

Isolation and Susceptibility Pattern of *Staphylococcus Aureus* in Locally Fermented Milk - Cereal Mixture 'Fura-da-nono' Commonly Sold in Wamba LGA Area of Nasarawa State

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Abstract

Fura is a locally fermented cereal based food commonly consumed in the Northern part of Nigeria, it is thick soft ball snack that is produced mainly from millet or sorghum mixed with spices such as ginger, pepper, black pepper and cloves to give it its desired taste. It is a semi-solid meal made from millet or sorghum and is used as staple food in most West African countries including Nigeria and Ghana. Fura is considered to be natural food since the raw material (millet) has been reported to have protein content up to 11% protein by weight and are rich in B vitamins such as niacin, B6 and folic acid, iron, potassium, zinc, magnesium and calcium content. They are also rich in some phytochemicals, including phytic acid which lower cholesterol and reduce the risk level of cancer in those who consume it. Moreover, cereals as functional foods provide dietary fibre, energy, protein, minerals, vitamins and anti-oxidants required for maintenance of human health. The aim of this study was to isolate *Staphylococcus aureus* in locally fermented milk-cereal mixture 'fura-da-nono' and to carry out susceptibility test on the isolate of *Staphylococcus aureus* recovered from 'fura-da-nono'. The study was conducted in Wamba Local Government Area of Nasarawa State using cross sectional study design. Two markets in Wamba Local Government area are selected based on convenience and availability of 'fura-da-nono'. These include Sisinbaki market and Wamba main market. Random sampling method was applied for the selection of 100 samples (24 samples from Sisinbaki market and 76 samples from Wamba main market). Each sample was collected in a sterile sample bottles and transported on ice to the Microbiology Laboratory Section of Diagnostic Laboratory Division, National Veterinary Research Institute, Vom, Jos, Nigeria for culture and microbiological examination. Chi-square test was used in determining the statistically significant difference between the occurrences of *Staphylococcus aureus* in locally fermented milk-cereal mixture (fura-da-nono) in areas all the samples. $P \geq 0.05$ is considered significant. The isolation of *Staphylococcus aureus* in locally fermented milk-cereal mixture (fura-da-nono) in the study area suggests that consumption of dairy products especially those that are produced using traditional methods constitute health hazard to consumers because the product can serve as medium for transmission of pathogenic microbes if is not prepared in hygienic way. The antibiotic susceptibility pattern of the *S. aureus* isolates revealed higher susceptibility to streptomycin, ciprofloxacin, gentamycin and cefixicin, while high levels of resistance to tetracycline were recorded. This could be of public health concern as tetracycline is one of the commonly used antibiotic in the study area to treat some infections. The findings from this study strongly suggests that selection pressure imposed by the use of antibiotics in human and in livestock production is a key driving force in the promotion of antibiotic resistance in *S. aureus* since this organism infect various hosts. This study revealed that Fura-da-nono sold in Wamba and Sisinbaki are unsafe for human consumption because they contain *Staphylococcus aureus* organisms.

Keywords: *S. aureus*; Milk; Nigeria

Introduction

'Nono' a locally fermented cow milk processed by the nomadic Hausas and Fulanis and constitutes a major part of the staple food in Northern Nigeria, often mixed with clumps made of millet or maize (fura) to make a preparation called "Fura-da-nono". The rate of consumption of nono is high in the Northern part of Nigeria compared to consumption of pasteurized milk due to the strong belief that locally processed raw milk and its by-product have nutritional advantages over the pasteurized ones. Milk meant for human consumption must be free of pathogenic organisms [1]. Raw milk-borne pathogens to humans, as it can be easily contaminated during milking and handling [2]. Specifically, human may be infected with milk borne pathogens through consumption of infected raw and unpasteurized milk products [1].

Source of microbial contamination might primarily be from the infected lactating animals, it could be also through the contamination of milk during milking, by the use of unsanitary utensils or milking equipment, or through use of contaminated, or from contaminated transportation vehicles and poor storage. Microbial contamination can also be through cross-contamination from previously fermented milk [3].

