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Original Research Article

A Survey on the Microbial Contaminants of Snuff Sold in Local Markets in Imo State, Nigeria

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Keywords

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and pooled
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A B S T R A C T

A survey on the microbial contaminants of snuff samples sold in local markets in Imo State, Nigeria was carried out using standard microbiological techniques. The mean total aerobic microbial counts ranged from $2.85x10^4 \pm 2.06 – 5.67x10^4 \pm 2.62$ and $1.00x10^3 \pm 0.00 - 2.40x10^3 \pm 1.67$cfu/g for bacteria and fungi respectively. The trend of the variations in bacterial counts for the three zones showed that Okigwe > Owerri > Orlu while Owerri > Okigwe > Orlu for fungi. Comparative analysis results however showed no statistical difference in counts recorded at various zones at P>0.05 for both. Among the bacterial isolates *Staphylococcus* recorded the highest %occurrence (43.5%), followed by *Micrococcus* (34.8%), while *Bacillus* and *Streptococcus* recorded the least (10.9%). *Penicillium* recorded the highest %occurrence among fungal isolates (50%), followed by *Aspergillus* (27.8%) while *Rhizopus* recorded the least (22.2%). Okigwe had the highest %occurrence of bacterial isolates across the zones (39.1%), followed by Orlu (32.6%) zone and Owerri zone the least (28.3%). Owerri zone recorded the highest among the fungal isolates in the zones (38.9%), followed by Okigwe (33.3%) while Orlu had the least (27.8%). Most snuffs sold in markets in Imo State were contaminated by potentially pathogenic microbes. Good manufacturing process and improvement in the sanitary conditions of the markets will help in reducing such contamination.

Introduction

Snuff is finely ground tobacco product that when in dry form is sniffed up or inhaled through the nose or tucked between the lower lip and gum (Ayo-Yusuf *et al*., 2005). Snuff is also available in wet form. Wet snuff sometimes called snus is rubbed inside the mouth instead. Tobacco snuff is in powdered form with potash and sweeteners as the main additives (Ayo-Yusuf *et al*., 2005). There are many microorganisms that are associated with snuff mostly transferred during production. Snuff can be traditional home made and commercial or individualized snuff product. According to Ogundero(1980), the local way of producing snuff involves pounding or crushing of the

cured leaves with locally made mortars, also depending on the scale of the operation, grinding stones may be used. People take snuff for different reasons: for medicinal purposes, cultural and traditional purposes, for smoking cessation program among others (Ureme *et al*., 2007 and Wilson, 1981). Some of the health effects of snuff use include: increase heart beat and raised blood pressure, development of oro-pharyngeal and upper respiratory tract cancer (gingival recession, caries, staining and abression), cardiovascular diseases and adverse reproductive outcome (Russel *et al*., 1980; Weintraub and Burt, 1987 and England *et al.*, 2003).

The principal content of tobacco is nicotine and has no use in medicine, though it is of value chiefly as an insecticide (Feyeraband and Russell, 1970). Snuff use is wide spread worldwide. According to Smith (2000), teen use of smokeless tobacco has increased, while use of all tobacco products by teen has decreased in the United States. Snuff is particularly popular amongst South African black women, with prevalence of 13.2% as compared to smoking prevalence of 5.4% in the same population group during 1998 (Ayo-Yusuf *et al*., 2000).

In Igbo communities of Nigeria, where tobacco is utilized for cultural and traditional purposes, nicotine has been associated with addiction in regular smokers and snuffers as noted by Ureme *et al*., (2007).

In Nigeria, cured tobacco is processed into snuff by small scale millers at various markets, using traditional milling or grinding stones, mortar and grinding machines (Ogundero, 1980). These milling methods and poor sanitary conditions prevailing around the snuff mills and the markets can expose the snuff to high levels of microbial contamination. Snuff is taken "raw" without further processing to reduce the microbial loads, mostly by elderly people with lower immunity and other health conditions. This study therefore aimed to ascertain the microbiological quality of snuff sold in Imo State.

