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Microbial Contamination of Locally-Prepared Snuff Sold at Eke-Awka Market, Anmbra State, Nigeria

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Abstract

Introduction: Snuff is one of the many products of tobacco. It is prepared by certain processes that involve fermentation and aging. Microorganisms have been found to play important roles in these processes of snuff production. Snuff has been recommended by physicians as a better substitute for cigarettes.

Methods: The microbial contamination of locally-prepared snuff sold in Eke-Awka Market, Anambra State was studied with a view to determining their suitability for use. The bacteria were isolated using nutrient agar, cetrimide agar and mannitol salt agar as the growth media while potato dextrose agar was the growth medium for the isolation of the fungi.

Results: The bacterial counts ranged between 3.0×10^2 cfu/g and 6.7×10^2 cfu/g while the fungal counts were between 1.0×10^2 cfu/g and 4.6×10^2 cfu/g. The bacteria were identified as *Corynebacterium bovis, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* and *Pseudomonas aeruginosa* while the fungi were *Aspergillus niger, Rhizopus stolonifer, Alternaria alternata, Geotrichum candidum, Penicillium italicum* and *Aspergillus flavus. Staphylococcus aureus* occurred most frequently (28.57%) among the bacteria in the samples while *Aspergillus niger* had the highest frequency of occurrence (25.00%) among the fungal isolates.

Conclusions: These microorganisms which may have entered the snuff from the air, soil, dust, storage containers and human handlers are known to cause diseases of man. Snuff should therefore be processed, packaged and handled hygienically to avoid its contamination with these organisms and the health risk they pose to the users.

INTRODUCTION

Snuff is a tobacco product which contains the chemical stimulant, nicotine. It is a product made from ground tobacco leaves and is an example of smokeless tobacco. Snuff is tobacco in the form of powder that can be inhaled or placed against the gums [1]. It is one of the oldest tobacco products known. There are two types of snuff, the dry snuff and moist snuff [2]. Dry snuff is produced as a dry powder while moist snuff is usually fine-cut, rather than ground and maintains high moisture content.

Snuff is generally inhaled or snuffed through the nose either directly from the fingers or by using specially made snuffing devices. When snuff is taken through the mouth, the tobacco releases its nicotine into the saliva, which is then absorbed through the mucous membrane in the mouth [1]. Users of smokeless products including snuff, face no known cancer risk in the oral region than smokers, and have a greater cancer risk than people who do not use any tobacco products [3]. As the primary harm from smoking comes from the smoke itself, snuff has been recommended as a way of reducing harm from tobacco [4].

Snuff is usually scented or flavoured. Tropical flavours are floral, mentholated (medicated), fruit and spice, either pure or in blends, camphor, cinnamon, rose, spearmint, bourbon, cherry, cola and whisky. Snuff comes in the range of texture and moistness, from very fine to coarse and from very dry to very moist. It has been found to be beneficial in some cases of hay fever because it may prevent allergens from getting to the mucous membrane within the nose [5]. It is also useful in opening the nasal cavities in those suffering from common cold. Medicated snuffs, flavoured with mentholated crystals, eucalyptus oils or camphor are recognized as being a great cure for a stuffy head [6].

Snuffing has become quite popular as a medication of long grief, pains and aches [7]. It has been reported that 12.6% of students between 14 and 19 years of age in England use snuff in a studied population [8]. The widespread use of tobacco in Nigeria is well known as a result of the high demand for snuff. There is corresponding maintenance of high supply by the snuff producing industries and importers. In most markets in the south eastern part of Nigeria especially Onitsha, Owerri, Enugu, Aba, Awka and Umuahia, snuff retailers abound.

The effect of occupational exposure to local powdered tobacco

on pulmonary function was studied on snuff industry workers in Onitsha and Enugu Markets [9]. The dust sampling result showed that chronic exposure to Nigeria snuff dust impairs lung function and the effect is progressive with time [1].
Local snuff powders can be contaminated from the production to the consumption by the consumers through exposure to soil and dust during the curing of the tobacco leaves [10]; the underlying microorganisms which contributed to the fermentation of the tobacco leaves; exposure to soil and dust while grinding locally [11]; re-use of unwashed storage containers and the powdery dust generated from the snuff [9].
Microbiological studies of snuff involve the isolation of both bacteria and fungi from snuff, therefore in this study, the bacteria and fungi in the snuff samples obtained from Eke-Awka Market Awka, Anambra State, Nigeria were isolated, characterized and identified.

