

Isolation And Characterization of Coagulase-Positive *Staphylococcus Aureus* From Traditionally Fermented Food Condiments Sold In Some Markes In Awka And Enugu, South East Nigeria

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Abstract: The presence of coagulase positive *Staphylococcus aureus* was examined in traditionally fermented food condiments 'Ogiri' from castor oil bean (*Ricinus communis*), 'Okpei' from mesquite seed (*Prosopis africana*) and 'Ugba' from African oil bean (*Pentaclethra macrophylla*). Samples of these condiments were purchased from ten different open markets in Enugu and Anambra states. *Staphylococcus aureus* was isolated using a selective medium; Mannitol salt agar. A total of 150 samples were examined and 26 of them (17.3%) were found to contain coagulase-positive *Staphylococcus aureus* and the prevalence rates of the organism in the two states examined revealed that Enugu state has a prevalence rate of 16% while Anambra state had 18.6%. Statistical analysis showed that the prevalence of the organism was significant ($P=0.05$) and that Enugu state food condiments were independent of Anambra state market food condiments under investigation.

Keywords: Coagulase-positive *Staphylococcus aureus*, Ogiri from (*Ricinus communis*), Okpei from (*Prosopis africana*), Ugba from African oil bean (*Pentaclethra macrophylla*), *Macrophylla*, fermentation, Anambra and Enugu.

I. Introduction

Traditionally fermented food condiments are often displayed in the Nigerian market under poor hygienic conditions. All fermented condiments are prone to microbial spoilage or contamination, the extent of which depends upon a number of factors such as poor personal hygiene, improper handling and the kinds of organisms present in the food. Certain environmental factors affect the keeping quality of these products such as temperature and humidity at which the food condiments are held (Blaiotta *et al.*, 2004).

Traditional diets in west African often lack variety and consist of large quantities of the staple food (cassava, yam, maize etc) with supplements of plantain, cocoyam, rice and beans depending of their availability and season (Ogunshe *et al.*, 2006). Soups eaten with the staples are essential component of the diet and may contain a variety of seeds, nuts, pulses and leaves (Campbell-Platt, 1980). Soups are the main sources of protein and minerals and one of the ways to improve the diet has been to improve the nutrient content of the soups.

Seeds of legumes may account for up to 80% percent of dietary protein and may be the only source of protein for some groups. Their cooked forms are eaten as meals and are commonly used in fermented form as condiments to enhance the flavour of food (Odufa, 1985; Achi, 1992). With high contents of protein, legume condiments can serve as tasty complements to sauces and soups and can substitute for fish or meat. The food flavouring condiments are prepared by traditional method of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate (Eka, 1980).

Fermented products remain of interest since they do not require refrigeration during distribution and storage. The traditional condiments have not attained commercial status due to the very short shelf life, objectionable packaging materials, stickiness and the characteristic putrid odour (Arogbu *et al.*, 1995). Fermented condiments often have a stigma attached to them: they are often considered as food for the poor.

The production of fermented vegetable proteins for use as food condiments is craft-based. Remarkably, in many areas of Nigeria today, they are still made in traditional ways with success depending upon observance of food manufacturing practices and control of environmental conditions during the manufacturing practices. Starter cultures are not normally used and therefore variation in the quality and stability of the product are often observed. Apart from 'dawadawa' prepared from the African locust bean, by far the bulk of the indigenous fermented condiments of Nigeria are to be found in the southern states of Nigeria. The north is very poor in food fermentations, which are practically confined to be staple sorghum porridges and to soured milks (Dirar, 1993). Interstate trade and relocation has widened the scope of the spread of food condiments throughout the country and beyond (Iwuoha and Eka, 1996). In Nigeria, these condiments are wrapped in banana leaves or any other leaf for ogiri and ugba while okpei is kept in bottles or plastic containers with lid covering them.