Materials and Methods

Samples collection

Snuff samples were collected from local markets in the three zones in Imo State, namely: Okigwe, Owerri and Orlu. A total of 12 samples, 4 from each zone were collected in sterile specimen bottles. Samples from each zone were then mixed together to form one-pooled sample. Three-pooled samples were used in the study, representing the three zones of the state.

Total aerobic microbial counts

The media of choice were nutrient agar, saboraud dextrose agar, and mannitol salt agar while Simon citrate agar, Triple Iron agar and Peptone water used for biochemical tests. All media used were prepared according to the manufacturer's instructions. Ten-fold serial dilution of the samples were carried out as described by ICMSF (1974), after which spread plate techniques as described by Cheesbrough (2005) was used for inoculation.

The plates were then incubated for 24 hours at 35^{o}C. Following the incubation, discrete bacterial colonies were obtained and identified using methods described by Fawole and Oso (2004) and Cheesbrough (2005). Fungal isolates were examined macroscopically and microscopically using the needle mouth technique and identified following the schemes of Alexopoulous and Mims (1981) and Barnet and Hunter (1972).

Statistical analysis

The values obtained for microbial counts were analyzed using ANOVA, Window's SPSS, and version 16.0.

Results and Discussion

The mean results of the total aerobic microbial counts for the snuff samples from various zones are shown in Table 1. The mean total aerobic bacterial counts ranged from $2.85x10^4 \pm 2.06 - 5.67x10^4 \pm 2.62$cfu/g. The trend of the variations in bacterial counts for the three zones showed that Okigwe > Owerri > Orlu. The fungal counts ranged from $1.00x10^3 \pm 0.00 - 2.40x10^3 \pm 1.67$cfu/g. The trend of the variations in fungal counts for the zones showed that Owerri > Okigwe > Orlu. Comparative analysis results however showed no statistical difference in counts recorded at various zones at $P>0.05$. Table 2 shows the percentage occurrence of each bacterial isolates. *Staphylococcus* recorded the highest percentage occurrence (43.5%), followed by *Micrococcus* (34.8%), while *Bacillus* and *Streptococcus* recorded the least (10.9%). Table 3 shows the percentage occurrence of the fungal isolates. *Penicillium* recorded the highest percentage occurrence among the isolates (50%), followed by *Aspergillus* (27.8%) while *Rhizopus* recorded the least (22.2%). Percentage distribution of the bacterial and fungal isolates in the zones showed that Okigwe zone had the highest percentage occurrence of the bacterial isolates across the zones (39.1%), followed by Orlu zone (32.6%) while Owerri zone recorded the least (28.3%). Owerri zone however recorded the highest percentage occurrence of the fungal isolates among the zones (38.9%), followed by Okigwe (33.3%) while Orlu had the least (27.8%), as shown in Figure.1.

The identification tests revealed the presence of *Bacillus, Staphylococcus, Micrococcus, Streptococcus* , *Penicillium*, *Aspergillus* and *Rhizopus* species, . Some of these isolates have been reported by previous authors (Tansey and Brock, 1972; Tansey, 1975; Rubinstein and Pederson, 2002 and Ayo-Yusuf *et al*., 2005).

Factors such as lack of proper heat treatment during the fire curing process of raw tobacco leaves can also produce contaminated products. Some of these fungi have been reported as potentially pathogenic to humans (Greenwood *et al*., 1992). The hygroscopic nature of dried tobacco leaves and snuff creates a suitable environment for the growth of microorganisms (Wightman, 1956 and Fletch *et al*., 1967). Some of these fungi might however be important in the fermentation of raw tobacco leaves and hence impacting the desired flavour. The high percentage occurrence of bacterial isolates in Okigwe compared to the other zones could be attributed to relative differences in the sanitary conditions of the markets in these zones. Owerri zone however recorded the highest percentage occurrence among the fungal isolates. The sanitary conditions as well as the nature and volume of activities around the markets could be important.

