

## Evaluation of Bacterial Population in the Saliva of Drug Addicts

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### Abstract

**Background:** The human mouth harbors over 700 microbial genera. The large number of them is normal bacteria; few of them are opportunistic pathogens.

**Objective:** The study was aimed to evaluate the bacterial population of drug addicted persons and compare it to that of a normal healthy person.

**Methods:** Five different samples of human saliva; alcohol drunken person, oral moist snuff user, cigarette, hashish and shisha smoker were investigated the involvement of bacteria in them. One sample was collected from normal non addict person. The bacterial population in drug addictive persons were compared that on normal non addictive person.

**Results:** The bacteria observed were: Gram negative cocci, mono cocci, streptococci, vibrio, gram negative rod, gram positive staphylococcus, gram positive streptococci and gram positive rod.

**Conclusion:** The order of bacterial population was; alcohol drunken person < oral moist snuff user < cigarette smoker < hashish smoker < shisha smoker < normal non addictive person. From the findings in this study it is concluded that all these drugs have some antibacterial activities.

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## Background

Saliva is a watery fluid, which contains about 99 percent water. A large difference is present in the concentration of dissolved organic and inorganic solid in the saliva of two individuals as well as in the single individual. Human Saliva performs many biologic functions that are very important in maintaining oral health [1].

The human mouth harbors over 700 microbial genera [2, 3]. The large number of them is normal bacteria; few of them are opportunistic pathogens, which are important for the growth of mouth microbial contagious diseases for example as dental caries and periodontitis [4]. These salivary bacteria reproduce the oral micro biota composition and can serve as a sign of the health and disease condition of oral cavity. For example, the bacteria present in the saliva counts have mostly been used for caries risk assessment [5-7].

There are nearly ten times as many bacterial cells in the human flora as there are human cells in the body. A number of them are present on the skin and as gut flora [8]. The human mouth provides an ideal environment for the existence and growth of bacteria. It provides a source of water and nutrients, as well as an average temperature [9]. Resident bacteria of the saliva adhere to teeth and gums to resist mechanical flushing from the mouth to stomach where they are destroyed by HCl [10].

The Saliva of a healthy man contains the bacteria like Streptococcus, Prevotella and Veillonella. Among them Streptococcus is the main species that accounted for 7 different species. Among the seven Streptococcus species, *S. salivarius* appeared as the most common species. Streptococcus and Prevotella are present in saliva of human from the ages of 32 to 35. While the saliva of those human whose ages are from five to sixty five containing the genera Rothia. Saliva of young children and older human shows higher bacterial diversity than that of young adults [11]. Anaerobic bacteria present in the oral cavity are Actinomyces, Arachnia, Bacteroides, Bifidobacterium, Eubacterium, Fusobacterium, Lactobacillus, Leptotrichia, Peptococcus, Peptostreptococcus, Propionibacterium, Selenomonas, Treponema, and Veillonella [11].

Streptococcus faecal is present in saliva is gram-positive cocci, while Eikenella and enterobacteriaceae are gram-negative bacilli, actinomyces and lactobacillus are gram-positive bacilli, veillonella is gram-negative cocci. Saliva also contain streptococcus (spirochete) [12].

The aim of this study was evaluate the bacterial population of drug addicted persons and compare it to that of a normal healthy person.

## Methods

All experiments were carried out according to the Scientific Procedures Issue-1 of Animal Bylaws-2008 approved by the legal bodies of the University of Malakand Khyber Pakhtoonkhwa, Pakistan. The Ethical Committee of the Department of Biochemistry granted approval for conducting this study under the said protocols (Procedures Issue-1 of Animal Bylaws-2008). Approval of the collection procedure was taken from University Review Board for the protection of human research prior from starting collection of saliva samples from volunteers. The sample of human saliva used in this study were collected from volunteers using different addictive substances like wine, oral moist snuff, hashish, shisha and cigarette. Five samples were collected from normal non addict persons (control) also. A total of thirty different saliva samples were evaluated for the involvement of bacteria present in them. The samples were categorized into six groups. In each category there were five volunteers of nearly same age with difference of  $\pm 6$  months. The samples were collected in sterilized glass vials and collection was made between 10 to 11 O'clock and inoculation to agar plates were made within two hours. The amount of alcohol etc in the saliva samples were not determined at the time of experiments. The details of which are given as follow:

- The saliva of wine drunken.
- The saliva of oral moist snuff user.
- The saliva of hashish smoker.
- The saliva of cigarette smoker.
- The saliva of shisha smoker.
- The saliva of normal, non-addict person (control).

