unable to ferment the sugar. The details of the other sugars are fermentation test is shown in table 11,(Figure 13.B)

NEGATIVE CATALASE

Figure.10. Catalase test: Catalase positive bacteria producing bubbles with H_2O_2

Figures.11. Coagulase test: Coagulase positive bacteria 'B 'cause coagulation of the blood serum, however in tube 'A' no coagulation was shown

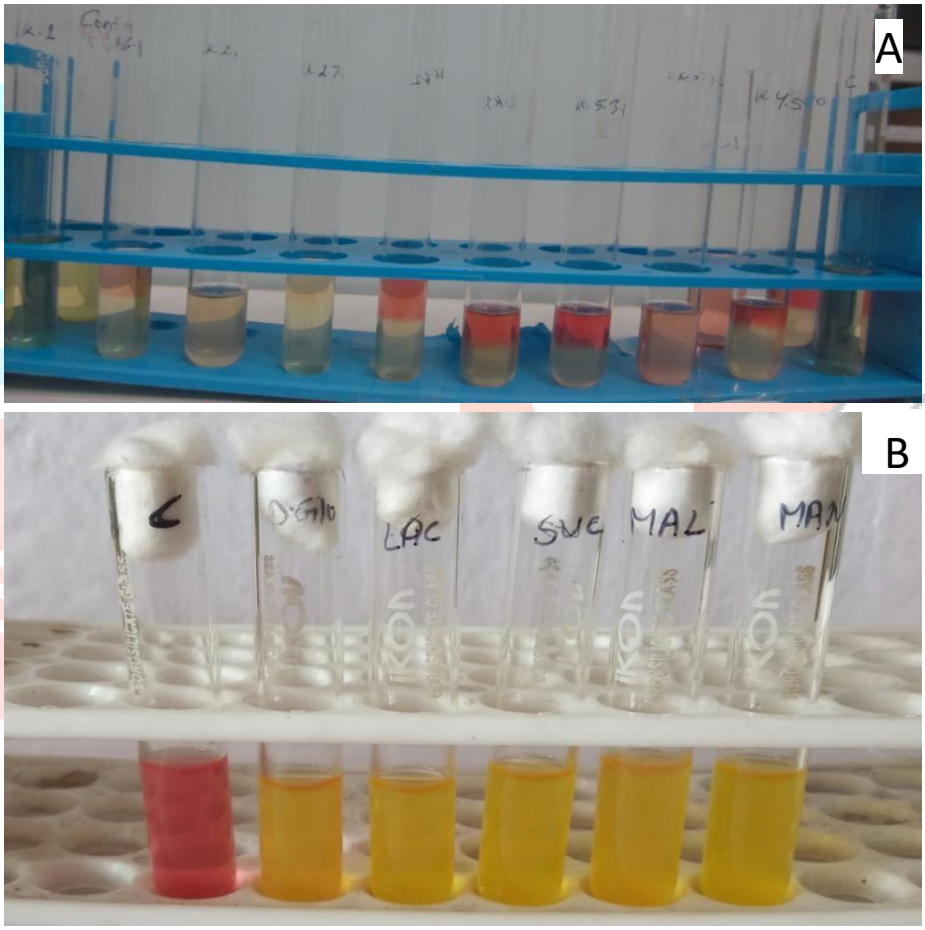

Figure.12. Biochemical test: (A) Result of Voges Proskauer test of cultures where red color formation showed the VP positive however no color formation showed the VP negative. (B) Sugar fermentation by different bacterial isolates. The red color indicates the negative control and yellow color indicates the sugar fermentation.

