Bacteriological Analysis of Fermented Cereal Flour Sold in Markets in Calabar Metropolis, Southern Nigeria

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Abstract

Fermented flour obtained from Maize, Sorghum and Millet is a popular product sold in Calabar. It is locally referred to as pap or ogi. . Different types of pap are sold in the market which include: white pap, yellow pap and reddish-brown pap. The aim of the study was to determining the presence of bacteria in pap samples in three markets in Calabar metropolis. The markets were Goldie, Marian and Watt market. A total of 80 pap samples were collected aseptically from the three different markets and were analyzed using standard microbiological and biochemical methods. The various pap samples were cultured on both Chocolate (CHOC) agar and MacConkey agar and incubated at 37°C overnight. *Staphylococcus aureus* (41.7%), non-coagulase *Staphylococcus aureus* (23.4%) and *Bacillus cereus* (27.8%) were isolated from the Pap samples purchased from the three markets.. Out of the 80 samples were examined for bacterial organisms, Staphylococcus aureus (41.7%) found in Pap samples purchased from Goldie market had the highest prevalence. However, the difference in the prevalence of *Staphylococcus aureus* contamination, non-coagulase *Staphylococcus* and *Bacillus cereus* infection by markets were not statistically significant (P>0.05). Therefore, there is a need for sanitary measures to be taken in the production of Pap, to minimize the rate of contamination during processing and storage and also, to ensure that pap sold to consumers are free of contamination.

Keywords: Fermented Cereal; Bacteriological; Market; Calabar Metropolis

Introduction

Pap otherwise called "Ogi" or "Akamu" is a product of fermented maize (*Zea mays*) widely eaten in Africa [1]. It is also made from sorghum (Sorghum vulgare) or millet (Pennisetum typoidum) [2]. Its smooth texture is almost like that of a hot blancmange and a sour taste that reminds one of yoghurt. The colour of ogi depends on the colour of the cereal used and includes: cream colour for maize ogi, reddish-brown colour for sorghum ogi [3]. Pap is often marketed as a wet cake formerly wrapped in leaves but presently in transparent polythene bags. This pap meal is mostly served as breakfast meals for both adults and weaning food for infants especially by low income earners who are not able to afford the more expensive weaning foods [4].

Most preparation of pap meal is from cereals such as; maize, guinea corn or millet readily available in all parts of the country.

Production of pap is carried out mostly by local producers [5]. This is made from wet corn starch or other cereals by

thoroughly washing the dry corn and soaking it for about 72-96 hours to make the corn soft enough to be blended. It is then washed and blended till smooth. The blended product is further sieved using a chiffon cloth while rinsing as necessary to remove shafts that might make the paste have a rough consistency. This pap paste is mixed with water and left to settle for about 3 hours [6]. After 3 hours, it is decanted and poured into a muslin bag and left to drain completely overnight to further give it its sour taste. The solidified pap is then cut and shaped into small portions and wrapped in transparent polythene bags for sale to consumers [7].

During processing, handling, there is a high risk of bacterial contamination which often makes the food products undesirable due to the presence of these organisms that cause food poisoning, spoilage or food intoxication [5]. Preliminary studies have shown that the processing and wet storage methods of this prepared paste permit bacterial growth, including other pathogens [8]. The machines used during processing may not be properly cleaned up after each usage. Water used in washing and grinding may contain microorganisms because of the high risk of environmental pollution.

Furthermore, not only is the storage cumbersome, which involves daily changing of the water used for storing the wet flour paste, but also very unreliable because of the prevalent epileptic electric power supply in Nigeria [8]. Food poisoning and infection can lead to fatal consequences in infected individuals, and the major risk factors are attributed to contaminated raw materials, poorly controlled fermentation conditions, poor personal/environmental hygiene and post processing handling [9].

Some are capable of producing heat resistant toxins (e.g *Staphylococcus aureus, Clostridium botulinum*). Most pathogens are mesophilic with optimal growth temperature ranging from 20°C to 45°C. However, certain food borne pathogens (i.e psychrotrophs) such as *Listeria monocytogenes*, are capable of growth under refrigerated conditions or temperatures less than 10°C [10]. Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in streets and other public places [11]. Street vended foods are not only appreciated for their unique flavours, convenience and the role which they play in the cultural and social heritage of societies. They have also become important and essential for maintaining the nutritional status of the populations [12].

Unsanitary handling of street foods by some vendors has been commonly found to be a source of contamination [13,14]. The vendors can be carriers of pathogens like *Escherichia coli, Salmonella, Shigella, Campylobacter* and *Staphylococcus aureus w*ho eventually transfer these foods

to the consumers. The hands of the food handlers have been found to be the most important vehicle for the transfer of organisms from faeces, nose and skin to the food.