The saliva samples of each category volunteers were mixed and inoculated to sterilized nutrient agar

plates and incubated for 24 hour in incubator at 37°C. Different colonies of bacteria were observed visually and were identified by using Gram staining. The bacterial populations in the selected samples were determined using hemocytometer.

## Results

Bacterial strains present in selected mixed saliva samples were observed under microscope. The determinations of the bacterial strain were done from their shapes while population from their number in hemocytometer. The various bacteria identified in the collected saliva samples are discussed as follow:

### *Types of Bacteria Present in the Saliva Sample of a Drunken Person*

Gram negative cocci, mono cocci, streptococci, vibrio, gram negative rod, gram positive staphylococcus, gram positive streptococci and gram positive rod were observed in the selected sample. The most abundant types of bacterial species were; gram positive cocci, gram negative cocci and gram positive streptococci.

It was noticed that the population of bacteria were minimum in the saliva sample of a drunken person as compared to the saliva sample of the person who did not used any addictive substances. The decreasing order of bacterial population in saliva of drug addicts was: Drunken person < oral moist snuff user < cigarette smoker < hashish smoker < shisha smoker. (Fig 1)

### *Types of Bacteria Present in the Saliva Sample of Oral Moist Snuff User*

Gram positive streptococci, gram positive rod, gram positive staphylococcus, gram negative cocci, monococci, streptococci, vibrio and gram negative rod observed in saliva sample of oral moist snuff user. However, the most abundant types of bacteria were gram positive cocci, gram negative cocci and gram positive rods.

It was noticed that the population of bacteria were lowered in the saliva sample of a person who used oral moist snuff from the saliva sample of that person who did not used any addictive substances. The decreasing order of bacterial population in saliva of addicts was: oral moist snuff user < cigarette smoker < hashish smoker < shisha smoker. But the population was greater from the population of drunken

person. (Fig 2)

### *Types of Bacteria Present in the Saliva Sample Hashish Smoker*

Gram positive staphylococcus, gram positive rod, streptococci, vibrio, gram negative rod, mono cocci and gram positive streptococci were observed in hashish smoker saliva. However, the most abundant types of bacteria were gram negative cocci.

It was noticed that the population of bacteria were lowered in the saliva sample of a person who smoked hashish from the saliva sample of that person who did not used any addictive substances. The decreasing order of bacterial population in saliva of addicts was: Hashish smoker < cigarette smoker < shisha smoker. While the increasing order of bacterial population in saliva of hashish smoker was: hashish smoker > oral moist snuff users > alcohol drunken person. (Fig 3)

### *Types of Bacteria Present in the Saliva of Cigarette Smoker*

Gram negative cocci, monococci, streptococci, vibrio, gram negative rod, gram positive staphylococcus, gram positive streptococci and gram positive rod were observed in the saliva sample of cigarette smoker. However the most abundant types of bacteria were gram negative cocci and gram negative rods.

It was noticed that the population of bacteria were lower in the saliva sample of a person who smoked cigarette from the saliva sample of that person who did not used any addictive substances and also from that person who smoked shisha. But the population of bacteria was high from the saliva sample taken from that alcohol drunken person, oral moist snuff user and hashish smoker. (Fig 4)

### *Types of Bacteria Present in the Saliva of Shisha Smoker*

Gram positive staphylococcus, gram positive rod, streptococci, vibrio, gram negative rod, mono cocci and gram positive streptococci were observed in the saliva of shisha smoker. However the most abundant types of bacteria were gram negative cocci.

