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Microbial Contamination of Locally Prepared Snuff Sold at Eke Igboji Market, Ikwo, Ebonyi State

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Abstract

Among the various things made from tobacco is snuff. The microbiological contamination of locally made snuff sold in Eke-Igboji market was investigated using standard microbiological procedure. Aliquots (0.1 ml) of the serially-diluted samples were plated out on the surface of nutrient agar, mannitol salt agar and potato dextrose agar in petri dishes. The plates also had 0.05mg/ml of ketoconazole to inhibit fungal growth and the plates were incubated at 28˚C for 24 hours while for the fungi 0.05 mg/ml of chloramphenicol was used to inhibit bacterial growth while it was incubation was at 28˚C for 72 hours.The bacteria identified were Corynebacterium sp, Micrococcus sp, Staphylococcus sp, Bacillus sp and Pseudomonas sp whereas the fungi were Aspergillus sp, Rhizopus sp, Alternaria sp, Geotrichum sp, Penicillium sp and Aspergillus sp. The bacterial count were between 2.9 x 10^2 cfu/ml and 6.8 x 10^2 cfu/ml while the fungal count was from 1.0 x 10^2 cfu/ml to 4.4 x 10^2 cfu/ml. The most common type of bacteria found was Staphylococcus spp (52.43%) and Corynebacterium sp was the least common (7.65%) while for fungi Aspergillus spp (27.00%) was found to be the most common while Geotrichum sp had the lowest frequency (5.40%).These microorganisms, which may have entered the snuff from the air, soil, dust, storage containers and human handlers are known to be harmful to humans. Therefore, in order to reduce the risk associated with the use of snuff by the snuff handlers, snuff must be processed and handled hygienically and the vendors should be educated on the health implications of taking a microbially contaminated snuff.

Keywords: Snuff, Staphylococcus sp, smokeless tobacco products, unhygienic, human health

Introduction

A tobacco product with nicotine as a chemical stimulant is called snuff. It is a tobacco that the leaves have been ground which is a typical example of a smokeless tobacco. It is usually in the form of tobacco powder that can be rubbed or inhaled (Donatelle and davis, 1999). It is among the first known tobacco products. Typically, snuff is inhaled or snuffed through the nose using either a specifically designed snuffing equipment or straight with the fingers. Smokeless tobacco" refers to a wide range of products for oral intake and nasal insufflation that contain unburned tobacco. These products are a global health problem due to their high level of addiction, wide variations in their content, production processes, and potential health concerns (Mutti *et al*., 2016; Siddiqi *et al*., 2020). Saliva contains nicotine released by the tobacco when snuff is consumed by mouth. Snuff ranges in texture and moisture content from extremely fine to coarse and from extremely dry to really wet. Due to its potential to keep allergens from reaching

the mucous membrane of the nose, it has been proven to be helpful in certain hay fever patients (Bofetta *et al*.,2008). It also helps those with common colds by opening their nasal canals. Snuffs that are medicated and flavored with camphor, eucalyptus oils, or mentholated crystals are known to be effective remedies for congested heads (Porter *et al*., 1997). Note that some of the more recent research (AlHebshi *et al*., 2017; Mehra *et al*., 2020; Zhang *et al*., 2020; Zhou *et al*., 2020) primarily concentrate on tobacco products that are not commonly available in the United States of America (Al-Hebshi *et al*., 2017; Rivera *et al*., 2020; Smyth *et al*., 2017; Tyx *et al*., 2020 Consequently, the microbial factor of these products remains to some extent, enigmatic. Snuffing has grown in popularity as a long-term medicine for pains and aches among the users. Due to the great demand for snuff, tobacco use is well known to occur in Nigeria.The industries and importers that produce snuff maintain a correspondingly high supply. From the point of production to the point of consumption, local snuff powders can become contaminated due to a number of factors, including the underlying microorganisms that caused the tobacco leaves to ferment, exposure to soil and dust during the curing process (Pauly and paszkiewicz, 2011), reuse of unwashed storage containers, and the powdery dust produced by the snuff (Maduka *et al*., 2009). The aim of this research is to conduct a microbiological assessment of snuff, as it is necessary to isolate the bacteria and fungi present in the snuff samples that were taken from the Eke- Igboji Market in Ikwo, Ebonyi State, Nigeria.

Materials and methods

Collection of Samples

Ten different samples of locally-ground snuff were obtained from different sellers at Eke- Igboji Market in Ikwo L.G.A. The samples were collected in sterile bijou bottles and conveyed to the Microbiology Laboratory of Alex Ekwueme Federal University Ndufu Alike Ikwo for microbial analysis.