Fermentation of fresh cow milk by bacterial organisms result in a product called "nono", [2]. Nono is a healthy liquid food, which is opaque white to milky in color and is consumed by the Saharrah tribe to West Africa Sub-region up to the Mediterranean region to the Middle East region, where it is called 'Dahi' or 'Lassi' [4]. Nono which forms two major parts of marketable food in Northern part of Nigeria is one of their popular consumed foods. It can be obtained from raw milk of cows, sheep or goats that is unpasteurized. Food borne illness can result from the consumption of this raw and unpasteurized milk product because the product can contain dangerous bacteria such as *Salmonella spp*, *Escherichia coli* and *Listeria spp* [5]. Nono, which is also called Nunu in some parts of Nigeria is rich in amino acids (proteins building blocks), calcium, phosphorus and vitamins A, C, E and B complex [4].

In Nigeria, locally processed cow milk products are processed mainly by Fulanis, where raw milk is turned into "Nono", Kindrimo and Manshanu. Nono is the Fulani word for cow's milk sold by Fulani women [4] and it is produced from unpasteurized cow milk collected in a container (calabash) and allowed to ferment naturally for 24 hours [6].

Fermentation of cereals for the production and preservation of food has been a long time practiced throughout Africa [7,8]. The fermentation process leads to food preservation and increased in the organoleptic properties of due to the production of lactic acid and other compounds that enhances the taste and flavor of the product [9]. Fura is an indigenous fermented cereal based foods commonly consumed in the Northern part of Nigeria, it is thick soft ball snack that is produced mainly from millet or sorghum with spices such as ginger, pepper, black pepper and gloves. It is a semi-solid dumpling meal made from millet or sorghum and is used traditionally as staple food in most West African countries including Nigeria and Ghana [8,10]. During the preparation of fura, the cereal grains, millet or sorghum are soaked in water and allowed to ferment overnight and then drained. Then allowed to dry, before grinding into fine powder and mixed with hot water and continuous stirring to form a smooth paste which are then molded into balls and allowed to ferment for 1-4 days at room temperature to give it the desired taste. The molded balls are pounded and re-molded and then sun-dried which can also be dry-milled into powder which is reconstituted in water to get fura meal. Also, the cooked dough balls can be broken and mixed with fermented milk (nono) to form fura-da-nono which can be a complete food providing energy and protein need of people [7,8]. Fura can be considered to be a natural food since the raw material (millet) has been reported to have protein content up to 11% protein by weight and are rich in B vitamins such as niacin, B6 and folic acid, iron, potassium, zinc, magnesium and calcium content. They are also rich in phytochemicals, including phytic acid which lower cholesterol and reduce the risk level of cancer. Moreover, cereals are functional foods since they provide dietary fibre, energy, protein, minerals, vitamins and anti-oxidants required for human health and well-being [11].

The aim of this study was to isolate *Staphylococcus aureus* in locally fermented milk-cereal mixture 'fura-da-nono' and to carry out susceptibility test on the isolate of *Staphylococcus aureus* recovered from 'fura-da-nono'.

Materials and Methods

Study area

The study was conducted in Wamba Local Government Area of Nasarawa State Nigeria. Wamba Local Government was purposively selected for the study because of the high population of Fulanis and availability of 'fura-da-nono' there. Wamba is situated at 8.93°

North Latitude and 8.6° East Longitude and it is 319 meters above the sea level. Nasarawa State shares boundaries with Kaduna State in the North, Plateau State in the East, Taraba and Benue States in the South while Kogi and the Federal Capital Territory flanks it in the West (Area from Bureau of Land and Survey, 2005). Wamba is a small city in Nigeria having 27, 137 inhabitants, it is located close to the beautiful Farin Ruwa falls one of the highest water falls in Africa. The source of the water falls is the Jos Plateau- the highest altitude in the country. Wamba falls under Southern Guinea Savannah; this zone has an average annual rainfall of about 1000-1500mm spread over six months (May-October). The most abundant woody species in this zone are; *Drypetes floribunda*, *Vitex doniana* and *Entoda abyssinica*. Here, it is common to see species that are predominant in the forest Savannah occurring in the Southern and Northern fringes of the zone. The common grass species found here include *Pennisetum*, *Andropogon*, *Hyparrhenia* and *ctenion* [12].