The finding that *Salmonella*, non-*typhi Salmonella*, and *E.coli* can survive on finger tips and other surfaces for a period of time and in some cases after washing, supports the reports of contamination of street vended food with toxigenic *S. aureus* [15,16]. Fermentation during pap production occurs in two distinct stages: (i) steeping of maize prior to obtaining pap gruel and (ii) souring of fermented pap [17]. During this process, the fermentation facilitates the multiplication of bacteria and their activities in softening the corn kernel [6]. During fermentation, there is an interaction of different kinds of organisms. This interaction can be beneficial to the final product by means of desirable biochemical changes like the production of aromatic compounds and other enzymatic activities.

Despite this, there is a risk of contamination of the food, as the interactions of microorganisms involved in the fermentation process may be detrimental causing spoilage [17]. More so, fermentation in the production of pap is largely done in a traditional formula and poorly controlled. This crude form of processing encourages a high level of bacterial contamination [6].

Some studies have identified some microorganisms and the level of contamination that are associated with commercially prepared fermented pap. In a study by Ekelene OE [18] in some parts of South Eastern Nigeria, the presence of bacteria isolates from the commercial and laboratory fermented ogi showed that *Lactobacillus spp*, and *Staphylococcus spp* were present in all samples while *Klebsiella spp* was isolated from markets varieties respectively. Therefore, this study aimed at determining the bacteriological survey of commercially prepared paps sold in markets in Calabar Metropolis, Southern Nigeria.

Materials and Methods

Study Setting

The study was carried out in Calabar South and Calabar Municipality Local Government Areas of Calabar, Cross River State.

Study Design

This study design was a cross sectional survey of Pap samples. Questionnaires were also used to obtain information about preparation, storage and hygiene practices of Pap vendors.
The sample size was 240.

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Sample Collection

Two hundred and forty (240) Pap Samples were purchased in the morning from Pap vendors from the different markets. Each Pap sample was labeled with masking tape for easy identification. The samples were taken to the University of Calabar Teaching Hospital Laboratory within 1 hours of sample collection for analysis. The Pap samples were unwrapped and put into sterile universal containers with proper labeling using permanent markers. Questionnaires were further used to assess hygiene practices of these Pap vendors.

Laboratory Analysis of Pap's Samples

Culture: About 1g of pap sample was added to 9mls of sterile physiological saline and a 10 fold solution was made. About 0.1ml was then transferred to MacConkey agar and Chocolate (CHOC) agar. A pool was made and streaked out using a sterile wire loop. The MacConkey plates were incubated at 37°C for 24 hours while the Chocolate (CHOC) agar plates were incubated in a Co_2 canister jar at 37°c for 24hours. At the end of incubation, plates were examined for bacterial growth and counted.

Identification of Bacterial Isolates

Identification of bacteria was based on morphological characteristics and biochemical tests carried out on isolated colonies.

Gram Staining

The Gram staining technique and Biochemical tests was carried out on all the isolates for the Identification of Bacteria.

Assessment of Pap Vendor's Preparation, Storage and Hygiene Practices

The preparation, storage and hygiene practices of pap vendors were assessed with the use of questionnaires. Information about their practices during these processes was obtained. Pap vendors were asked about the source of water used for preparation and storage, refrigeration practice, storage method (whether wet or dry storage method), number of times water used to store pap was changed.

Results

Distribution of Microorganisms in Pap

Table 1 shows the occurrence of bacterial isolates based on location of samples. Of the 80 pap samples examined from the study area; 54 (67.5%) had significant bacterial growth while 26 (32.5%) had insignificant growth.

Table 2 shows the distribution of bacterial isolates detected from samples of pap purchased from pap vendors in markets in Calabar Metropolis. Of the three genera of bacteria isolated, *Staphylococcus aureus 48* (41.7%) was the highest followed by *Bacillus species* 32 (27.8%), and lastly non-coagulase *Staphylococcus aureus* 27 (23.4%). However, the difference in the prevalence of Staphylococcus aureus contamination, non-coagulase staphylococcus and Bacillus cereus contamination by markets was not statistically significant (P>0.05)

Markets	No of Samples Examined	No (%) with Significant Growth	No (%) with Insignificant Growth
Goldie	27	22 (81.4)	5 (18.5)
Marian	26	15 (57.6)	12 (45.1)
Watt	27	17 (65.3)	9 (34.6)
Total	80	54 (67.5)	26 (32.5)

Table 1: Distribution of bacteria based on markets.