Staphylococcus sps recorded the highest percentage occurrence of 43.5% among bacteria. This could be as a result of the hardy nature of the genera which enables them to withstand the low water activity and high salt content of snuff. Though *Staphylococcus* species don't grow outside the body, they are however hardy and though not spore formers, they may remain alive in a dormant state for several months when dried in dust, pus, or sputum (Greenwood *et al*., 1992). Possible species

of *Staphylococcus* that are pathogenic to man include *S. aureus, S. epidermidis* and *S. saprophyticus* (Prescott *et al*., 2005).

Micrococcus sps recorded the percentage occurrence of 34.8%. This high percentage occurrence of the organism in snuff samples could be attributed to their resistance to desiccation and nutrient deprivation (Greenwood *et al*., 1992 and Tolaro, 2005). The organism, being a normal flora of the human body may have been introduced into the snuff by the handlers in the course of processing and packaging the snuff (Ehiri *et al*., 2001 and Nwanze *et al.*, 2010).

Bacillus sp recorded a percentage occurrence of 10.9%. As a spore former and ubiquitous organism, *Bacillus* could have been introduced into the snuff from the environment during the processing of tobacco leaves to snuff. The spores could be resistant to the conditions of curing (Bhoola *et al*., 1992; Imamura *et al*., 1994; Nicholson and Setlow, 1990; Rubinstein, 2000 and Rubinstein *et al*., 2001). Bacillus has potential health implications (Ayo-Yusuf *et al*., 2000 and Prescott *et al*., 2005).
Streptococcus sp recorded the least percentage occurrence of 10.9% in the samples. This organism is a commensal in human body which could have been the sources of the organism in the snuff. Some strains of the organism have been implicated in dental caries (Greenwood *et al*., 1992).

This could be a serious health concern since some snuff users either sniff up or tuck it between the lower lip and the gum (Tansey 1975 and Ayo-Yusuf *et al*., 2000). Also, some content of tobacco and snuff might have enhanced the growth of *Streptococcus* sps as reported by Keen and Johnson (1999) and Lindermeyer *et al*. (1981). The markets where the snuff are sold were in poor sanitary conditions and most of the snuff vendours were observed to also sell other items, including perishables, thus giving room for possible cross contamination.
Rhizopus sps recorded percentage occurrence of 22.2%. *Rhizopus* species are spore formers and these spores could illicit allergic reactions in humans. Some of such fungi and actinomycetes in tobacco and its products and their health implications have been reported (Kurup *et al*., 1983 and Verweij *et al*., 2000).

Contamination by these fungi and their spores could have resulted from processing with contaminated materials such as dirty pestles, mortars, stones, machines, and even cellophane papers. The level of activities going on around the environment of the processing also tend to boast the microbial load of the resulting products and the selective effect of the process on the ecology of the surviving microbial populations (Thatcher and Clark, 1973).

Aspergillus sps recorded percentage occurrence of 27.8%. They can be found in hay, soils, and compost piles (Eicker, 1972). These spores and ubiquitous nature of *Aspergillus* can explain their relative high percentage occurrence in snuff. Some *Aspergillus* species could be dangerous, having been implicated in food contamination (aflatoxins), aspergillosis, increased incidence of severe asthma, sinusitis and chronic bronchitis (Chester *et al*., 1971; Fisher *et al*., 1981 and Willey *et al*., 2008). Also, in Imo State, most snuff users are elderly people, with lower immunity and other health conditions. This opportunistic pathogen is reported to infect immunocompromised individuals with high mortality (Willey, 2008). However, this may not be significant as a few spores may not be enough to establish respiratory mycosis (Greenwood *et al.*, 1992).

Table.1 Mean Total Aerobic Microbial Counts for Different Zones (cfu/g)

Location	Bacterial counts	Fungal counts
Okigwe	$5.67x10^4 \pm 2.62$	$1.2x10^3 \pm 0.28$
Owerri	$4.95x10^4 \pm 2.28$	$2.4x10^3 \pm 1.67$
Orlu	$2.85x10^4 \pm 2.06$	$1.0x10^3 \pm 0.00$

No statistical difference was observed among counts from different zones for both bacteria and fungi at $P>0.05$