METHODS

Collection of Samples

Ten different samples of locally-ground snuff were obtained from different sellers at Eke-Awka Market in Awka, Anambra State. The samples were collected in sterile bijou bottles and conveyed to the Microbiology Laboratory of Nnamdi Azikiwe University Awka for microbial analysis.

Isolation of Microorganisms

A ten fold serial dilution of each of the samples was prepared using sterile distilled water. 0.1 mL aliquots of the serially-diluted samples (102) were spread-plated on the surface of nutrient agar (NA) and mannitol salt agar (MSA) contained in petridishes to isolate the bacteria. The plates also had 0.05 mg/mL of ketoconazole to inhibit fungal growth. The plates were incubated at 28°C for twenty-four hours in an inverted position. The colonies that developed were counted, subcultured and stored on sterile nutrient agar slants for characterization and identification. The same procedure was used in the isolation of the fungi except that potato dextrose agar (PDA) was used as the growth medium while chloramphenicol at a concentration of 0.05 mg/mL was used to inhibit bacterial growth. Incubation was at 28°C for three days after which the fungal colonies that developed were counted, subcultured and stored in sterile PDA slants for identification tests.

Characterization and Identification of the Bacterial Isolates

The bacterial colonies were characterized on the basis of their cultural and morphological characteristics. Gram staining, motility, indole, methyl-red, voges proskaeur, catalase, coagulase, oxidase, spore, citrate utilization and sugar fermentation tests were carried out as described by Onuorah et al [12]. They were identified according to the scheme of Cheesbrough [13].

Characterization and Identification of the Fungal Isolates

The fungal isolates were characterized and identified based on their cultural and microscopic features. The microscopic examination was carried out using lactophenol cotton blue staining and slide culture tests.

Lactophenol Cotton Blue Staining

A fragment of the test fungus was placed on a clean microscopic slide and two drops of lactophenol cotton blue solution were introduced. The slide was covered with a coverslip avoiding bubbles and viewed under the microscope. The characteristic features were recorded and compared with the identification scheme of Cheesbrough [13].

Slide Culture Test

A fragment of the fungal mycelia was introduced on a grease-free slide containing prepared sterile sabouraud dextrose agar with a sterile wire loop. The slide was incubated at 280C for twenty four hours after which it was stained with lactophenol cotton blue dye. The slide was after that covered with a coverslip and viewed under the microscope.

RESULTS

The microbial counts of the snuff samples are presented in Table 1. The bacterial counts were between 3.0×10^2 cfu/g and 6.7×10^2 cfu/g while the fungal counts ranged between 1.0×10^2 cfu/g and 4.6×10^2 cfu/g. The microorganisms isolated from the snuff samples are shown in Table 2. They were identified as *Pseudomonas aeruginosa*, *Corynebacterium bovis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Aspergillus niger*, *Rhizopus stolonifer*, *Alternaria alternata*, *Geotrichum candidum*, *Aspergillus flavus* and *Penicillium italicum*.

Table 1: Microbial Counts of the Snuff Samples

Sample	Total Bacterial Count, $\times 10^2$ cfu/g	Total Fungal Count, $\times 10^2$ cfu/g
1	6.6	4.2
2	5.4	3.3
3	6.5	4.1
4	6.7	4.6
5	3.1	1.4
6	4.7	2.4
7	5.6	3.6
8	4.9	2.2
9	3.4	1.3
10	3.0	1.0

Table 2: Microorganisms Isolated From the Snuff Samples

Bacteria	Fungi
Corynebacterium bovis	*Aspergillus niger*
Micrococcus luteus	*Rhizopus stolonifer*
Staphylococcus aureus	*Alternaria alternata*
Staphylococcus epidermidis	*Geotrichum candidum*
Bacillus subtilis	*Aspergillus flavus*
Pseudomonas aeruginosa	*Penicillium italicum*

The frequency of occurrence of the bacteria in the snuff samples is shown in Table 3. *Staphylococcus aureus* occurred most frequently (28.57%) while *Corynebacterium bovis* had the lowest frequency of occurrence (5.71%).

