Isolation and Characterization of *Listeria monocytogenes* from Kunu, a Locally Produced Beverage Marketed in Different Markets in Abia State of Nigeria.

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**Abstract:** This study was carried out to determine the level of contamination of kunu beverage by *Listeria monocytogenes*, monitor the level of antibiotic resistance among *Listeria monocytogenes* isolates. The percentage composition of crude proteins, crude fats, ash, carbohydrates were quantitatively determined. The rate of occurrence of the isolates of *Listeria monocytogenes* was almost 100% in a total sample size of eighty-seven (87). The bioloads of *Listeria monocytogenes* in kunu samples studied ranged from 11.0 x 10^6 cfu/ml – 41.0 x 10^6 cfu/ml. Antibiogram of *Listeria monocytogenes* showed that Ampicillin resistance was profound. The security of kunu as a food product is hampered due to listerial contamination. Government and Non-Governmental Agencies should intensify actions to reduce if not eradicate food-borne listeriosis in all parts of the world.

**Key words:** *Listeria monocytogenes*, food security, kunu, antibiotic resistance, Nigeria.

**INTRODUCTION**

Kunu Zaki is a cereal-based beverage in Nigeria. The beverage is also known and marketed as kunu in all parts of Nigeria. The cereals used in its production are millet, sorghum, and maize in decreasing order of preference. (Gaffa et al., 2002)

The traditional production process involves: steeping the grain in a local household utensils such as calabashes, and earthen ware vessels and grinding of the steamed grain with ginger in grinding machines to pulverize the grains for enzymatic actions. (Adeyemi and Umar, 1994; Onuorah S.I. et al., 1987)

Boiling of the slurry and gelatinization follows this. Mixtures of sweet potato, tuber paste and malted rice which are already grounded, are also added during boiling. The mixtures are left at room temperatures for 2-4 days, and filtered using local sieve.

The filtrate, which is the kunu is served with or without sugar and either cold or warm

The contents and microbiological quality of this product have been studied and reported. (Onuorah et al., 1987; Gaffa et al., 2002; Gaffa et al., 2002). An improved method of production involves washing grains thoroughly with clean water, steeping in water (60-70°C) with 0.5% sodium metabisulphate for three (Onuorah et al., 1987) hours, wet grinding with other clean ingredients, liquefaction and saccharification at 60 – 70°C for six hours. This is followed by filtration with sterile cloth materials, bottling and pasteurization at 60°C for 30 minutes.(Gaffa et al., 2002)

Bacterial contamination of both traditional and improved kunu beaverages has been widely reported in all parts of Nigeria. (Chukwu et al., 2003).

A major obstacles in the consumption of kunu is the outbreak of listeriosis, a food borne disease which manifests as meningitis among infants, and miscarriages among pregnant women. (Kayser, 2001) The disease called listeriosis, is caused by *Listeria monocytogenes*, a gram positive, facultative anaerobe which occur singly or in pairs, also in short chains. (Murray et al., 2002)

The organism is isolated from soil, water, vegetations, animal meat, poultry materials, milk, cheese, turkey etc. (Nester et al., 2004; Charpenter and Courvalin, 1999). Numerous raw, processed, cooked food items have been contaminated with *Listeria monocytogenes* at levels above ten organisms per gram. (McLauchin and Riegel, 2002; Low and Donachie, 1997).

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The presence of a single cell of the organism is grossly intolerable in terms of public health and food security. (Willey et al., 2008). This study investigated the contamination of kunu a lactic acid fermentation product in Nigeria by *Listeria monocytogenes* and also investigated the level of antibiotic susceptibility Patterns of the isolates from the kunu beaverages studied.

**MATERIALS AND METHODS**

**Study Area:** The study area for the work was Aba, a commercial city in Eastern Nigeria. The major occupation of the people is trading, and the major source of domestic water is bore holes. It is a city with widespread report of hepatitis virus infection, and a wide range of bacterial infections as a result of dense population and poor hygiene.

**Sample Collection:**
87 samples of kunu beaverages were purchased from eight (Kayser, 2001) different markets in Aba, Abia State – Nigeria. They were labeled at point of purchase and transported to the microbiology laboratory of Abia State University, Uturu – Nigeria for laboratory studies within 3 – 4 hours of collection.

**Isolation and Characterization of *Listeria monocytogenes* from Kunu Beaverages:**
The samples were plated on a *listeria selective* medium (Oxoid Ltd, Basingstoke, Hants, England, CM 0856). The wire Loop was flamed before collecting a loopful of sample, and after streaking to avoid contamination from the laboratory.

