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Microbial Composition of Smokeless Tobacco Products from Karnataka

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ABSTRACT

Background: Though there is a lot of literature available about presence of carcinogenic chemicals in these products, there is lack of information regarding the microbial composition of these products.

Aim & Objective: The study was conducted with the objective of assessing microbial composition of smokeless tobacco products and factors associated with higher colony count.

Methodology: This cross-sectional study included a total of 43 samples obtained from four districts of Karnataka namely Udupi, Bengaluru, Bidar and Belagavi. Microbial populations were assessed using quantitative aerobic culture and colony forming units per gram was reported for each sample. The data was entered into excel and analysed using Statistical Package for Social Sciences version 16.0. Results have been expressed as frequencies and percentages. Univariate analysis was done to study association between background characteristics and higher colony counts.

Results: All except three samples showed bacterial contamination. Two samples showed pathogenic bacterial growth, while the other 38 samples had non-pathogenic bacteria. There was no association between background characteristics and colony counts.

Conclusions: Microbial contamination of smokeless tobacco products is common. There is a need to further investigate various factors associated with microbial contamination so that necessary interventions can be implemented.

Key-words: Smokeless tobacco, microbial, bacterial

INTRODUCTION

Globally, India is the second largest producer of tobacco products. [1] As per Global adult tobacco survey (GATS-2) report, 28.6% of adults used smokeless tobacco products. [2] India also has one of the largest rates of SLT use and is more common among individuals from lower socioeconomic status, and vulnerable population such as migrants and labourers including women.[3] Inspite of ill effects to health, SLT products are commonly sold in India in rural as well as urban areas.[4]

Carcinogenicity and other adverse health effects of SLT products are mainly attributed to presence of tobacco specific nitrosamines (TSNA) and aromatic hydrocarbons. These nitrosamines are formed by reaction of alkaloids with nitrites which are produced by the nitrite reducing bacteria abundantly present in these products. [5-7] Further, these SLT products are often manufactured in unorganized sectors in India in the pretext of creating more employment.[8] Microbial contaminations may occur during processing and manufacturing of tobacco products.[9-11]

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There is a lacuna of studies assessing the microbial composition of smokeless tobacco products in India. There is a strong need to gain an understanding of the types and numbers of microorganisms that may be present in commercially available smokeless tobacco products to know entire spectrum of diseases that could be caused by SLT. The current study is planned to provide a baseline microbial profile of smokeless products and factors associated with higher colony count.

METHODOLOGY

An approval was obtained from institutional ethics committee (IEC number: 29/2020). The state of Karnataka is divided into four administrative divisions and samples of smokeless tobacco products were procured from one district under each of the administrative zones by convenience sampling namely Udupi, Bengaluru, Bidar and Belagavi during February-March 2020. The tobacco products were collected by research assistants by visiting two-three shops selling tobacco products in a given area.

After noting down the basic information on the packs, physical appearance of the powder was documented. One gram of tobacco product was weighed and dissolved in 10ml of sterile brain heart infusion (BHI) broth. The broth was then vortexed for 30 seconds. 10-fold dilutions were made from the broth for quantitative culture of the same. 50 µl of undiluted and diluted sample were cultured. Plates were incubated at 37^0C for 24 hours. Number of bacterial colonies were noted down. Colony count / gram was calculated using the formula: Colony Forming Units/gram = Number of colonies x Dilution factor x Volume of broth used to dissolve / Weight of the sample x Volume of inoculum. Bacterial identification was done for bacterial isolates other than aerobic spore bearing bacilli using Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) VITEK®MS system (BioMerieux, Inc, Durham, NC).

Data was entered in Microsoft Excel and exported to SPSS Version 16.0. The results have been presented as frequencies and proportions. Fisher's exact test was done to find the association between various background characteristics and colony count Multivariate logistic regression was performed using all variables to identify the factors more likely to be associated with higher colony counts. A p value of <0.05 was considered significant.

RESULTS

The study included a total of 43 samples consisting of chewable products and snuff. Out of the 43 SLT products, 37 were chewing forms and six were snuff packs. Among the packs, 15 were from Bengaluru, 12 from Udupi, 11 from Belagavi, and five from Bidar. Overall, the samples belonged to 28 different brands. Among the various products, 12(27.9%) were manufactured outside the state and 31(72.09%) were manufactured within the state of Karnataka.

The tobacco samples cultured were in different physical forms namely, loose brown flakes 19(44%) followed by loose black flakes 14(32%), snuff 6(13%) and filter pouch 5(11%).

Table 1: Colony count in different type of tobacco products based on macroscopic appearance (n=43)

Colony Count	Type of Tobacco Product			
	Loose brown flakes	Loose black flakes	Fine snuff	Filter pouch
No Growth	1 (5.56)	0 (0)	0 (0)	2 (40)
10^2	0 (0)	1 (7.14)	0 (0)	1 (20)
10^3	5 (27.78)	1 (7.14)	3 (50)	2 (40)
10^4	10 (55.56)	3 (21.43)	3 (50)	0 (0)
10^5	2 (11.11)	9 (64.29)	0 (0)	0 (0)
Total	18 (100)	14 (100)	6 (100)	5 (100)

On examination, three samples (9.4%) did not show any bacterial growth. Majority of samples did show some bacterial contamination ranging from 10^2 to $>10^5$ as shown in table 1. Bacterial isolates were mainly aerobic spore bearing bacilli which are environmental flora and non-pathogenic except for two samples from which *Escherichia coli* and *Coagulase negative staphylococcus* were grown. There was no growth of any fungal isolates.