Isolation of Microorganisms

A ten fold serial dilution of each of the samples was prepared using sterile distilled water. Aliquots (0.1 ml) of the serially-diluted samples were plated out on the surface of nutrient agar (NA) and mannitol salt agar (MSA) in petri dishes. The plates also had 0.05mg/ml of ketoconazole to inhibit fungal growth. The plates were incubated at 28˚C for 24 hours while for the isolation of the fungi dextrose agar (PDA) with 0.05 mg/ml of chloramphenicol was used to inhibit bacterial growth. Incubation was at 28˚C for 72 hours . The isolates were subcultured and stored for identification tests.

Identification of the Bacterial Isolates

Gram staining, motility, indole, methyl-red, voges proskaeur, catalase, coagulase, oxidase, spore, citrate utilization test and sugar fermentation tests were conducted according to Cheesbrough, 2006.

Identification of the Fungal Isolates

The cultural and microscopic characteristics of the fungal isolates were used to identify and characterize them. The Lactophenol cotton blue staining and slide culture assays were used for the study.

Lactophenol Cotton Blue Staining

On a sterile microscope slide, a piece of the test fungus was positioned, and two drops of lactophenol cotton blue solution were added. Under a microscope, the slide was covered with a coverslip to prevent bubbles. The distinctive characteristics were noted and contrasted with Cheesbrough's identification scheme.

Test of Slide Culture

Using a sterile wire loop, a piece of the fungal mycelia was placed onto a greaseless slide that held sterile sabouraud dextrose agar. After twenty-four hours of incubation at 28^{o}C, lactophenol cotton blue dye was used to stain the slide. A coverslip was placed over the slide and viewed under the microscope.

RESULTS

Table 1 shows the microbiological counts of the snuff samples. The bacterial count were between 2.9 x 10^2 cfu/ml and 6.8 x 10^2 cfu/ml while the fungal count was from 1.0 x 10^2 cfu/ml to 4.4 x 10^2 cfu/ml. The microbes isolated includes Corynebacterium sp, Bacillus sp, Pseudomonas sp, Staphylococcus sp, Micrococcus sp, Rhizopus sp, Alternaria sp, Geotrichum sp, Aspergillus sp and Penicillium sp.

Table 1: Microbial Counts of the snuff samples

Sample	Total bacterial count x 10^2 cfu/g	Total Fungal Count x 10^2 cfu/g
A	6.5	4.0
B	5.3	3.1
C	6.4	3.9
D	6.6	4.4
E	3.0	1.3
F	4.6	2.2
G	5.5	3.3
H	4.8	2.0
I	3.2	1.1
J	2.9	1.0

Table 2: Micrograms isolated from the snuff samples

Bacteria	Fungi
Corynebacterium sp	Aspergillus sp
Micrococcus sp	Rhizopus sp
Staphylococcus sp	Alternaria sp
Bacillus sp	Geotrichum sp
Pseudomonas sp	Aspergillus sp
	Penicillium sp

Table 3 shows the frequency of bacterial presence in the snuff. The most common type of bacteria found was Staphylococcus spp (52.43%), whilst Corynebacterium sp was the least common (7.65%).

Table 3: Frequency of Occurrence of the Bacteria in the snuff samples

Bacteria	Number isolated	Occurrence %
Corynebacterium sp	3	7.65
Micrococcus sp	4	10.21
Staphylococcus sp	19	52.43
Bacillus sp	4	16.20
Pseudomonas sp	5	13.51
Total	**35**	**100**

The frequency of incidence of the fungi in the snuff samples reported in Table 4 showed that Aspergillus spp (27.00%)was found to be the most common, while Geotrichum sp had the lowest frequency(5.40%).

Table 4: Frequency of Occurrence of the Fungi in the snuff samples

Bacteria	Number of isolated	Occurrence
Aspergillus sp	8	27.00
Rhizopus sp	5	21.47
Alternaria sp	2	8.53
Geotrichum sp	2	5.40
Penicillium sp	3	14.68
Aspergillus sp	4	22.92
Total	**24**	**100**

