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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL FLORA ISOLATED FROM ORAL CAVITY OF SNUFF/ CIGARETTE USERS AND NON - USERS

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ABSTRACT

Exposure to snuff and smoke has impact on the oral flora of the human beings. The aim of the present study was to identify the human micro flora of oral cavity of snuff/cigarette users' and non-users. A total of 70 samples were collected from lateral sites of mouth from male students of University of Swabi. Bacterial isolates were identified by culturing on a blood agar medium, mannitol salt agar and subject to required biochemical tests. After the identification their antibiotic susceptibility pattern was studied. Among total of 70 samples 56 were positive for different bacterial growth, the predominant isolates were S*taphylococcus aureus* (41%) and *Staphylococcus epidermidis* (55.3%) whereas *Streptococcus pneumonia* (3%) was isolated from snuff users only. Overall Gram-positive bacteria including *Staphylococcus aureus*, *staphylococcus epidermidis* and *Streptococcus pneumoniae* were identified among the 60% of snuff users and 28.9% of cigarette users and 11.11% non user. The antibiotic susceptibility pattern results showed that The antibiotic susceptibility pattern results showed that the *Staphylococcus aureus* was found highly resistant to penicillin 23 (100%) and fully susceptible to Linzolid 23(100%). Similarly *Staphylococcus epidermidis* was found highly resistant toward penicillin 28(90.3%) and fully susceptible to two antibiotics i.e. Ciprofloxacin and Linzolid 31(100%). *Streptococcus pneumonia* was found highly resistant toward Ciprofloxacin and penicillin 2(100%) and fully susceptible to Amoxicillin, Amikacin, Fosfomycin, Levofloxacin and Linzolid. The result of present study revealed that the bacteria are involved with substantial risks of oral diseases.

Keywords: Oral Hygiene, Snuff users/ smoking, Pathogenic micro flora,

INTRODUCTION

The environment of the human's mouth allows the growth of characteristic microorganisms. Microorganisms found in mouth are referred to as oral microflora or oral microbiota or oral microbiome. The oral micro flora forms an association with the host which provides nutrients, water and suitable temperature for the microorganisms to grow (Saleem *et al*., 2017). The oral microflora is comprised of over 600 prevalent species, with distinct subsets predominating at different habitats (Aas *et al*., 2007). Various species of the genus *Streptococcus, Lactobacillus, Lactococcus, Enterococcus, Staphylococcus, Corynebacterium, Veillonella* and *Bacteroids* are commonly found in the oral cavity (Wang *et al.,* 2012). The most dominant species are from genera Staphylococcus and Streptococcus (Saleem *et al*., 2017). Different oral structures and tissues are colonized by definite microbial communities (Aas *et al*., 2005). These species have ability of adhering to the soft and hard tissues (Saleem *et al*., 2017). Streptococcus and Staphylococcus bacteria in oral cavity have the ability to shift their lifestyle from normal flora to pathogenic one (Saleem *et al*., 2017).

Tobacco in its various forms is a risk factor for many systemic diseases, gingivitis, and periodontal disease (Mujahid *et al.,* 2014). Tobacco smoke consist a major class of organic chemical compounds that contains chemical a sphyxiants, ciliastic compounds, irritants, co-carcinogens and carcinogens. Its use is known to be related with carcinoma of the oesophagus, larynx, lungs, and lips, emphysema, chronic bronchitis, coronary artery disease. Tobacco habit come across all over the world is mostly in the form of tobacco smoking, tobacco chewing, and tobacco snuff. Tobacco is used in the form of cigars and cheroots (5%), cigarettes (30%), bidis (34%), hookah (9%), chewing tobacco (19%), and snuff (2%) (Bharati *et al.,* 2013). Snuff is a form of dry or moist tobacco which is fermented into powdered form. Snuff/chewing tobacco are mostly taken orally that maybe in dry or moist form containing approximately 20-55% of moisture (Boffetta *et al*., 2008). Snuff is widely used in many different countries after its discovery since 17th century. Today, there is a consensus of opinion that smoked and smokeless tobacco consists of at least 5, 300 different chemicals (Rodgman and Perfetti, 2016).