TableNo.11. Representing the biochemical and physiological characteristics of the producing bacterial isolates

	Microscopy			Sugar Fermentation Test					Growth at/in			
Bacterial isolates	Gram's reaction	Endospore	Motility	Dextrose	Sucrose	Maltose	Mannitol	Lactose	37^0C	7%NaCl	V.P	TentativeIdentification
ER5	+	-	-	+	+	+	+	+	+	+	+	*S. aureus*
ER8	+	-	-	+	+	+	-	-	+	+	+	*S. aureus*
ER9	+	+	-	±	-	±	-	-	+	+	±	*Micrococci* Spp.
ER10	+	-	-	+	+	+	+	+	+	+	+	*S. aureus*
ER15	+	+	+	+	+	+	+	-	+	+	+	*Bacillus* Spp.
ER28	+	+	-	+	-	+	-	-	+	+	+	*Cornybacterium*
ER36	+	-	-	+	+	+	+	+	+	+	+	*S. aureus*
ER45	+	-	-	+	+	+	+	+	+	+	+	*S. aureus*
ER55	+	+	-	±	-	±	-	-	+	+	±	*Micrococci* Spp.
ER65	+	+	+	+	+	+	+	-	+	+	+	*Bacillus* Spp.

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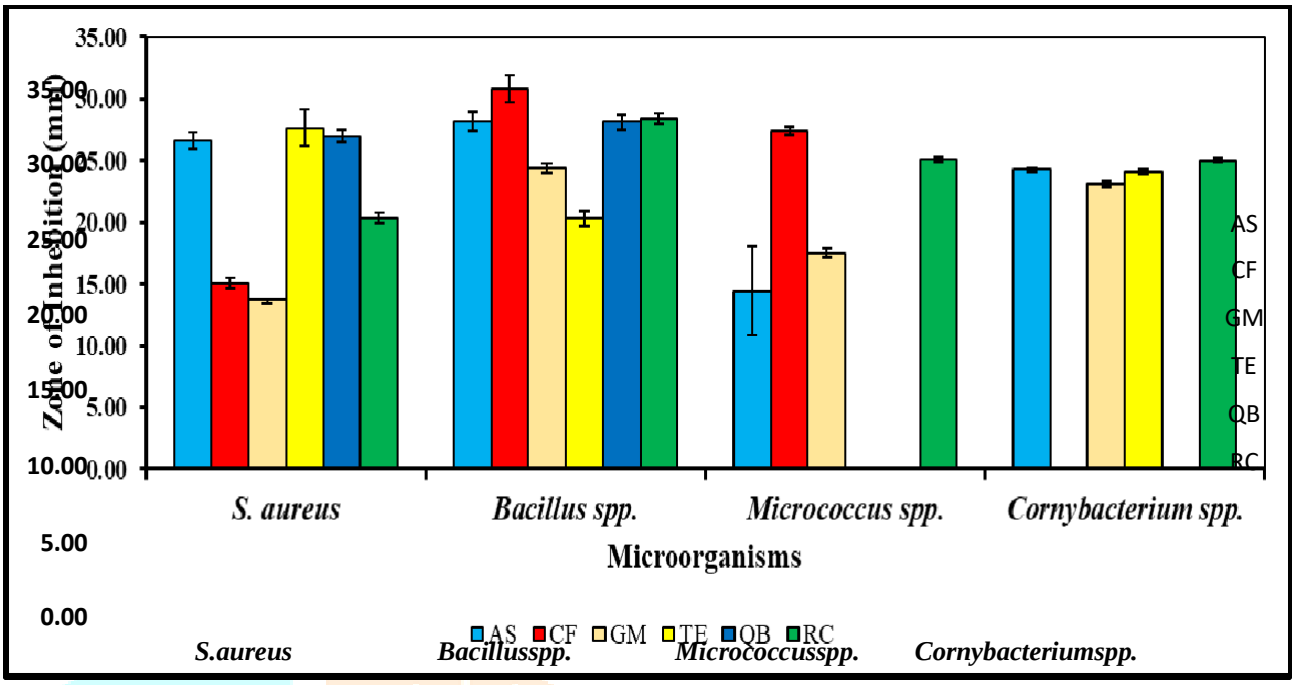

Figure15. Mean zone of inhibitions of different antibiotics against different bacteria of oral microflora of non-smoker

Table12. Antimicrobial profile of different bacteria of oral micro flora of Non-smoker Individual