Study design

Cross sectional study was carried out. Two markets in Wamba Local Government Area are selected based on convenience and availability of 'fura-da-nono'. These include Sisinbaki market and Wamba main market.

Sample collection

Sisinbaki market and Wamba main market were identified in Wamba Local Government Area for the samples collection. Random sampling method was applied for the selection of 100 samples (24 samples from Sisinbaki market and 76 samples from Wamba main market). Each sample was collected in a sterile sample bottles and transported on ice to the Microbiology Laboratory Section of Diagnostic Laboratory Division, National Veterinary Research Institute, Vom, Jos, Nigeria for culture and microbiological examination.

Culture and isolation of *Staphylococcus aureus*

Five hundred microliters of each sample was enriched in 4.5 milliliters of peptone broth and incubated at 37°C for 24 hours. A loop full of the broth culture was streaked on blood agar (BA) and incubated at 37°C for 24 hours. The colonies were examined for typical morphological characteristics on blood agar. Presumptive *Staphylococcus aureus* colonies were purified onto blood agar and nutrient agar. The cultured plates were incubated at 37°C for 24 hour and were further characterized by biochemical tests.

Morphological characteristics

The smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained using Grams' stains. The stained smear was examined under microscope. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes [13].

Biochemical examination

Biochemical tests were performed to confirm *Staphylococcus aureus* using Catalase, Oxidase and Coagulase test [14];

Antibiotic susceptibility test

Six isolates confirmed by biochemical test were tested for their susceptibility against 8 conventional antibiotic agents. Antibiotic agents tested were: ciprofloxacin (15 µg), gentamicin (10 µg), cefoxitin (30 µg), tetracycline (30 µg), chlorphenicol (10 µg), erythromycin (5 µg), oxacillin (1µg) and streptomycin (30 µg). Colony was picked from each isolate and inoculated into 5 mls each of peptone water and incubated at 37°C for 5 minutes. The turbidity of each isolate in the peptone water was measured in a Nephelometer to get a 0.5 MacFaland standard. Each isolate culture was spread evenly on plate. The culture was allowed to absorb into the medium for about 10 min. Subsequently, each antimicrobial disc was picked with a sterile forceps and placed on the media containing the medium at an appropriate distance from each other. The plates were later incubated at 37°C for 24 hours. The diameter of each of the zone of inhibition of each antibiotic was measured to interpret the test culture as resistance or sensitive [15].

Materials

'Fura-da-nono', petri dish, inoculation loop, bunsen burner, sample bottle, incubator, micro pipette, ruler, forceps, media, animal plasma, hydrogen peroxide, lugol's iodine, crystal violet, dilute carbol fuchsin, acetone, autoclave, normal saline, slides, microscope, immersion oil, antibiotics (Ciprofloxacin, Gentamicin, Cefoxitin, Tetracycline, Chloramphenicol, Erythromycin, Oxacillin and Streptomycin).

Statistical analysis

The Chi-square test was used to determine the statistically significant difference between the occurrences of *Staphylococcus aureus* in locally fermented milk-cereal mixture (fura-da-nono) in the different sampling areas. $P \geq 0.05$ is considered insignificant [16].

Results

Out of the 100 samples of 'fura-da-nono' analyzed 6(6%) *Staphylococcus aureus* were confirmed by biochemical test.

Location (market)	Number examined	No. positive	% prevalence	P-value 2
Sisinbaki	24	2	8.3	0.45
Wamba	76	4	5.3	

Table 1: Prevalence of *staphylococcus aureus* isolated from locally fermented milk-cereal mixture (fura-da-nono) based on location.