Staphylococcus aureus is a ubiquitous bacterium: being both a human and zoonotic commensal, it is regarded as potentially hazardous in foods due to the production of heat-stable enterotoxins (SEs) (Sandel and Mckillip, 2004). SEs are classified as members of the pyrogenic toxin super antigen family because of their similar biological activities and structural relatedness (Omoe *et al.*, 2002). Enterotoxin-producing *Staphylococcus aureus* plays an important role as causative organism of food intoxications. In many countries, *Staphylococcus aureus* is considered to be the second or third most common pathogen causing outbreaks of food poisoning only outnumbered by *Salmonella spp* and in competition with *Clostridium perfringens* (Anon, 1990, Bean and Giriffin, 1990).

Staphylococcal enterotoxin (SE) are resistance to proteolytic enzymes and the human intestinal tract and are heat stable protein with a molecular weight of approximately 27-311 KDA (Betley *et al.*, 1992; Balaban and Rasooly, 2000) thus

cases of illness might occur although no viable bacteria can be isolated from the suspected food stuff. The primary reservoirs of *Staphylococcus aureus* are the skin and the mucus membranes, especially of the nasopharyngeal region of birds and mammals. The pathogen is found in 30 -80% of the human population and one to two-thirds of these carriers harbour enterotoxigenic strains (Wieneke *et al.*, 1993). Thus, unhygienic treatment of food has to be considered as a major risk of contamination, and staphylococcal food poisoning is often associated with highly handled food (Catteau, 1993). This research aims at isolation and characterization of coagulase-positive *Staphylococcus aureus* from traditionally fermented food condiments sold in some markets in Awka and Enugu, South East Nigeria.

II. Materials And Methods

Collection of samples

Fermented foods condiments were purchased from ten different open markets in Enugu and Anambra states (five markets each) in which five samples of Ogiri Igbo, Ugba, and Okpei were bought for each of the markets. Ogiri Igbo and Ugba were wrapped in banana leaves prior to purchase while okpei was in a small plastic container covered with a lid and brought to the laboratory in a polythene bag. Visual observation on each of the condiments before analysis showed no sign of spoilage. The basic substrate used to make the samples are as follows:

- * Ogiri made from castor oil bean (*Ricinus communis*)
- * Okpei made from mesquite seed (*Prosopis africana*)
- * Ugba made from African oil bean (*Pentaclethra macrophylla*)

Media used

Two media namely, Mannitol Salt Agar (MSA) (oxid) and Nutrient Agar (oxid) containing 7% sodium chloride for the slant culture. All the media, chemicals and reagents used in the study were of analytical grade. The media were measured out and prepared according to manufacturer's instructions.

Microbiological analysis

One gram each of fermented sample (Ugba, Ogiri and Okpei) was taken aseptically and was thoroughly mashed with clean sterile laboratory mortar and pestle and suspended in 9ml of sterile distilled water in a sterile test tube and the content was thoroughly mixed by shaking and using sterile glass rod. Ten-fold serial dilution was thereafter prepared. About 0.1ml of (10^{-3} , 10^{-4} , and 10^{-5}) dilutions were plated on Mannitol Salt Agar (MSA) then incubated at 37°C for 24h. Plates were observed for growth., colonies obtained were purified and preserved on nutrient agar slants containing 7% sodium chloride and kept in a refrigeration temperature (4°C) until use.

Characterisation and Identification of Bacterial Isolates

The cultural characteristics of the isolates on the agar plates were observed. The identification procedures for the bacteria were carried out according to the methods of Cowan and Steel (1996), and Cheesbrough, (2003) which are as follows:

Confirmatory Tests

Series of confirmatory tests were done to identify the organisms isolated, these include: Gram Staining (Cheesbrough, 2003), biochemical tests, catalase test (Harrigan and Maclance, 1986), coagulase test (Cowan and Steel, 2003) and sugar fermentation test (Harrigan and Maclance, 1986). Specifically growth in 17% NaCl according to Cheesbrough (2003) was used to confirm coagulase-positive *S. aureus*.