Tobacco in filter pouches showed less colony count as compared to tobacco which was not packaged in filter pouches. Majority of black large flakes had colony count of $>10^5$.

All the snuff products showed bacterial growth whereas three of the chewable products did not show any growth. Seven chewable products showed a growth of more than 10^5 organisms. The colony count in samples ranged from no growth to $8.0x10^5$ CFU/gm.

As shown in table 2, univariate analysis found that colony count of more than 10^3 per gram(p=0.002) was more likely to be associated with mode of use of tobacco product. Multivariate logistic regression showed that none of the background characteristics were significantly associated with colony count.

DISCUSSION

Our study included microbiological analysis of 43 smokeless tobacco products including 28 different brands. The study found that 40 out of 43 products had bacterial contamination. Though majority of the products were found to have non-pathogenic bacteria namely Bacillus species, two products showed the presence of E. coli and Coagulase negative S. aureus. All the six fine snuff products showed microbial contamination. However, there was no association between the type of product, mode of use, date & place of manufacture or date of expiry with the microbial growth in the samples.

Table 2: Association between background characteristics of smokeless tobacco products and colony count (n=43)

Characteristics	Colony count		Total	p value
	<10^3 CFU*/gm (n=17) (%)	>10^3 CFU*/gm (n=26) (%)		
Mode of use				
Loose black flakes	2 (11.76)	12 (46.15)	14	0.002
Loose brown flakes	6 (35.29)	12 (46.15)	18	
Filter pouch	5 (29.41)	0 (0)	5	
Snuff by nose	4 (23.53)	2 (7.69)	6	
Type of tobacco				
Plain tobacco	8 (47.06)	22 (84.62)	30	0.07
Khaini	3 (17.65)	1 (3.85)	4	
Zarda	2 (11.76)	1 (3.85)	3	
Snuff	4 (23.53)	2 (7.69)	6	
Place of manufacturing				
Karnataka	13 (76.47)	17 (65.38)	30	0.51
Outside Karnataka	4 (23.53)	9 (34.62)	13	
Date of packaging				
Mentioned	4 (23.53)	13 (50)	17	0.11
Not mentioned	13 (76.47)	13 (50)	26	
Date of expiry				
Mentioned	3 (17.65)	8 (30.77)	11	0.48
Not mentioned	14 (82.35)	18 (69.23)	32	

*Fisher's exact test was done to assess the factors likely to be associated with colony count >10^3CFU/gm

A similar study by Mehra R et al from Delhi also reported that all the 22 products studied had bacterial contamination. The study found that the most commonly isolated bacteria were Pseudumonas aeruginosa, Streptococcus faecalis, E. coli and Klebsiella pneumonia.[12] A study by Han J et al, Rubeinstein I et al from United States of America and Yusuf OAA et al from South Africa also found that the predominant species were from the class of Bacillus. [13-15] A study done in Nigeria exclusively among snuff products showed that the colony counts ranged from 3.0x10^2 CFU/gm to 6.7x10^2/gm while our study showed higher bacterial counts. The study also found that Staphylococcus aureus was the most frequently occurring bacteria in the snuff products. [16] A study by Tyx RE et al from France reported that 33 bacterial families from Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes were identified from the samples. [11] The observed difference in the type of bacteria in various studies, can be attributed to type of tobacco product analysed and possible geographical variations in practices related to manufacture of tobacco products.

Since the products were collected during summer, impact of changing weather and storage conditions on bacterial populations could not be studied. As the testing for SLT microbial contamination involves resources, we could test limited number of samples. However, as the samples were drawn from four different administrative zones of the state, the findings are fairly representative.

Future studies can be conducted with higher number of samples from different geographic locations across different seasons. Further research needs to be conducted with greater number of brands and different types of tobacco products including smoked products to get a better understanding of the microbial populations in smokeless tobacco products.

CONCLUSION

The study assessed the microbial composition of smokeless tobacco products. Majority of the products had bacterial contamination. Two samples were found to have pathogenic bacteria. With majority of the samples showing bacterial contamination of varying degrees, it sheds light on the lack of uniform safety regulations in place during the manufacturing of these products.

RECOMMENDATIONS

The microbiological profile of SLT products needs be scrutinized and levels should be stipulated by the regulatory bodies so that a uniform process is followed during their manufacturing. The National Tobacco testing laboratories could play a key role in addressing this issue and protecting people's health. The National Tobacco Control Program should communicate the health risks due to microbial contamination of SLT products in addition to oral cavity problems, cancers and other non-communicable diseases to the masses.

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