<i>Staphylococcus aureus</i>			
Antibiotics	R (%)	I (%)	S (%)
CIP	1/6 (16.7)	0/6 (0.00)	5/6 (83.3)
GN	0/6 (0.0)	2/6 (33.3)	4/6 (66.7)
FOX	2/6 (33.3)	0/6 (0.0)	4/6 (66.7)
TE	6/6 (100.0)	0/0 (100.0)	0/0 (0.0)
C	1/6 (16.7)	3/6 (50.0)	2/6 (33.3)
E	1/6 (16.7)	5/6 (83.3)	0/6 (0.0)
OX	5/6 (83.3)	1/6 (16.7)	0/6 (0.0)
S	0/6 (0.0)	0/6 (0.0)	6/6 (100.0)

Table 2: Antibiotic susceptibility pattern of *Staphylococcus aureus* isolates from locally fermented milk-cereal mixture (Fura-da-nono).

KEY: Gentamicin (GN), Ciprofloxacin (CIP), Cifoxitin (FOX) Tetracycline (TE), Chloramphenicol (C), Erythromycin (E), Oxacillin (OX), Streptomycin (S).

<i>Staphylococcus aureus</i> isolates identification number	Total number of antibiotics to which isolate was resistant	Antibiogram (resistant antibiotics)	Percentage resistance (%)
13	2	TE, OX	25.0
22	2	TE, OX	25.0
25	5	FOX, TE, C, E, OX	62.5
30	2	FOX, TE	25.0
39	1	TE	12.5
54	3	CIP, TE, OX	37.5

Table 3: Antibiotic resistance profile of six (6) *Staphylococcus aureus* isolates against 8 antibiotics.

KEY: Gentamicin (GN), Ciprofloxacin (CIP), Cifoxitin (FOX), Tetracycline (TE), Chloramphenicol (C), Erythromycin (E), Oxacillin (OX), Streptomycin (S).

<i>Staphylococcus aureus</i> isolates ID	Total number of antibiotics to which isolate was susceptible	Antibiogram (susceptible antibiotics)	Percentage susceptible (%)
13	4	CIP, FOX, S, GM	50.0
22	4	CIP, N, FOX, S	50.0
25	3	CIP, N, S	37.5
30	4	CIP, C, OX, S	50.0
39	3	CIP, FOX, S	37.5
54	4	N, FOX, C, S	50.0

Tables 4: Antibiotic susceptibility profile of six (6) *Staphylococcus aureus* isolates against antibiotics.

KEY: Gentamicin (GN), Ciprofloxacin (CIP), Cifoxitin (FOX) Tetracycline (TE), Chloramphenicol (C), Erythromycin (E),

Discussion

Contamination of food by the pathogenic microorganisms most especially *Staphylococcus aureus* remains a significance threat to achieving sustainable public health in Nigeria and the world over. The isolation of six (6) *Staphylococcus aureus* with prevalence of 6% from processed 'fura-da-nono' in this study is quite worrisome because this organism is associated with unhygienic processes. It is most likely that the isolation of this organism might have been attributed to the use of non-portable water, dirty utensils and processing the food in an unhygienic environment by the 'fura-da-nono' vendors. The detection of *Staphylococcus aureus* in ready to consume 'fura-da-nono' in this study constitute a very serious public health concern because the organism is known to be responsible for a wide range of diseases such as food intoxication, toxic shock, pneumonia, meningitis amongst others in both humans and animals due to its capacity to release heat stable toxin [17].

The nutritive content of 'fura-da-nono' made it auspicious for the proliferation of *Staphylococcus aureus* microorganism; it is therefore necessary to observe all hygienic process during milking, collection, fermentation and processing. The findings of this study lend credence to report documented by Usman and Mustapha [18] that had a prevalence of 12.6% isolate of *Staphylococcus aureus* from fermented milk in Zaria. From the outcome of this study, it is noted that the isolation of *Staphylococcus aureus* from 'fura-da-nono' is a strong indication that despite the fact that the product is a delicacy in Nigeria, it can serve as a vehicle for the transmission of pathogenic microorganisms if not handled hygienically during preparation, storage and marketing.