Antibiotics	***S. aureus***	***Bacillus spp.***	***Micrococcus spp.***	***Corynebacterium spp.***
Ampicillin	26.66±0.65	28.16±0.73	14.41±3.58	24.26±0.15
Cefotaxime	15.1±0.44	30.80±1.06	27.45±0.33	-
Gentamicin	13.73±0.37	24.41±0.46	17.5±0.29	23.08±0.22
Tetracycline	27.66±1.45	20.33±0.60	-	24.08±0.22
Levofloxacin	27±0.5	28.11±0.59	-	-
Ciprofloxacin	20.36±0.45	28.36±0.45	25.08±0.22	25.00±0.15
Here'-' indicates the resistant isolate or nor zone of inhibition				

Table13. Antimicrobial profile of different bacteria of oral micro flora of smoker Individual

Antibiotics	***S. aureus***	***Bacillus spp.***	***Micrococcus spp.***	***Corynebacterium spp.***
Ampicillin	13.67±0.45	15.17±0.53	1.42±0.58	11.27±0.15
Cefotaxime	-	-	-	-
Gentamicin	0.73±0.57	11.42±0.46	4.50±0.29	10.08±0.22
Tetracycline	14.67±1.45	7.33±0.60	-	11.08±0.22
Levofloxacin	-	15.12±.59	-	-
Ciprofloxacin	7.37±0.45	-	12.08±0.42	12.01±0.35
Here'-' indicates the resistant isolate or nor zone of inhibition				

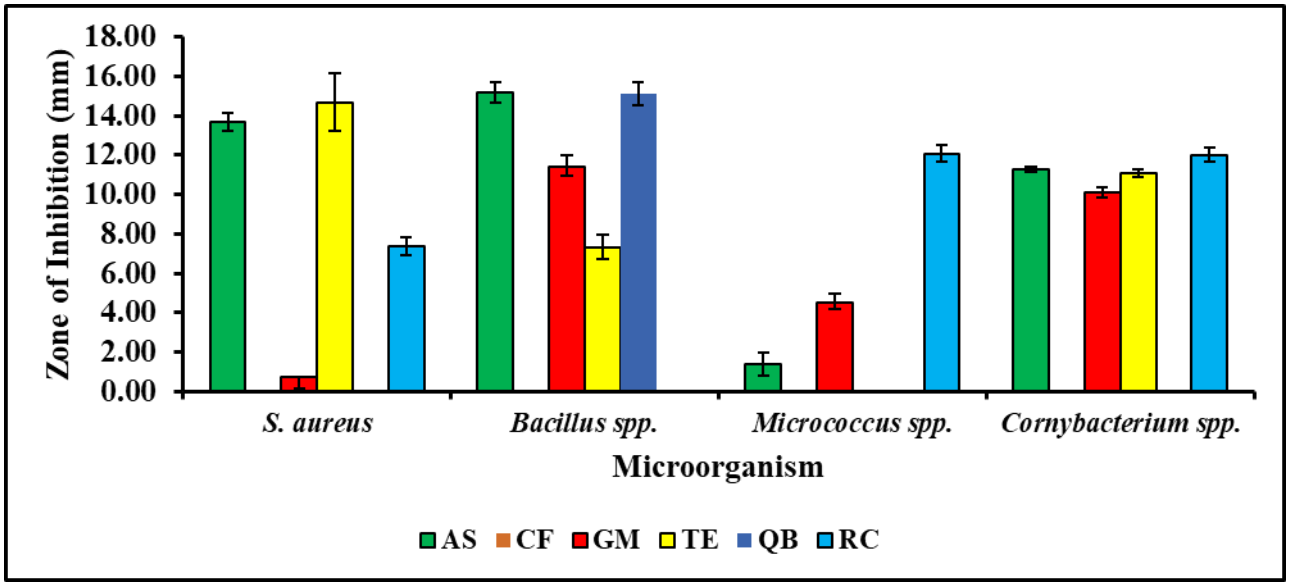

Figure.16. Mean zone of inhibition of different bacterial isolates from smoker group against different antibiotics

Antibiotic Susceptibility Testing:

Antibiotic susceptibility test of the test organisms was performed by Kirby- Bauer disk diffusion method in compliance with Clinical and Laboratory Standards Institute (CLSI 2020) guidelines using Mueller-Hinton Agar Standard Media.