It was noticed that the population of bacteria were lower in the saliva sample of a person who smoked shisha from the saliva sample of that person who did not used any addictive substances. But the





Figure 1: Alcoholic person saliva slide

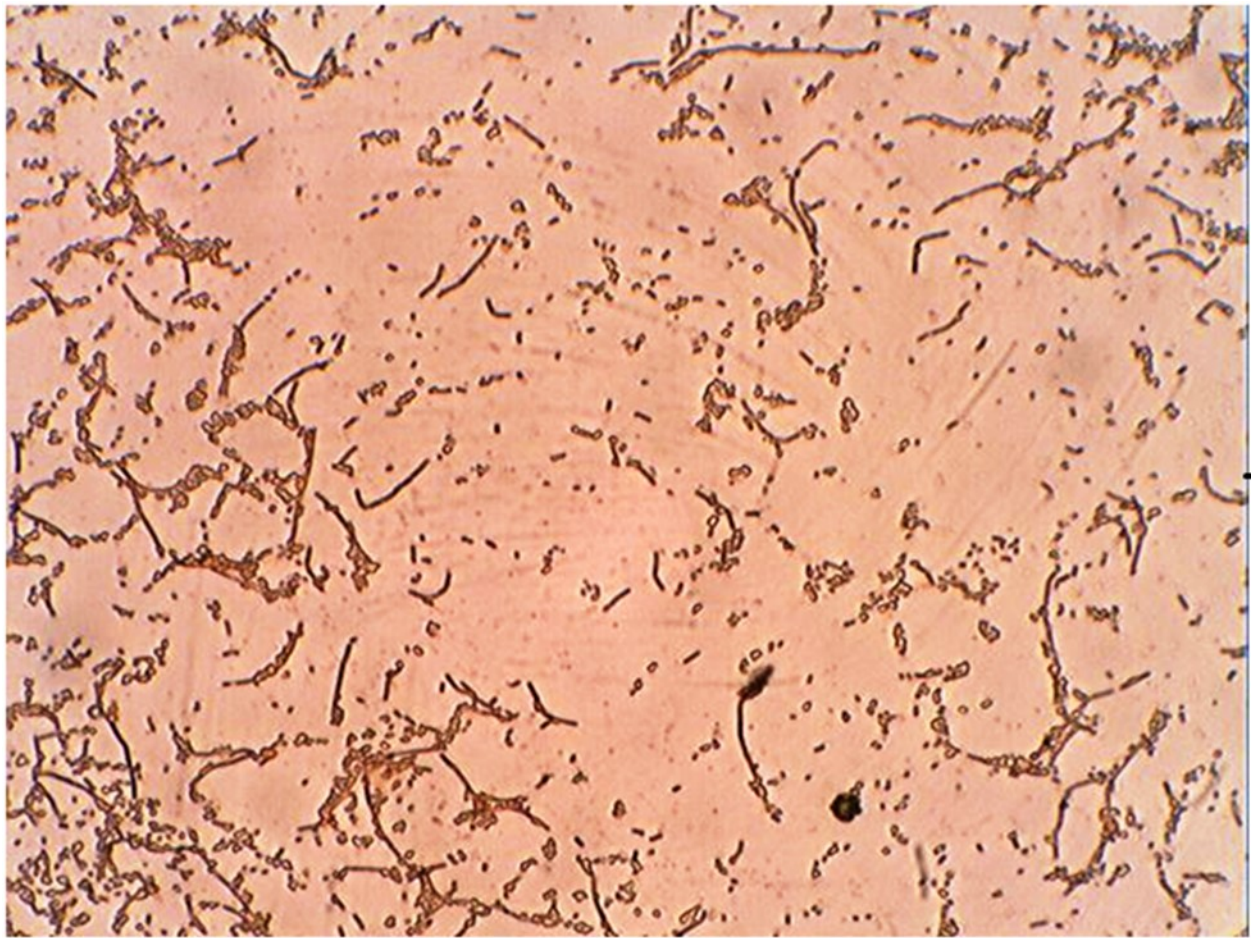


Figure 2: Oral moist snuff user saliva slide



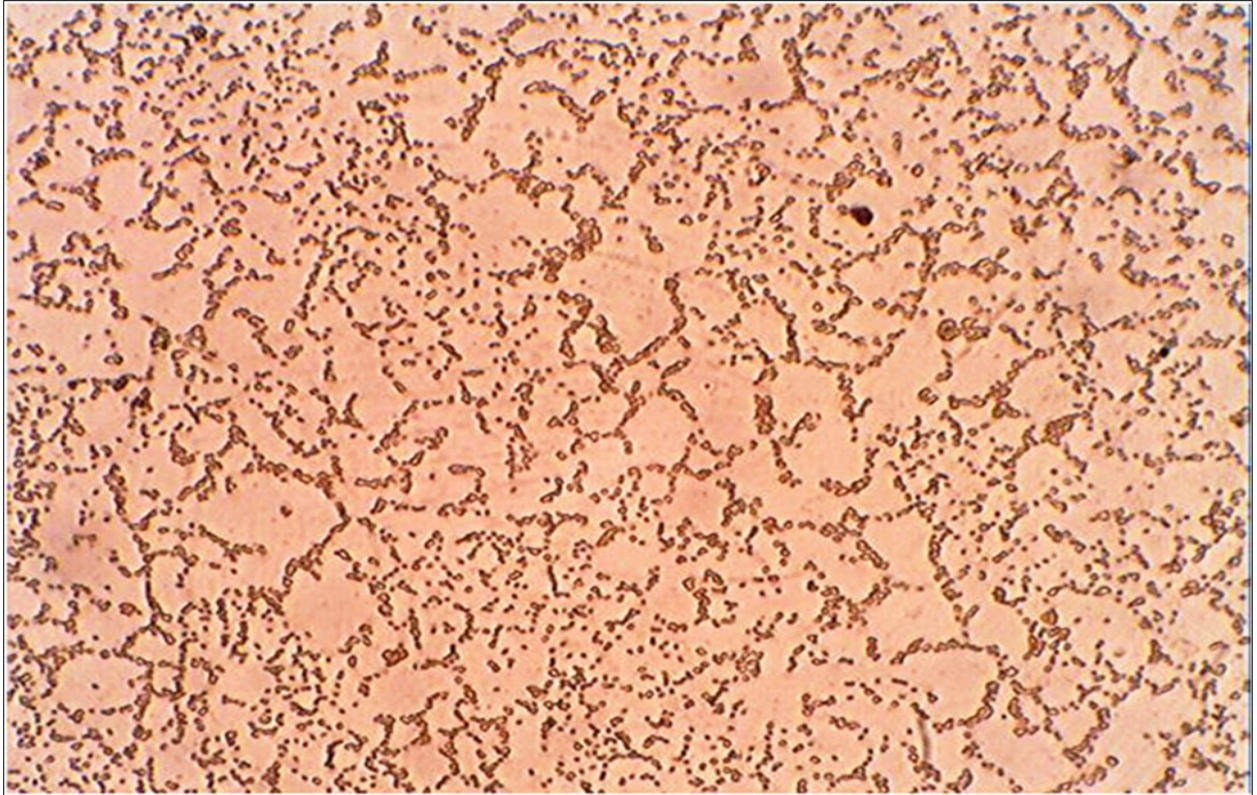


Figure 3: Hashish smoker saliva slide

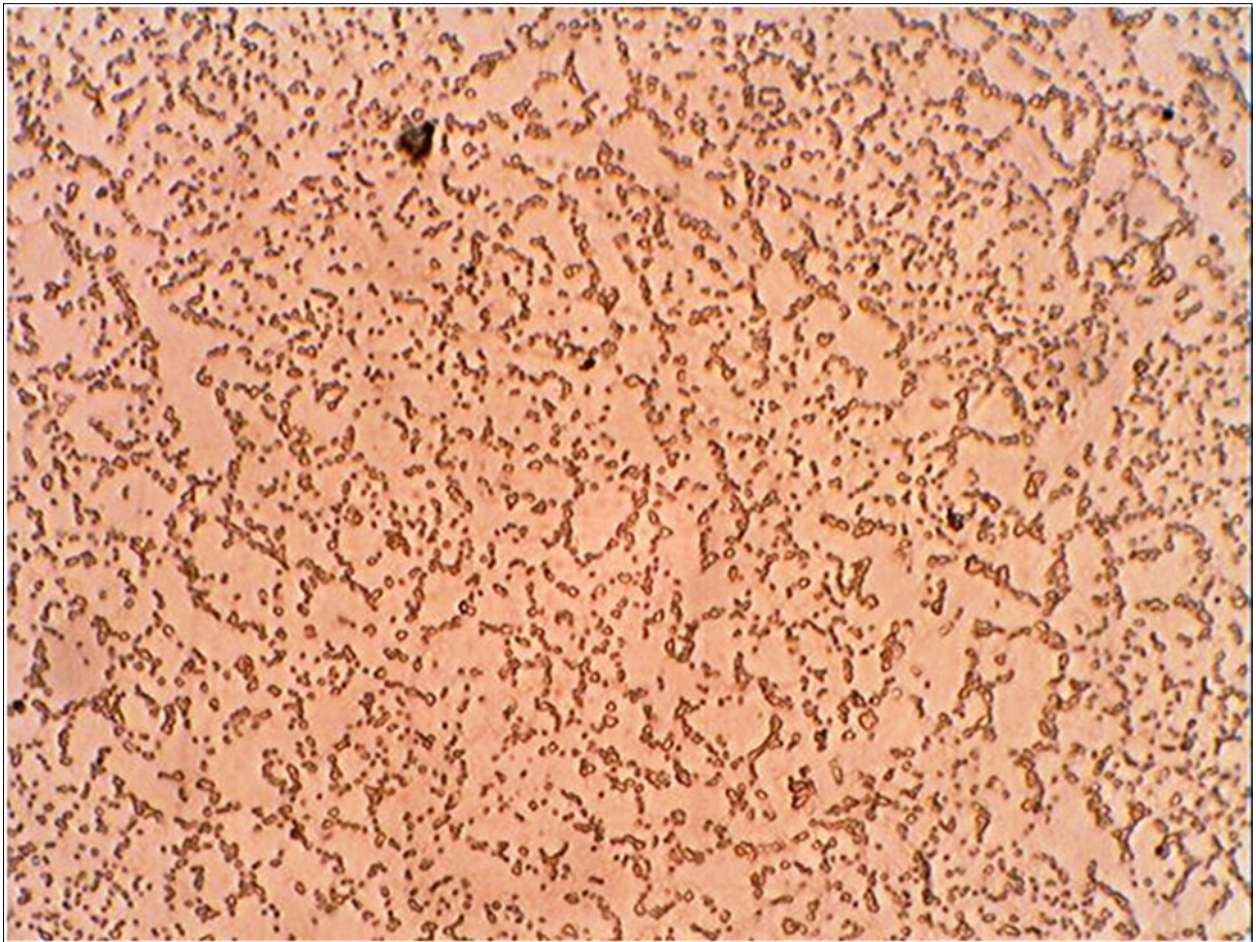


Figure 4: Cigarette smoker saliva slide



populations of bacteria were high from the saliva sample taken from alcohol drunken person, oral moist snuff user, hashish smoker and cigarette smoker. (Fig 5)

#### *Types of Bacteria Present in the Saliva of a Normal Non Addictive Person*

Gram positive staphylococcus, Gram negative cocci, monococci, streptococci, vibrio, gram negative rod, gram positive staphylococcus, gram positive streptococci and gram positive rod were observed in saliva of normal non addictive person.

It was noticed that the population of bacteria were higher in the saliva sample normal non addictive person as compared to alcoholic, oral moist snuff user, hashish, cigarette and shisha smokers. (Fig 6)

#### **Discussion**

In this study thirty different human saliva samples were investigated for population and types of bacteria present. Saliva has an important role in the human body and mouth. Use of different type of addictive substances affects the types and population of bacteria in the human saliva. The bacteria which were observed are gram negative cocci in large amount, gram positive cocci in moderate amount and gram negative rods in small amount.