This observation is suggestive of the extent to which people consume the milk product without an idea of whether it's contaminated or wholesome, and this may constitute a major public. This has again underscores the urgent need to think of the introduction of 'Hazard Analysis and Critical Control Points (HACCP) at all stages of milk processing to reduce these risks to a safe level.

Multiple antibiotic resistances in pathogenic bacteria in food and food producing animals and environmental sources is recognized as a global public health threat. The findings of this study noticed that the antibiotic susceptibility of the six (6) *Staphylococcus aureus* isolates showed variable responses to different antibiotic agents, with most of the exhibiting multiple resistance. Similar findings have previously been reported by Bronzware., *et al.* [19] and White., *et al.* [20], who documented that multiple antibiotic resistances in pathogenic bacteria in food and food producing animals and environmental sources is surging globally, it is also poses danger to public health. It was noted with concern in this study that some of the isolates of *S. aureus* isolated from processed fura-da-nono were resistant to more than 3 classes of antibiotics. The indiscriminate usage of antibiotics in the treatment of animals and their incorporation in animal feeds could presumably account for majority of the increase in antibiotic resistance to humans and animal bacterial isolates. These might probably be linked to prolong and pervasive use of this antibiotic thereby resulting in *S. aureus* acquiring resistance to most commonly used antimicrobials [21,22].

The absolute resistance of *Staphylococcus aureus* to tetracycline (100%) recorded in this study is not unexpected because the drug is the most commonly available antibiotic that is used as a growth promoter and prophylaxis in livestock management in Nigeria as reported by Aliyu., *et al.* [16]. Okpo., *et al.* [23] also recorded a very high resistance of *Staphylococcus aureus* to oxytetracycline in their study. The notably high resistance of *Staphylococcus aureus* to oxacillin, erythromycin and chloramphenicol is a point of worry due to the fact that these drugs are not commonly used for the treatment illnesses in small and large animals in Nigeria. This could possibly be due to cross contamination of the 'fura-da-nono' during processing by handlers with the drug-resistant strains of the pathogen as reported by Aliyu., *et al.* [16]. This finding is similar to that of Aueyiagu and Isiyaku [2] who reported multidrug resistance among *Staphylococcus aureus* from dairy products in Jos.

Results of the antibiogram indicated that ciprofloxacin, gentamicin, streptomycin and ciprofloxacin could be prescribed as drugs of choice in diseases associated with food intoxication caused by *Staphylococcus aureus*.

Conclusion

The detection of *Staphylococcus aureus* in fura-da-nono in the study area suggests that consumption of dairy products especially those that are produced using traditional unhygienic methods constitute health hazard to consumers because the product can serve as medium for transmission of pathogenic microbes. The antibiotic susceptibility pattern of the *S. aureus* isolates revealed higher susceptibility to streptomycin, ciprofloxacin, gentamicin and ciprofloxacin, while high levels of resistance to tetracycline were recorded. This is of public health concern because tetracycline is a commonly used antibiotic in humans in the study area. The findings from this study suggest that selection pressure imposed by the use of antibiotics in human and in livestock production is a key driving force in the promotion of antibiotic resistance in *S. aureus*. This study revealed that Fura-da-nono sold in Wamba and Sisinbaki are unsafe for human consumption because they contain *Staphylococcus aureus* organisms.

Recommendation

'Fura-da-nono' producers should be enlightened on how to undertake hygienic milking, proper fermentation and pasteurization of their products to reduce the presence of *Staphylococcus aureus*. This can be achieved through workshops, seminars, Fulani day, herd and farm visit by the relevant authorities.

Assessment of the quality of 'fura-da-nono' should be carried out from time to time to safeguard public health. Hygienic and healthy sanitary practices such as washing of utensil, hands, covering of food and cleanliness on the part of the seller should be maintained through the chain of production until it gets to final consumers. Indiscriminate use of antibiotics in Veterinary practice should be discouraged.

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