Among all the isolates tetracycline was found most effective with the zone of inhibition (ZOI) of 27±1.45mm against S. aureus. In the same way *Bacillus* isolates also showed sensitivity against all the antibiotics used in the present study. *Micrococcus* sp. showed resistance against tetracycline and levofloxacin. In the same way, *Corynebacterium* sp. was resistant against cefotaxime and levofloxacin, however, the most effective antibiotic against *Corynebacterium* was Ciprofloxacin with the ZOI of 25.00±0.15mm (Table 13, figure 15).

In the smoker group, the zone of inhibition was ranged between 0.73±0.57-15.17±0.53 mm, however, the same range for non-smokers are 13.73±30.80mm. It means normal microflora of smoker's gut carries more resistant microflora than the non-smokers. For S. aureus Tetracycline was found most effective antibiotic with ZOI of 14.67±1.45mm. Cefotaxime was found resistant for all the organisms. For *Bacillus* isolates ciprofloxacin was found non-effective, however, Ampicillin and Levofloxacin was found more effective. *Micrococcus* was found resistant against cefotaxime, tetracycline and levofloxacin. *Corynebacterium* was found resistant against cefotaxime and levofloxacin, however, ciprofloxacin was found most effective with ZOI of 12.01±0.35mm (Table 14, figure 16).

Conclusion- The present study indicated that despite the normal appearance of Gingival in smokers, cigarette smoke could change the structure of Human gingival mucosa in a dose-dependent manner. These changes may alter the virulence of bacteria and host-pathogen interactions and finally, contribute to the development of the oral disease. Although early studies based on traditional targeted molecular

methods yield conflicting findings concerning the effects of smoking on sub gingival micro flora associated with oral disease.

Discussion

This study was aimed to determine the prevalence of smoker and non-smoker at Chhatrapati Sivaji Subharti Hospital in Meerut and to determine antimicrobial resistance of *S. aureus* by disc diffusion method. The overall prevalence of *S. aureus* colonization among Smoker was 75. The detection of *S. aureus* isolates was performed by antibiotic sensitivity testing against Cefotaxcitin disc (30 mcg), Ampicillin disc, (20 mcg), Levofloxacin (5 mcg), Ciprofloxacin (5 mcg.) Gentamicin (10 mcg) by Kirby Bauer disc diffusion method on Muller Hinton agar plate, with 16-18 hours incubation at 37ºC according to latest CLSI guidelines (CLSI 2019). A total of 100 people were included in the study. In this study out of 100 person cases, 50 patients showed significant bacterial growth making an overall prevalence of 7.3%. It was found that the most common etiological agent causing oral infection was *Staphylococcus aureus* (47%) *Micrococcus* spp. (24%), *Bacillus* spp. (13%) and *Corynebacterium* (16%). The incidence of oral cavity infection varies among different studies. The present studies show that the incidence rate is very high of *Staphylococcus aureus* 47%. in the present study, the incidence of smoker and nonsmoker to age shows that age group shows that age groups between 19 to 25 years give 38%, in addition, age groups among 25 to 35 years gives 38% and age groups stuck between 35 to 60 shows 24% prevalence. In addition, age groups among 25 to 35 years gives 38% and age groups stuck between 35 to 60 shows 24% prevalence My research showed a significantly higher number of bacteria in swab samples Incubated for 12 hours at 37ºC from smokers and as compared to non–smoker users (P = 0.005). So, during its initial growth period (12 hours), the reproduction of bacteria exhibited a significant difference by producing higher bacterial growth in smokers and tobacco chewers compared to non–tobacco users. This result supported my hypothesis, that cigarette consumers contained more bacterial growth in their oral cavity compared to non–smoker users, because both smokers and -smoker exhibited a high amount of bacterial growth.

Limitations

Molecular detection of genes responsible for causing oral infection could not be done due to limited resources.

Acknowledgement

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