Table.2 Percentage Occurrence of Each of the Bacterial Isolates

ZONES	*Bacillus*	*Micrococcus*	*Staphylococcus*	*Streptococcus*	%Occurrence [b]
Okigwe	1	8	7	2	39.1
Owerri	2	5	5	1	28.3
Orlu	2	3	8	2	32.6
%Occurrence [a]	10.9	34.8	43.5	10.9	100

a = % Occurrence of individual isolate across the zones
b = % Occurrence of total isolates from each zones
Numbers represent the frequency of occurrence of individual isolate in the zones

Table.3 Percentage Occurrence of Each of theFungal Isolates

Location	*Rhizopus*	*Penicillium*	*Aspergillus*	*% O*ccurrence [b]
Okigwe	1	2	3	33.3
Owerri	2	3	2	38.9
Orlu	1	4	0	27.8
%Occurrence [a]	22.2	50	27.8	100

a = % Occurrence of individual isolate across the zones
b = % Occurrence of total isolates from each zones
Numbers represent the frequency of occurrence of individual isolate in the zones

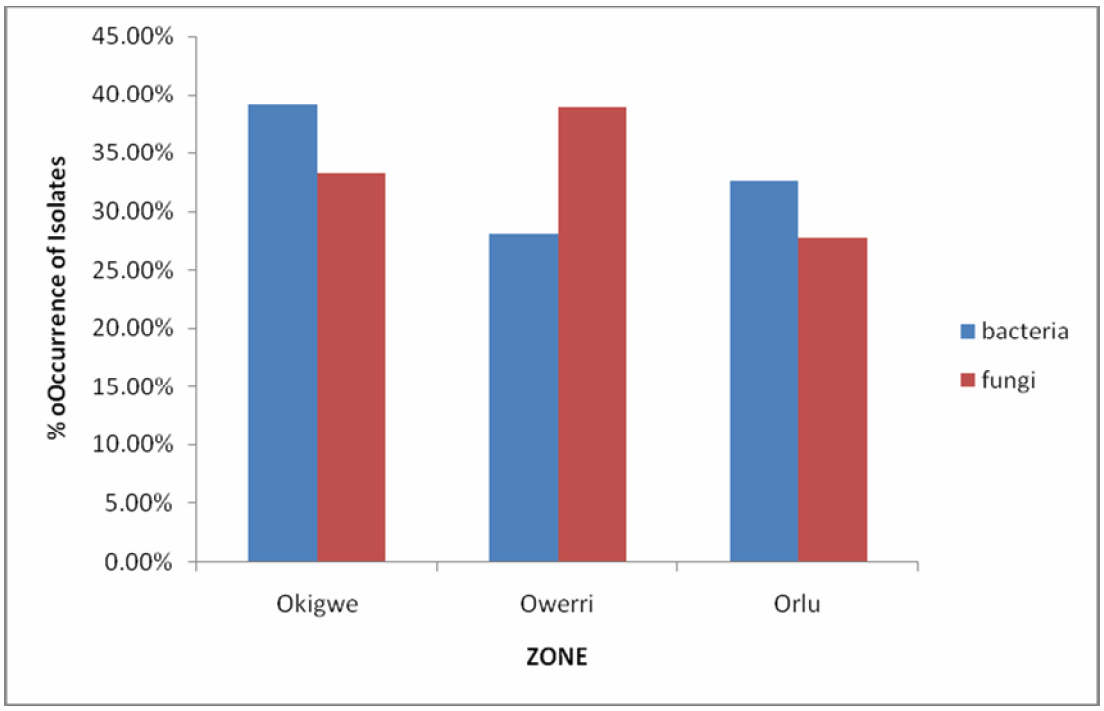

Fig.1 Percentage Distribution of Bacterial and Fungal Isolates in Different Zones

Penicillium sps recorded the highest percentage occurrence of 50%. This could equally be attributed to their resistant spores and ubiquitous nature. Snuff has low water activity and is hygroscopic in nature. *Penicillium* species have been reported to grow under such conditions (Willey *et al*., 2008). As *Penicillium* species are normal microbiota of external ear, they may have been introduced into the snuff in the course of processing and handling of the snuff (Willey *et al*., 2008).

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