Red and white wine have phenolic compounds, which show high antibacterial activities. Wine phenolic are divided in to flavonoids and non-flavonoids. These wines are made from red and white grapes [13]. A few phenolic compounds show anti-microbial and anti-pathogenic activity such as resveratrol, hydroxyl tyrosol, quercetin and a number of phenolic acids [14]. Many Studies carried out on alcohol which shows the anti-microbial activity against many pathogens [15]. In the saliva of alcohol drunken man the most abundant types of bacteria were gram positive cocci and gram positive streptococci. While the populations of the bacteria was low as compared to the population of bacteria present in the saliva of those persons who used oral moist snuff, smoked hashish, smoked cigarette, smoked shisha and from those persons who did not used any addictive substances. This was due to the antibacterial activities of the reported components present in wine.

Many snuffs are made from dried leaves of

tobacco and are used in the treatment of nausea and travel sickness. Some other activities reported for *Nicotiana tabacum* are: analgesic activity, anesthetic activity, angiogenesis inhibition, antibacterial activity, anti convulsant activities, anti-estrogenic effect, antifungal activity, antiglaucomic activity, antioxidant activity, antistress effect, antiviral activity, aromatase inhibition, arrhythmogenic effect, carcinogenic activity, bronchoconstrictor activity [16]. In the saliva of a man who used oral moist snuff, gram positive cocci, gram negative cocci and gram positive rods were present in large amount. While the population of the bacteria present in saliva of those persons who used oral moist snuff were lesser than that of the persons who smoked shisha, hashish, and cigarette and from those who did not used any addictive substances. However, the population was greater than from the saliva of a person who drank alcohol. From the findings in this study it is concluded that snuffs have some bactericidal activities as the bacterial population was low as compared to saliva of control.

Cannabis sativa is mainly used for hashish belonging to the family Cannabinaceae have been screened for their antimicrobial activity against two Gram positive organisms (*Bacillus subtilis*, *Staphylococcus aureus*), two Gram negative organisms (*Escherichia coli*, *Pseudomonas aeruginosa*) and two fungi namely *Aspergillus niger* and *Candida albicans* using the cup plate agar diffusion method by Mechoulam and Lander [17]. The oil of the seeds of Cannabis sativa exerted pronounced antibacterial activity (21 to 28 mm) against *Bacillus subtilis* and *Staphylococcus aureus*, moderate activity (15 mm) against *Escherichia coli* and high activity (16 mm) against *Pseudomonas aeruginosa* [18]. Saliva of hashish smokers contained the gram-negative cocci in large amount while the population of the bacteria in the saliva of the hashish smokers are lesser than those persons who smoked shisha, cigarette and from those persons who did not used any addictive substances. However the populations of the bacteria were greater from those persons who drank alcohol and used oral moist snuff.

Tobacco contains nicotine which shows interference with microorganisms and pathogens which is a vital finding. The nicotine present in saliva seriously