Habitual use of snuff and smoked tobacco is rising day by day. Smokeless tobacco used daily varies widely depending on age, sex, origin, and socioeconomic status (Boffetta *et al*., 2008). Smokeless tobacco products are commonly considered to be less toxic than smoked products regardless of the fact that they are associated with many adverse health effects including, oral lesions, nicotine addiction, oral and pancreatic cancer, and cardiovascular diseases (Stepanov *et al*., 2008). Many of the health effects are assigned to chemical carcinogens produced present in smokeless tobacco (Stepanov *et al*., 2008). Smokeless tobacco is divided into dry snuff, moist snuff and chewing tobacco. Dry snuff is associated with oral cancer while moist snuff has no risks of oral cancer. Use of snuff for 30 minutes causes a gradual rise in blood nicotine concentration due to a sustained level of concentration that continues for up to 2 hours. Systemic dose of snuff and chewing tobacco is estimated at 2 to 3 mg (Smyth *et al*., 2017).When oral cavity is constantly exposed to tobacco for long time it evidently influence and brings about alteration in oral microflora (Nagarajappa and Prasad, 2010).

Keeping all facts in mind the overall purpose of the study was to examine the bacterial population from oral cavity of snuff users and non-users, to find out the distinct number of microbial population in both users and non-users and to find out their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Area of study

The samples and data were collected from male students at University of Swabi (from Nov 2017 to April 2018). Data was collected using planned questionnaire. The questionnaire includes questions about age, snuff/cigarette use, time and number of snuff/cigarette use, drug use, type of drug, and hygiene,

Sample Collection

A total of 70 samples were collected from male students (19-30 years age) at University of Swabi. Samples were collected from both snuff/cigarette users and non-users with the help of sterile swabs during day time. During the sample collection process data regarding age, type of drug, hygiene, the median of starting it and the average number of use per day were also collected from tobacco user and nonuser through planned questionnaire.

Isolation and identification of bacterial flora

For the isolation and identification of bacterial flora from oral cavity of snuff/cigarette users and non users, the collected samples were inoculated onto blood agar medium with 5% of sterile sheep blood and subsequently sub-cultured on mannitol salt agar (MSA) plates.

Antibiotic Susceptibility Pattern

According to the Clinical Laboratory Standards Institute (CLSI) guidelines antimicrobial sensitivity test was performed by the Kirby- Bauer disc diffusion method. The antibacterial agents tested for sensitivity were Penicillin (1U), Ciprofloxacin (5µg), Levofloxacin (5µg), Amoxycillin (10µg), Fosfomycin (50µg) and Amikacin (30µg) and Gentamicin (> 590µg).

Statistical Analysis

Chi square test was employed using SPSS version (16.0) to analyzed bacterial growth among snuff users, Cigarette users and non users.

RESULTS

Snuff / Cigarette Users and non-Users

Total of 70 Samples were collected among these samples Snuff or cigarette users were 64.29% while non-users were 35.71% (categorically 60% were snuff users, 28.89% were smokers and both tobacco product users and 11.11% was other types of drug user. While data collection it was observed that snuff users were in high number in university in comparison to cigarette user and non user (Fig. 1**)**

Identification of Bacterial Isolates

Among total of 70 samples, 56 samples (both users and non-users) had bacterial growth while the remaining 14 samples produced no growth. Among total samples 23(41%) strains of *Staphylococcus aureus* were isolated, 5 (21.7%) from non user, 10(43.4%) from cigarette users 8(34.7%) from snuff user, whereas *Staphylococcus epidermidis* was present in 31(55.3%) samples i.e. 18 (58%) non user, 1(3.2%) cigarette user and 12(38.7%) snuff user. while 2 (3)% of *Streptococcus pneumonaie* were present in oral cavity of snuff users and absent in non user and cigratte user (Table 1).

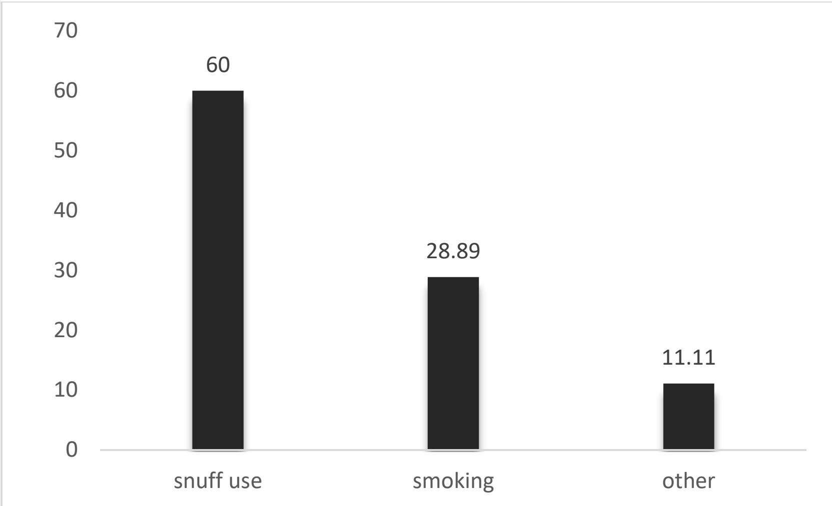

Fig.1. Percentage of Snuff, Cigarette and Other Dugs/ Non User.