III. Results

Three different fermented food condiments were examined for the presence of coagulase positive *Staphylococcus aureus*. For each type of condiment, fifty (50) samples were examined. The assays performed on the one hundred and fifty samples demonstrated the presence of coagulase positive *Staphylococcus aureus* in 26 samples (17.3%). Preliminary examination of cultured plates revealed that not all fermented condiments samples showed the presence of Gram positive *Staphylococcus aureus*.

The morphology and biochemical characteristics of the bacterial isolates are shown in Table 1. The prevalence rate of *Staphylococcus aureus* in the 150 samples investigated was 17.30% as seen in Table 2. The prevalence rate of *Staphylococcus aureus* in condiments from Enugu state markets was 16% as shown in Table 3. The prevalence rate of *Staphylococcus aureus* in condiments from Anambra state markets was 18.6 % as shown in Table 4.

The result of the statistical analysis on the prevalence of the organism in the 150 samples using chi square shows that coagulase positive *Staphylococcus aureus* prevalence rate was significant in the investigated fermented condiment since $F_{cal} = -0.6724 < F_{tab} = 9.55$, (Table 5). Contingency table to show that Enugu and Anambra state food condiments were independent showed that $X^2_{cal} = 0.1861 < X^2_{tab} = 3.81$ (Table 6).

Table 1: Biochemical characteristics of *Staphylococcus aureus* isolated from the condiments (Ogiri, Okpei and Ugba) in Enugu and Anambra markets.

Biochemical Tests	Results
Grams Reaction	+
Catalase	+
Coagulase	+
Growth in 3%, 5%, 7%, NaCl broth	+
Glucose	A/G
Maltose	A/G

Lactose	A
Sucrose	A/G
Dulcitol	A
Fructose	A/G
Mannitol	A/G
Kes: + = Positive A = Acid production A/G = Acid/Gas Production	

Table 2: Prevalence of Coagulase Positive *Staphylococcus aureus* in the 150 samples of fermented food condiments investigated.

Fermented food condiments	No of samples Examined	No of Positive samples	% of Positive samples
Ogiri-Igbo	50	9	18
Ugba/ukapaka	50	12	24
Okpei	50	5	10
Total	150	26	17.3

Table 3: Prevalence of Coagulase Positive *Staphylococcus aureus* in condiments from Enugu state markets

Market	No of samples Examined	No of Positive samples	% of Positive samples
Nkwo Eziagu	15	5	33.3
Ugwuoba	15	4	26.6
Oji River	15	2	13.3
New Market	15	1	6.6
Afia Ogige	15	0	0.0
Total	75	12	16

Table 4: Prevalence of Coagulase Positive *Staphylococcus aureus* in condiments from Anambra state markets

Market	No of samples Examined	No of Positive samples	% of Positive samples
Eke Awka	15	3	20
Nkwo Amaenyi	15	3	20
Agu Ukwu	15	6	40
Ose Onitsha	15	2	13.3
Awka-Etiti	15	0	0.0
Total	75	14	18.6

Table 5: Anova Table for the prevalence of coagulase-positive *Staphylococcus aureus* in Enugu and Anambra state markets

Source of Variation	SS	d.f	Ms	F ratio
SST	-1250.0	2	-625	-0.6724
SSE	2900	3	966.67	

Hypothesis

H₀: Coagulase positive *Staphylococcus aureus* prevalence is significant.

H₁: Coagulase positive *Staphylococcus aureus* prevalence is not significant.

$\alpha = 5\%$ F1 - α ; r-1, N-r

F 0.95, 2, 3 = 9.55

Table 6: Contingency Table showing the incidence of Enugu and Anambra state food condiments

Status Market	Positive	Negative	Total
Anambra	14	61	75
Enugu	12	63	75
Total	26	124	150

Hypothesis

H₀: Enugu and Anambra state markets are independent.