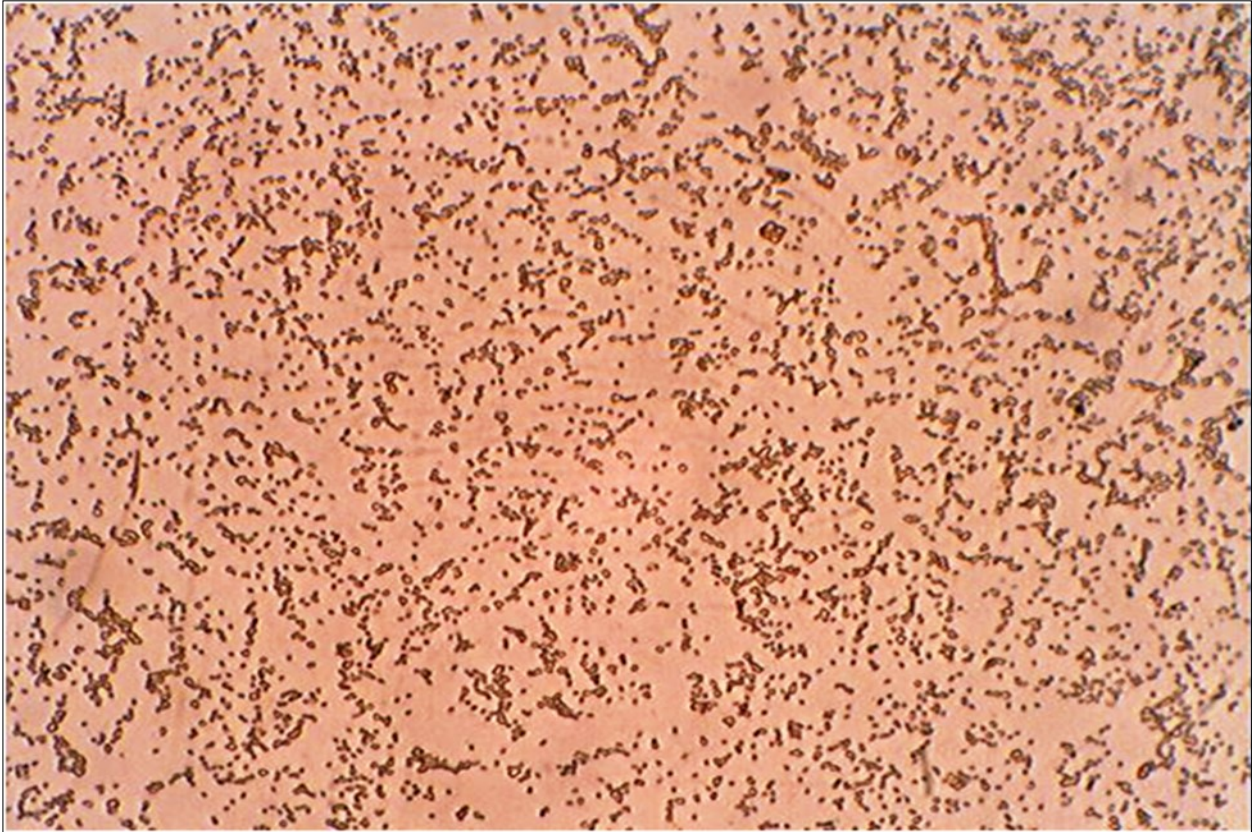


Figure 5: Shisha smoker saliva slide

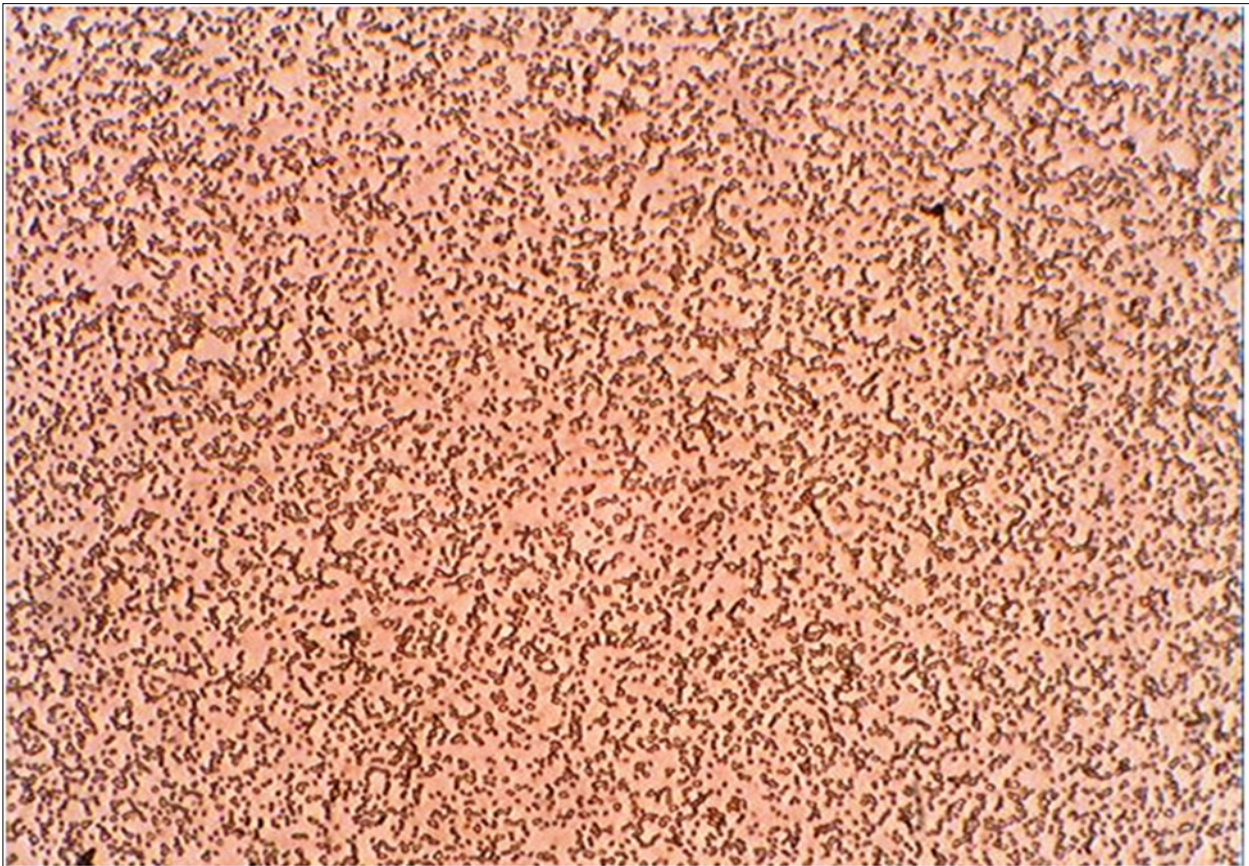


Figure 6: Normal non-addictive person saliva slide



affects the amount of micro flora colonization in the mouth. Tobacco is also used for shisha smoking and has anti-bacterial and anti-microbial activities [18]. Gram negative cocci and gram negative rods were present in abundant amount in the saliva of those persons who smoked cigarette while population of the bacteria were lesser from those persons who smoked shisha and from those who did not used any addictive substances. However, the population was greater from those persons drinking alcohol, smoking hashish and oral moist snuff user. Gram negative cocci were the abundant bacteria present in the saliva of those persons who smoked shisha while population of the bacteria in the saliva of those persons who smoked shisha were lesser than from those persons who did not used any addictive substances. However population of bacteria were greater than from those persons who drink alcohol, used oral moist snuff, smoked cigarette and smoked hashish. Gram negative cocci, mono cocci, streptococci, vibrio, gram negative rod, gram positive staphylococcus, gram positive streptococci and gram positive rod were present in large number in the saliva of those person who did not used any addictive substances while the population of bacteria were greater than from those persons who drink alcohol, used oral moist snuff, smoked cigarette, smoked hashish and smoked shisha.