Table 1. Percentage and occurrence of bacterial isolates in oral cavity of Snuff/ Cigarette Users and Non Users.

Groups	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Streptococcus pneumonae*	P value
Non User	5 (21.7%) ± 0.5	18 (58 %) ± 0.76	0 (0%) ± 0	0.001
Cigarette User	10 (43.4%) ± 0.6	1(3.22%) ± 0.1	0 (0%) ± 0	
Snuff User	8 (34.7%) ± 0.43	12(38.7%) ± 0.32	2 (100%) ± 0.3	
Total (n=56)	23	31	2	

Table 2. Antibiotic Susceptibility pattern of Bacterial isolates (S, susceptible; R, resistant).

Antibiotics	*Staph. aureus* (n = 23)		*Staph. epidermidis* (n = 31)		*Streptococcus pneumonia* (n = 2)	
	S	R	S	R	S	R
Ciprofloxacin	18 (78.2%) ± 0.32	5 (21.7%) ± 0.21	31 (100%) ± 0.7	0 (0%) ± 0	0 (0%) ± 0	2 (100%) ± 0.4
Penicillin	0 (0%) ± 0	23 (100%) ± 0.4	3 (9.6%) ± 0.04	28 (90.3%) ± 0.3	0 (0%) ± 0	2 (100%) ± 0.6
Levofloxacin	13 (56.5%) ± 0.41	10 (43.4%) ± 0.3	30 (96.7%) ± 0.5	1 (3.2%) ± 0.11	2 (100%) ± 0.01	0 (0%) ± 0
Amoxicillin	5 (21.7%) ± 0.23	18 (78.2%) ± 0.06	27 (87%) ± 0.47	4 (12.9%) ± 0.05	2 (100%) ± 0.05	0 (0%) ± 0
Fosfomycin	10 (43.4%) ± 0.04	13 (56.5%) ± 0.01	30 (96.7%)± 0.3	1 (3.2%) ± 0.7	2 (100%) ± 0.35	0 (0%) ± 0
Linzolid	23 (100%) ± 0.12	0 (0%) ± 0	31 (100%) ± 0.45	0 (0%) ± 0.21	2 (100%) ± 0.5	0 (0%) ± 0
Gentamicin	12 (52.1%) ± 0.54	11 (47.8%) ± 0.03	29 (93.5%)± 0.53	2 (6.4%) ± 0.04	1 (50%) ± 0.02	1 (50%) ± 0.1
Amikacin	9 (39.1%) ± 0.06	14 (60.8%) ± 0.07	27 (87%) ± 0.44	4 (12.9%) ± 0.08	2 (100%) ± 0.2	0 (0%) ± 0

Antibiotic Susceptibility pattern of Bacterial isolates

Antibiotic susceptibility Pattern was tested against all 56 isolates i.e. 23 of *Staphylococcus aureus*, 31 of *Staphylococcus epidermidis* and 2 of *Streptococcus pneumonae* using eight commonly used antibiotics i.e Ciprofloxacin, Penicillin, Levofloxacin, Amoxicillin, Fosfomycin, Linzolid, Gentamicin and Amikacin. The antibiotic susceptibility pattern results showed that the *Staphylococcus aureus* was found highly resistant to penicillin 23 (100%) followed by Amoxicillin 18(78.2%), Amikacin 14 (60.8%), Fosfomycin 13(56.5%), Gentamicin 11 (47.8%), Levofloxacin 10 (43.4%), Ciprofloxacin 5(21.7%) and fully susceptible to Linzolid 23(100%). Similarly *Staphylococcus epidermidis* was found highly resistant toward penicillin 28(90.3%) followed by Amoxicillin and Amikacin 4 (12.9%), Gentamicin 2 (6.4%) Levofloxacin and Fosfomycin1 (3.2%), whereas susceptible to Ciprofloxacin and Linzolid 31(100%). *Streptococcus pneumonia* was found highly resistant toward Ciprofloxacin and penicillin 2(100%) followed by Gentamicin 1(50%) and fully susceptible to Amoxicillin 2(100%), Amikacin 2(100%), Fosfomycin 2(100%), Levofloxacin and Linzolid 2(100%) (Table 2).