H₁: Enugu and Anambra state markets are not independent

Let $\alpha = 5\%$

$X^2_{0.95, 1} = 3.81$

Since $X^2_{cal} = 0.1861 < x^2_{tab} = 3.81$. By this it is concluded by accepting H₀ which states that Enugu market food condiments are independent of Anambra market food condiments (Snedecor and Cochran,1976).

IV. Discussion

This work studied the presence and characteristics of coagulase-positive *Staphylococcus aureus* in fermented food condiments bought from ten different markets in Enugu and Anambra states. The high prevalence rate of coagulase positive *Staphylococcus aureus* detected in the samples examined (17.3%) and from the statistical analysis, it can be concluded that prevalence is significant since $F_{cal} - 0.6724 < F_{tab} = 9.55$; it is concluded by accepting H₀ which showed that coagulase-positive *Staphylococcus aureus* prevalence is significant in the investigated fermented food condiments (Lindman, 1999) (Table 5). Also Since $X^2_{cal} = 0.1861 < x^2_{tab} = 3.81$. it is concluded by accepting H₀ which states that Enugu market food condiments are independent of Anambra market food condiments (Snedecor and Cochran,1976) (Table 6). This showed that this organism is very common in nature and that there is a great likelihood that fermented food condiments may become contaminated.

From the study, Ugba samples showed the highest prevalence of 24% followed by Ogiri-Igbo (18%) and then Okpei (10%) which is in line with the speculation of Onoue *et al.*, (1986). It is speculated that the contamination is due to poor personal hygiene, improper handling during manufacture, inadequate storage conditions and from infected handlers.

From the result, Agu-Ukwu market in Nri, Anambra state had the highest number of positive samples (40%) followed by samples in Nkwo Eziagumarket in Enugu state (33.3%) and Afor Ugwuoba market samples in Enugu state (46.6%). Two markets, one from each of the two states showed no positive samples.

The contamination of these products occurred during mixed fermentation activities of different organisms randomly introduced into food through water, materials, equipments used, leaves for wrapping, air and human handlers who have skin lesions containing *S. aureus*, or by sneezing or coughing (Jay, 1986), improper food storage or preparation (Onoue *et al.*, 1986; Trantor, 1990).

The prevalence of coagulase positive *Staphylococcus aureus* in samples from Anambra state was higher (18.6%) than that of Enugu state (16%). This is due to poor sanitary condition in the manufacturing process and handling. Anambra people are so business conscious and they are usually in hurry to prepare these condiments thereby introducing contaminants in the process. Traditional fermented food condiments (Ogiri, Okpei, Ugba etc) are made under primitive conditions which result in introduction of contaminants such as coagulase positive *Staphylococcus aureus* which result to poor quality. These condiments have not attained commercial status due to the very short shelf life, objectionable packaging materials, stickiness and the characteristic putrid odour (Arogba *et al.*, 1985). Health symptoms related to consumption of fermented food condiments with high load of coagulase positive *Staphylococcus aureus* may lead to food poisoning which is presented as gastrointestinal symptom observed during intoxication and vomiting with or without diarrhoea.

Generally, symptoms are short in duration (approximately 24 – 48 hours). However, severe dehydration can result, necessitating intravenous fluid supplementation. Most cases of Staphylococcal intoxication result from improper food storage or preparation (Trantor, 1990). The presence of *S. aureus* in foods commonly indicates contamination that has been directly introduced into the food by workers who have skin lesions containing *S. aureus*, or by sneezing or coughing (Jay, 1986).

If traditionally prepared food condiments are well done under a septic technique, they could be exported to earn the country some foreign exchange now that newer sources of foreign exchange are being sort by Nigeria. More research is therefore needed to upgrade the fermentation processes of traditionally fermented food condiments in Nigeria perhaps by the use of starter cultures.

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Appendix Iv: Anova Table

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Appendix V: Contingency Table

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