

Effect of Smoke on Normal Human Mouth Microflora

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Abstract: *Cigarette smoking is a public health problem. It decreases the commensal population of normal flora in the oral cavity leading to an increase of pathogenic microbes. It causes oral cancer, periodontitis, colour change on the teeth, halitosis and other health implications. The study was designed to determine the changes caused by tobacco smoking on the microbial profile and oral health conditions of cigarette smokers. One hundred and twenty subjects comprising 60 tobacco smokers and 60 non smokers were enrolled for the study. Oral swabs were collected from the oral cavity of the subjects using sterile swab sticks under standard aseptic methods. The specimens were subjected to microscopy and culture. Organisms were identified using standard microbiological techniques. Higher rates of microbes 86.7% were recovered from the oral cavity of smokers than non smokers 33.3%. There was a statistically significant effect of tobacco smoke on the oral flora of smokers ($\chi^2 = 299.0$, $P = 0.0002$). *Staphylococcus aureus* 13(25.0%) and *Klebsiella pneumoniae* 10(19.2%) were more prevalent among smokers, while *Klebsiella pneumoniae* 4(20.0%) and *Pseudomonas aeruginosa* 4(20.0%) were the most prevalent bacterial isolates among the control subjects. Smokers had a diverse microbial colonization than non smokers. Smoking may have altered bacterial acquisition and oral mucosal colonization in favor of periodontal pathogens. The campaign against smoking should therefore be intensified as this may help to improve the oral health conditions of smokers. The oral microbiota has been observed to be influenced by cigarette smoking and linked to several human diseases. However, research on the effect of cigarette smoking on the oral microbiota has not been systematically conducted in the Chinese population.*

Keywords: Oral Microbiota, Cigarette Smoking, 16S rRNA Gene Sequencing, China, Saliva

I. INTRODUCTION

The human oral cavity is inhabited by over 600 bacterial species, known collectively as the oral microbiome, these bacteria are involved in a wide variety of functions, and many are important in maintaining oral health. Oral dysbiosis leads locally to periodontitis, dental caries and potentially to head and neck cancer (Wade, 2013; He et al., 2015)[1].[2] There is also increasing evidence of a role for oral dysbiosis in systemic diseases of the lung, digestive tract and cardiovascular system, yet factors that influence the oral microbiome are poorly understood. Cigarette smoke is a source of numerous toxicants (WHO, 2012) that come into direct contact with oral bacteria; these toxicants can perturb the microbial ecology of the mouth via antibiotic effects, oxygen deprivation or other potential mechanisms[3]. Loss of beneficial oral species due to smoking can lead to pathogen colonization and ultimately to disease; this contention is strongly supported by the well-established role of smoking in the onset and progression of periodontitis. Previous studies have shown alterations in the abundance of selected oral bacteria in smokers compared with non-smokers, however, results across these studies are largely inconsistent, possibly due to small sample sizes in some, use of different sampling sites in the mouth and use of different laboratory methodologies, some of which impose limitations on bacterial profiling.

To improve our understanding of the influence of smoking on the oral microbiome, we conducted a comprehensive assessment of oral microbiome community composition and individual taxon abundance, by

bacterial 16S rRNA gene sequencing, in 1204 individuals from two large US national cohorts[3]. Strengths of our study include the availability of detailed data from both cohorts on individual smoking history and potential demographic confounding factors. In addition, the large sample size available from each cohort provided us with excellent statistical power for discovery in combined analyses, as well as the opportunity to independently replicate results in each cohort[4].



II. MATERIALS AND METHODS

The basic components of most cigarettes are tobacco, chemical additives, a filter, and paper wrapping. The user burns the tobacco and inhales the smoke. Smokers are exposed to a toxic mix of over 7,000 chemicals, including more than 70 that can cause cancer, when they inhale cigarette smoke

2.1. Preparation of Agar Plate

Agar plates are the standard solid support material for growing microorganisms. Microbial growth media contains nutrients and an energy source to fuel the microbes as they grow, and agar to keep the media in a semi-solid, gel-like state. Nutrient Agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

Composition of Nutrient Agar

0.5% peptone – this provides organic nitrogen.

0.3% beef extract/yeast extract – the water-soluble content of these contribute vitamins, carbohydrates

Nitrogen

0.5%NaCl

5% agar .

Preparation of Nutrient Agar

1. Suspend 28 g of nutrient agar powder in 1 litre of distilled water.
2. Heat this mixture while stirring to fully dissolve all components.
3. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.
5. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.
6. Replace the lid of each Petri dish and store the plates in a refrigerator.

2.2. Agar Well Diffusion Method

A cork borer is also used to punch holes on an agar plate and to perform well diffusion assays in microbiology to study bioactivity. Agar well diffusion method is widely used to evaluate the antimicrobial activity of microbial extracts .Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 μ L) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested .



2.3 Preparation

1. Half the cigarette was burned and the Ash was collected in the dilution tube containing saline.
2. Half the cigarette was dipped in the dilution tube containing methanol
3. The remaining part was kept in the dilution tube containing saline .

2.4 Sample Loading

Sample A and Sample B are the samples taken from two different humans from their mouth with the help of sterile cotton swab. Using quadrant method, wells were made in the nutrient agar plate with sterile cork borer. The quadrant were numbered and the samples were loaded in the wells precisely. After loading the samples we incubated the petri dishes containing samples in the incubator at 37°C for 24 hours.

III. RESULT

After incubation the petri dishes were taken out from the incubator and we see that only methanol mixed with the tobacco sample had the inhibition zone around the well other two zones were not seen.



IV. CONCLUSION

We conclude that Ash and tobacco in the cigarette doesn't have any antimicrobial property which affects the normal human mouth microflora.

REFERENCES

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