### Conclusion

The microbial study of human saliva samples were conducted, various types of bacteria were detected and effects of different types of addictive substances on the population of the bacteria present in saliva were also observed. The bacteria observed were: Gram negative cocci, mono cocci, streptococci, vibrio, gram negative rod, gram positive staphylococcus, gram positive streptococci and gram positive rod. The bacteria always grow in suitable environments like proper pH, temperature and availability of the nutrients. The pH and secretion rate of saliva are affected due to the using of different addictive substances like alcohol, oral moist snuff, cigarette shisha and hashish. The order of bacteria population was as; Drunken person < oral moist snuff user < cigarette smoker < hashish smoker < shisha smoker < normal non addictive person. From the findings in this study it is concluded that all these drugs have some antibacterial activities. However the already reported health problem associated with the

use of these drugs should be kept in mind while using these substances. Also the bacteria present in the human saliva played an important role in human body and in the oral cavity and by using, drinking and smoking different types of addictive substances the types as well as the numbers and population of the bacteria are affected. Therefore it should be necessary and will be fruit full if these addictive substances are avoided and thus chemistry of saliva, especially the population of bacteria would not be affected.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contribution

FAK and JU carried out practical work; MZ conceived the idea, did the literature survey and drafted the manuscript. All authors read and approved the final manuscript.

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