Markets	No (%)of Pap Samples With *Staphylococcus Aureus*	No (%) of Pap Samples With Non- Coagulase *Staphylococcus*	No (%) of Pap Samples With *Bacillus Cereus*	Total No (%) of Pap Samples Examined
Goldie	20 (47.6)	7 (17.6)	11 (26.1)	38
Marian	13 (32.5)	11 (26.5)	13 (32.5)	37
Watt	15 (45.4)	9 (27.2)	8 (24.2)	32
Total	48 (41.7)	27 (23.4)	32 (27.8)	107

Table 2: Distribution of bacteria in pap samples from market.

Information gathered from pap vendors through the use of questionnaires, showed that 90% of pap vendors made use of community borehole water for both preparation and storage of pap compared to the 10% that made use of private borehole for both storage and preparation. The difference between those that used community bore hole and those that used private bore hole in pap preparation was statistically significant (P< 0.05).

Majority of Pap vendors (93%) reported practicing wet storage methods with the rest reporting that they made use of dry storage methods. The difference between those that used wet and dry methods of storage was statistically significant (P< 0.05). Up to 76% of respondents change water once, 16% changed water used for Pap storage more than once and the remaining percent do not change at all (6%). A good number (66%) practiced refrigeration of Pap as a method of storage while the remaining 33% did not. However, the difference between those that used refrigerator for storage and non-refrigeration was statistically insignificant (P > 0.05) (Table 3).

Variable	No (%) of Pap Vendors
Source of Water For Preparation (n=30)	
community borehole	27(90)
private borehole	03(10)
Storage Method (n=30)	
Wet	28 (93)
Dry	02(6)
Source of Water For Storage (n=30)	
Community borehole	27(90)
Private borehole	03(10)
Number of Times Water is Changed (n=30)	
Once	23(76)
more than once	05(16)
do not change	02(6)
Refrigeration (n=30)	
Yes	20(66)
No	10 (33)

Table 3: Vendors practice in pap preparation and storage.

Discussion

This study isolated *Staphylococcus aureus,* non-coagulase *Staphylococcus aureus* and *Bacillus cereus* from three markets; Goldie market, Watt market and Marian market in Calabar Metropolis. Of the 80 samples analyzed, the overall prevalence of *Staphylococcus aureus* was 41.7%, which was higher than 5.0% prevalence of *Staphylococcus aureus* isolated from Uturu and Okigwe markets in Imo state, Nigeria [18]. This was also higher than the 4.2% prevalence of *Staphylococcus aureus* isolated in Ebonyi state, Nigeria [19]. This prevalence was lower than the 58.3% prevalence of *Staphylococcus aureus* isolated in Bauchi state, Nigeria [20].

The prevalence of *Bacillus cereus* was 27.8% and was seen to be higher than the prevalence of 8.3% of *Bacillus cereus* in a study on pap in Bauchi State, Nigeria [20]. *Staphylococcus aureus (*47.6%) had the highest prevalence found in samples from Goldie market, followed by *Bacillus cereu (26.1%)s* and non-coagulase *Staphylococcus aureus (17.6%)*with the least prevalence from both Watt and Marian market respectively. However, the difference was not statistically insignificant (P>0.05). Goldie market is a small market located behind the University of Calabar. Students and other residents patronize this market greatly. This market is known for its poor drainage system and overpopulation due to the small size of its geographic space. The presence of a high rate of microorganisms from this market could be attributed to the poor drainage system through the environment. These findings from the study are similar to the previous studies carried out on pap samples by Ogbonnaya JA, et al. [1,20] which reported the same microorganisms in their study.

Several authors have highlighted the importance of adequate nutritional quality and hygiene during the preparation of foods and also the link between infection and nutrition [7] reported the presence of pathogenic bacterium such as *Staphylococcus aureus* in pap and other food. The common storage of pap was wet storage [13]. This method of storage has a high connection to the microbial load which is due to the fact that water used for storage, most of the time, is unclean and harbours microorganisms. A good percentage of pap sellers in the market made use of community borehole water for both production and storage of pap produce. The difference between those that used community borehole and private bore holes for preparation and storage was statistically significant (P < 0.05). The isolation of the microorganisms from the pap samples confirmed that it could serve as a vehicle for the transmission of potentially pathogenic microorganisms [9].

Conclusion

*Staphylococcus aureus (*41.7%), non-coagulase *Staphylococcus aureus* (23.4%) and *Bacillus cereus* (*27.8%) were isolated from pap samples purchased from markets in Calabar Metropolis, Calabar, cross River State, Nigeria. *Staphylococcus aureus (*41.7%) was found to be the highest isolates from samples obtained from Goldie market the hygienic practices of those selling pap in markets was very

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poor.

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