The plates were incubated at 37°C ± 2°C in anaerobic condition for 24 – 48 hours. The emergent growth colonies were gram stained and subjected to array of biochemical tests such as blood agar haemolysis, catalase test, motility test, methyl red, voges-proskauer tests. Other biochemical tests conducted include: glucose, lactose, mannitol, maltose, sucrose fermentation tests.

Isolates which were β-haemolytic, catalase-ve, motile, methyl red +ve, voges-proskaeur +ve, and produces acid in the fermentation of glucose, lactose, mannitol, maltose, sucrose were identified as *Listeria monocytogenes*. The procedures adopted for the isolation and characterization of the *Listeria monocytogenes* have been previously reported. (Chukwu et al., 2006; Onwuchekwa and Okereke, 2004; Nwachukwu and Azu, 2004).

**Serial Dilution and Plate Count:**
The bacterial load in kunu samples analysed were obtained using procedures which were previously reported. (Nwachukwu and Azu, 2004).

**Antibiotic Susceptibility Testing:**
Seven (Chukwu et al., 2003) antibiotics, which represent the commonly used and abused antibiotics in the study Area, were used. The antibiotics were already impregnanted into paper discs at different concentrations and marketed by optun laboratories, Nigeria Ltd, Aba, Abia State, Nigeria. The antibiotics and their concentrations were as follows: Ampicillin (25µg/disc), Chloramphenicol (30µg/disc), Erythromycin (15µg/disc), Tarivid (5µg/disc), Zinnat (30µg/disc), Augumentin (30µg/disc), and Peflacine (25µg/disc). Antibiotic susceptibility testing was carried out using the procedures reported by Adetunji and Adegoke. (2008)

**Determination of Food Nutrients in Kunu Samples:**
The determination of food nutrients such as crude protein, crude fat, ash, carbohydrates, total soluble solids was done using procedures previously reported by James (1995). The determination of pH of kunu samples was done using pH metre (Beudeux, USA) in accordance with methods previously reported. (Brown and Booth, 1994). Determination of nutrients concentration and other related parameters were carried out at National Root Crop Research Institute, Abia State – Nigeria.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Improved Kunu (% Content)</th>
<th>Traditional Kunu (% Content)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>(81.40)</td>
<td>(93.9)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Crude protein</td>
<td>(4.78)</td>
<td>(3.86)</td>
<td>0.05*</td>
</tr>
<tr>
<td>Crude fat</td>
<td>(0.32)</td>
<td>(0.33)</td>
<td>0.44</td>
</tr>
<tr>
<td>Ash</td>
<td>(1.30)</td>
<td>(1.90)</td>
<td>0.49</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>(4.81)</td>
<td>(3.76)</td>
<td>0.04*</td>
</tr>
<tr>
<td>pH</td>
<td>(5.50)</td>
<td>(5.0)</td>
<td>0.45</td>
</tr>
<tr>
<td>Total Soluble Solids</td>
<td>(7.80)</td>
<td>(6.41)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Chi-square statistics was used. P values were set at 0.05 and values* < 0.05 are significant.
Prevalence of Occurrence of *Listeria monocytogenes* from Kunu Samples from Different Markets in Nigeria

<table>
<thead>
<tr>
<th>Markets</th>
<th>No of Kunu Samples</th>
<th>Percentage Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>12 (100)</td>
</tr>
<tr>
<td>B</td>
<td>14</td>
<td>14 (100)</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>10 (100)</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>7 (100)</td>
</tr>
<tr>
<td>E</td>
<td>12</td>
<td>8 (66.67)</td>
</tr>
<tr>
<td>F</td>
<td>11</td>
<td>10 (90.90)</td>
</tr>
<tr>
<td>G</td>
<td>13</td>
<td>12 (92.30)</td>
</tr>
<tr>
<td>H</td>
<td>14</td>
<td>14 (100)</td>
</tr>
</tbody>
</table>

Numbers in brackets are in percentage.

N = sample size = 87

The bioloads of *Listeria monocytogenes* in samples designated A, B, C, D, E, F, G, H, I, J were observed at 25.0 x 10^5 cfu/ml, 33.0 x 10^5 cfu/ml, 30 x 10^5 cfu/ml, 30 x 10^5 cfu/ml, 14 x 10^5 cfu/ml, 27 x 10^5 cfu/ml, 41.0 x 10^5 cfu/ml, 11 x 10^5 cfu/ml, 18 x 10^5 cfu/ml and 33.0 x 10^5 cfu/ml respectively (Table 2).