DISCUSSION

The environment of the human's mouth allows the growth of characteristic microorganisms. These microorganisms found in mouth are referred to as oral microflora or oral microbiota or oral microbiome. Various species of the genus *Streptococcus, Lactobacillus, Lactococcus, Enterococcus, Staphylococcus, Corynebacterium, Veillonella and Bacteroids* are commonly found in the oral cavity (Wang *et al.*, 2012). The most dominant species are from genera staphylococcus and streptococcus (Saleem *et al*., 2017). While a study carried in Bagdad, Iraq shows different result in contrast to our study, which shows that the predominant isolate was *Streptococcus* sp. in both groups but with higher percentage in non-smokers (42.6%) compared to smokers (31.6%) (Saleh *et al.*, 2016). In our study total of 70 Samples were collected among these samples Snuff or cigarette users were 64.29% while non-users were 35.71% (categorically 60% were snuff users, 28.89% were smokers and both tobacco product users and 11.11% was other types of drug user.-Among total of 70 samples, 56 samples (both users and non-users) had bacterial growth while the remaining 14 samples produced no growth. Among Total samples 23(41%) strains of *Staphylococcus aureus* were isolated, 5 (21.7%) from non user, 10(43.4%) from cigarette users 8(34.7%) from snuff user, whereas *Staphylococcus epidermidis* was present in 31(55.3%) samples i.e. 18 (58%) non user, 1(3.2%) cigarette user and 12(38.7%) snuff user. while 2 (3)% of *Streptococcus pneumonaie* were present in oral cavity of snuff users and absent in non user and cigratte user. The bacterial was affected due to constant use of tobacco products. Similar study has been carried out by Saleem *et al. (*2017*)* which shows that 21.7% of bacterial isolates was from Streptococcus species, 19.6% of bacterial isolates were of Pseudomonas species and Staphylococcus aureus. Similarly Enterococcus faecium and Citrobacter species each isolated in 10.9% of bacterial isolates. A similar research for comparative study between the smoke users and non-users was conducted by Saleh *et al*. (2016). The identified bacteria were *Staphylococcus* and *Streptococcus* species.

In our study Antibiotic susceptibility Pattern was tested against all 56 isolates i.e. 23 of *Staphylococcus aureus*, 31 of *Staphylococcus epidermidis* and 2 of *Streptococcus pneumonae* using eight commonly used antibiotics i.e Ciprofloxacin, Penicillin, Levofloxacin, Amoxicillin, Fosfomycin, Linzolid, Gentamicin and Amikacin. The antibiotic susceptibility pattern results showed that the *Staphylococcus aureus* was found highly resistant to penicillin 23 (100%) followed by Amoxicillin 18(78.2%), Amikacin 14 (60.8%), Fosfomycin 13(56.5%), Gentamicin 11 (47.8%), Levofloxacin 10 (43.4%), Ciprofloxacin 5(21.7%) and fully susceptible to Linzolid 23(100%). Similarly *Staphylococcus epidermidis* was found highly resistant toward penicillin 28(90.3%) followed by Amoxicillin and Amikacin 4 (12.9%), Gentamicin 2 (6.4%) Levofloxacin and Fosfomycin1 (3.2%), whereas susceptible to Ciprofloxacin and Linzolid 31(100%). *Streptococcus pneumonia* was found highly resistant toward Ciprofloxacin and penicillin 2(100%) followed by Gentamicin 1(50%) and fully susceptible to Amoxicillin 2(100%), Amikacin 2 (100%), Fosfomycin2 (100%), Levofloxacin and Linzolid 2(100%). The findings of this study was similar to studies conducted on different types of oral micro flora in Indian population by Mussarat *et al.* (2014) and Deepak *et al*. (2014). Antibiotic resistance is one of the world's most pressing public health problems. The antibiotic resistant organisms can threaten communities with new strains of infectious diseases. These diseases are then difficult to cure and more expensive to treat. Treatment failures may arise due to the resistance offered by pathogen against effective broad-spectrum antibiotics. These treatment failures by antibiotic are harmful. They are hard to treat infections and may results in high death rates (Khushal, 2004).

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