Table 3: Frequency of Occurrence of the Bacteria in the Snuff Samples

Bacteria	Number Isolated	Occurrence, %
Corynebacterium bovis	2	5.71
Micrococcus luteus	3	8.57
Staphylococcus aureus	10	28.57
Staphylococcus epidermidis	8	22.86
Bacillus subtilis	5	14.29
Pseudomonas aeruginosa	7	20.00
Total	35	100.00

The frequency of occurrence of the fungi in the snuff samples is presented in Table 4. *Aspergillus niger* had the highest frequency of occurrence of 25.00% while *Geotrichum candidum* had the lowest frequency of occurrence of 8.33%.

Table 4: Frequency of Occurrence of the Fungi in the Snuff Samples

Bacteria	Number Isolated	Occurrence, %
Apergillus niger	6	25.00
Rhizopus stolonifer	4	16.67
Alternaria alternata	3	12.50
Geotrichum candidum	2	8.33
Penicillium italicum	4	16.67
Aspergillus flavus	5	20.83
Total	24	100.00

DISCUSSION

Bacteria and fungi were isolated from the snuff samples examined in significant numbers. More bacteria were isolated from the samples than fungi (Table 1). More than one million bacteria were reported to be found in a gram of tobacco [14]. The bacteria were *Corynebacterium bovis, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* and *Pseudomonas aeruginosa* while the fungi were *Aspergillus niger, Rhizopus stolonifer, Alternaria alternata, Geotrichum candidum, Aspergillus flavus* and *Penicillium italicum* (Table 2). Rubinstein and Pederson [15] and Yang et al. [16] isolated bacteria, yeasts and molds in their different types of snuff. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were also identified in bacteriological examinations of the sputum samples of a patient with chronic bronchitis. The patient used snuff and it was theorized that the snuff might have been the source of the pathogens.

A study was undertaken on twenty one samples of previously unopened packs of snuff [16]. They isolated *Bacillus subtilis, Staphylococcus aureus, Staphylococcus albus* and *Pseudomonas aeruginosa*. Varma et al. [17] also reported the isolation of nine species of *Aspergillus* in stored leaves of chewing tobacco. Approximately eighteen of the Aspergilli were found to be mycotoxigenic.

Studies over years of snuff consumption have proved that it is regularly contaminated by microbial vegetative cells, endospores and microbial toxins which can be detrimental to human health [10]. These organisms isolated from the snuff samples studied may have entered the produce as a result of poor management which could be during or after processing. The processing of tobacco into snuff may have been done in an unhygienic environment and manner.

In Nigeria, snuff is produced locally by mechanical grinding during which aseptic conditions are not maintained so that microorganisms may enter the produce. *Staphylococcus aureus* was the most frequently isolated bacterium from the samples (Table 3) while *Aspergillus niger* was the predominant fungus isolated. Welty [18] isolated *Alternaria sp* and *Aspergillus sp* from flue-cured tobacco which can be used in the production of snuff. *Rhizopus sp* and *Geotrichum sp* are widely distributed in the soil and plants. Micrococci are found in the soil and on dust particles, storage containers, grinding equipments and skin and because they air-borne, they can easily contaminate snuff.

Corynebacterium sp commonly inhabit soil and plant surfaces while Bacillus spores can also be seen in the soil [19]. Staphylococcus may enter through the dust and handlers while the fungal spores may gain entrance through the air and soil. These organisms isolated from the snuff can be pathogenic to man. Staphylococcus can cause gastroenteritis, food poisoning, skin and wound infections [19]. Some species of Bacillus such as Bacillus anthracis are known pathogens of man and can cause anthrax which is a respiratory disease. *Bacillus pumilus* and *Bacillus licheniformis* and *Bacillus subtilis* can cause opportunistic infections in man [15] while *Aspergillus sp* can cause pulmonary and invasive Aspergillosis especially in people that are immune-compromised [20]. Snuff must therefore be processed and handled hygienically to minimize the incidence of these organisms thereby reducing the health risk they pose to its users.

The snuff samples studied contained some bacteria and fungi which are known to be pathogenic to man. It is therefore important that snuff should be processed, packaged and handled hygienically. The employment of the western snuff production and preservation processes adopted in developed countries by snuff producers in Nigeria is recommended.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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