The antibiogram of *Listeria monocytogenes* from kunu sample analysed was studied. Ampicillin resistant listeria monocytogenes were isolated, from this study. Chloramphenicol, Erythromycin, Tarivid, Zinnat, Augumentin, Peflacin, mm; millimeter.

RESULTS AND DISCUSSION

Quantitative analyses of kunu showed that the product contains moisture, crude protein, crude fat, ash, and carbohydrate. The improved kunu products significantly showed higher content of moisture (81.40%) than the traditional product (93.9%) (P = 0.03).

The improved kunu also had higher contents of crude protein, and carbohydrate than the traditionally produced kunu products (P = 0.04 – 0.05). The pH values of both brands ranged between 5.0 – 5.50 (Table 1).

Randomized sampling from 8 different markets in Aba showed that samples from A, B, C, D, E, F, G, H had 100%, 100%, 100%, 100%, 66.7%, 90.90%, 92.30% and 100% prevalence rate of *Listeria monocytogenes* respectively. (Table 2).

The antibiogram of *Listeria monocytogenes* from kunu sample analysed was studied. Ampicillin resistant listeria monocytogenes were isolated, from this study. Chloramphenicol, Erythromycin, Tarivid, Zinnat, Augumentin, and Peflacin resistant and susceptible isolates of *Listeria monocytogenes* were observed in different samples of kunu. However,ampicillin resistance was more profound in most of the samples of kunu studied. (Table 4)
The results obtained in this study have shown that *Listeria monocytogenes* is common contaminants of a Nigeria local beaverage sold and marketed as kunu. The findings suggests that the public health qualities of the product is doubtful. The production quite unwholesome for human consumption.

This work is similar to that of Bille, (1999) Gaffa and Ayo (2002) Chukwu et al, (2006) who independently reported the isolation of *Listeria monocytogenes* in ready-to-eat dairy products. This is the first report of *L. monocytogenes* contamination in a local beaverages like kunu. The findings in this study are similar to the previous cases reported in Nigerian processed meats and ready-to-eat dairy products like cooked salami, meat loaf, suya, cheese (gouda), unpasteurized (raw) milk sold as fura-de-nunu, ice cream which are contaminated with *Listeria monocytogenes*.

This study investigated the bioload of *listeria monocytogenes* in the various brands of kunu, and discovered that the listerial load varied from $1.1 \times 10^5$ cfu/ml – $4.1 \times 10^5$ cfu/ml. The bioload is apparently high, and may be justified by the fact that the raw materials including water used in the production process might be heavily contaminated. It is worthy to note that both improved and traditional kunu have no quality control measures and critical control points, as such the contamination by highly pathogenic *Listeria monocytogenes* is also justified.

This high bioloads recorded is similar to the cases reported in kunu production in the studies in Northern Nigerian isolated mainly enteric nautical pathogens Northern Nigeria, (Gaffa et al., 2002; Chukwu et al., 2006). In this study, a commercially made antibiotic susceptibility disk was used to check the antibiotic susceptibility pattern of *Listeria monocytogenes* isolates Ampicillin resistance was profound among ten isolates which were randomly selected. This could be explained by the fact the organism may have acquired genes for Ampicillin resistance in the chromosome or extrachromosomal plasmids. This is in contrast with usual scientific report that Ampicillin is the drug of choice for listeriosis, caused by *Listeria monocytogenes*.

The observation of ampicillin resistant *Listeria monocytogenes* of public health importance, as we suggest that resistance occurred as a result of abuse of ampicillin. Ampicillin is among the antibiotics sold in motor parks, streets, without the prescriptions of doctors, pharmacists. The study showed that Peflacine, Tarvid and Chloramphenicol (in decreasing order) remained most active against this bacterial pathogen. This might be explained by the fact that these groups of drugs especially Peflacine, Tarvid are expensive.

This later observation of drug resistance is similar to the work of David and Odeyemi (2006) who investigated the antibiogram of environmental isolates of *listeria monocytogenes* in Ado-Ekiti, Nigeria and discovered that chloramphenicol, fluoroquinolones which are broad spectrum antibiotics were significantly effective against this organism.

In Nigeria, regulatory agencies like National Agency for Food and Drug Administration and Control (NAFDAC) need to intensify their campaign against the sales of traditionally manufactured kunu, which serve as a food vector for transmission of listeriosis.

International Agencies such as the World Health Organization should direct their research funding attention to listeriosis transmission through food products in developing countries.

The global attempt to ensure the security of food products should also be directed towards the production of wholesome local beaverages in developed and developing countries.

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