DISCUSSION

Significant number of bacteria and fungi were identified from the snuff samples that were studied. Table 1 shows that more bacteria than fungus were identified from the samples. A gram of tobacco was discovered to contain more than a million bacteria (Roth *et al*.,1994). The fungi isolated includes Aspergillus sp, Rhizopus sp, Alternaria sp, Geotrichum sp, Aspergillus sp and Penicillium sp while the bacteria included Corynebacterium sp, Micrococcus sp, Staphylococcus sp, Bacillus sp and Pseudomonas sp. This corroborates the work of Huang *et al*., 2010 who identified bacteria, molds and yeasts from tobacco leaves. Studies carried out over many years of snuff use have shown that it is frequently contaminated with endospores, microbial toxins, and vegetative cells, which can be detrimental to human health (Pauly and paszkiewicz, 2011). It is possible that improper handling during or after processing let the microorganisms isolated from the study's snuff samples enter the final product. The unhygienic methods and conditions that were applied when processing the tobacco to manufacture snuff could be responsible for these microbial contamination. In Nigeria, snuff is produced locally by mechanically grinding which aseptic conditions are not adhered to thereby allowing bacteria to enter the finished product. From table 3, Aspergillus sp was the most commonly isolated fungi from the samples, while Staphylococcus sp was the most commonly isolated bacteria (Table 3). According to Saleem *et al*.,(2018), a more thorough isolation of various species of Aspergillus was reported to be the predominant genera in Pakistani smokeless tobacco. Similarly, taxa from the genera Alternaria and Aspergillus were discovered in metagenomic interrogations of American snuff products (Rivera *et al*., 2020). Corynebacterium sp. frequently lives on the surfaces of plants and soil but Bacillus spores are resident in the soil.. While fungal spores can enter through the soil and air, Staphylococcus sp can enter through dust and handling. These isolated microorganisms have detrimental effects on human health as Staphylococcus sp causes diseases ranging from food poisoning, wound infections and gastroenteritis while Bacillus sp (*Bacillus anthracis*) are recognized aetiologic agents for respiratory illnesses like anthrax. Smokeless tobacco product consumption leads to a variety of pathologies, many of which include a disruption of the environment in the oral cavity. Although we do not go into further detail about the mechanisms underlying the significant roles played by these bacteria in tooth decay and periodontal disease, we do highlight their collective response to nicotine exposure. Remarkably, oral commensal streptococci, such as *S. gordonii* and *S. mutans*, exhibit dysregulated gene expression in response to

nicotine exposure (El-Ezmerli and Gregory 2019; Huang *et al*., 2014; Wagenknecht *et al*., 2018). Therefore, in order to reduce the risk associated with the use of snuff by the snuff handlers, snuff must be processed and handled hygienically. Some of the germs and fungus found in the snuff samples under study are known to be harmful to humans. These possibilities are conceivable when you take into account that using smokeless tobacco involves keeping tobacco in the mouth for prolonged periods of time, allowing the chemical (nicotine) and microbiological content to seep into the oral environment. Therefore, it's intriguing to think about how much of the microbial species and metabolites found in smokeless tobacco products (STP) enter the mouth cavity. Since no research on these factors has been published to date, this option needs to be looked into. According to Wilkins *et al*., (2019), smoking toxicities seem to be important in the suppression of some species and enrichment of others, which can lead to dysbiosis in the GI tract and the potential development of a number of systemic disorders in the host. According to a recent study looking at the impact of electronic cigarettes on user microbiomes, the diversity of the gut and buccal cavities is similar to that of people who never smoke or who never use tobacco products. Presently, advances in technology are making it possible to use more sophisticated technologies in investigations of the STP microbiological component. Research in smokeless tobacco microbiology is bridging information gaps that were previously unfilled because of inadequate techniques. Snuff should consequently be prepared, packaged, and handled with utmost hygiene. Nigerian snuff makers are advised to use the western snuff production and preservation methods that have been embraced by developed nations.

REFERENCES

Al-Hebshi N.N, Alharbi F.A, Mahri M., Chen T. (2017). Differences in the bacteriome of smokeless tobacco products with different oral carcinogenicity: compositional and predicted functional analysis. *Genes* 8(4).

Boffetta P., Hecht S., Gray N., Gupta P., Straif K.(2008). Smokeless tobacco and cancer. *Lancet Oncol*. 9(7):67-75

Cheesbrough M.(2006). District laboratory practice in tropical countries. 2nd ed. India: Cambridge University Press.

Donatelle J.R, Davis G.L (1999). Health. The Basis. 3rd ed. Allyne and Bacon Publications USA Pp 45-102.

El-Ezmerli N.F, Gregory R.L (2019). Effect of nicotine on biofilm formation of Streptococcus mutans isolates from smoking and nonsmoking subjects. *J Oral Microbiol* 11(1):166-275.

Huang J., Yang J., Duan Y., Gu W., Gong X., Zhe W, Su C.,Zhang K.Q (2010). Bacterial diversities on unaged and aging flue-cured tobacco leaves estimated by 16S rRNA sequence analysis. *Appl Microbiol Biotechnol*. 88(2):553- 562

Maduka S.O, Osim E.E, Nneli R.O, Anyabolu A.E(2009). Effect of occupational exposure to local powdered tobacco (snuff) on pulmonary function in south eastern Nigerians. *Niger J Physiol Sci*. 24(2):195-202.

Mehra R., Mohanty V., Balappanavar A.Y, Kapoor S. (2020). Bacterial contamination of packaged smokeless tobacco sold in India. *Tob Prev Cessat* 6:11.

Mutti S., Reid J.L, Gupta P.C, Pednekar M.S, Dhumal G., Nargis N., Hussain A.G, Hammond D.J.T. (2016). Perceived effectiveness of text and pictorial health warnings for smokeless tobacco packages in Navi Mumbai, India, and Dhaka, Bangladesh: findings from an experimental study. *Tob Control* 25(4):437–443.

Pauly J.L and Paszkiewicz G.(2011). Cigarette smoke, bacteria, mold, microbial toxins, and chronic lung inflammation. *Journal of oncology*. 1-11

Porter R., Teich M.(1997). Drug and Narcotics in history. Cambridge: Cambridge University Press, Hindawi Publishing Corporation Pp 10 - 55.

Rivera A.J, Tyx R.E, Stanfill S.B, Watson C.H (2020). Microbial communities and gene contributions in smokeless tobacco products. *Appl Microbiol Biotechnol* 104(24):1–17.

Roth D.S, Cowart W.A, Jenkins C.B, Boyle D.M.(1994). Sterilization process in the manufacturing of snuff. US Patent. 5(374):149.

Rubinstein I. and Pedersen G.W.(2002). Bacillus species are present in chewing tobacco sold in the United States and evoke plasma exudation from the oral mucosa. *Clin Diagn Lab Immunol*. 9(5):1057-60.

Saleem S, Naz SA, Shafique M, Jabeen N, Ahsan SW (2018). Fungal contamination in smokeless tobacco products traditionally consumed in Pakistan. *J Pak Med Assoc* 68(10):1471–1477.

Siddiqi K., Husain S., Vidyasagaran A., Readshaw A., Mishu M.P, Sheikh A. (2020). Global burden of disease due to smokeless tobacco consumption in adults: an updated analysis of data from 127 countries. *BMC Med* 18(1):222.

Smyth E.M, Kulkarni P., Claye E., Stanfill S., Tyx R., Maddox C., Mongodin E.F, Sapkota A.R (2017). Smokeless tobacco products harbor diverse bacterial microbiota that differ across products and brands. *Appl Microbiol Biotechnol* 101(13):5391–5403.

Tyx R.E, Rivera A.J, Keong L.M, Stanfill S.B (2020). An exploration of smokeless tobacco product nucleic acids: a combined metagenome and metatranscriptome analysis. *Appl Microbiol Biotechnol* 104(2): 751–763.

Wagenknecht DR, BalHaddad AA, Gregory RL (2018). Effects of nicotine on oral microorganisms, human tissues, and the interactions between them. *Curr Oral Health Rep* 5(1):78–87.

Wang F, Men X, Zhang G, Liang K, Xin Y, Wang J, Li A, Zhang H, Liu H, Wu L (2018). Assessment of 16S rRNA gene primers for studying bacterial community structure and function of aging flue-cured tobaccos. *AMB Express* 8(1):182.

Wilkins LJ, Monga M, Miller AW (2019). Defining dysbiosis for a cluster of chronic diseases. *Sci Rep* 9(1):12918.

Zhang Q, Geng Z, Li D, Ding Z (2020). Characterization and discrimination of microbial community and co-occurrence patterns in fresh and strong flavor style flue-cured tobacco leaves. *Microbiology open* 9(2): 965.

Zhou J, Yu L, Zhang J, Liu J, Zou X (2021). Dynamic characteristics and co-occurrence patterns of microbial community in tobacco leaves during the 24-month aging process. *Ann Microbiol* 71(1):1–13.

Zhou J, Yu L, Zhang J, Zhang X, Xue Y, Liu J, Zou X (2020). Characterization of the core microbiome in tobacco leaves during aging. *Microbiology Open* 9(3):984.

