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Bacteriological Quality of Commercially Sold Yoghurt in Calabar Municipality, Cross River State, Nigeria

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Abstract
Background: *Yoghurt production involves the fermentation of the lactose content of milk to yield lactic acid, CO_2, acetic acid, diacetyl, acetaldehyde, amongst others, using Streptococcus thermophilus and Lactobacillus bulgaricus. Fermenting organisms produce lactase, which hydrolyzes lactose to simple sugars, glucose and galactose, making it more accessible to lactose-intolerant patients.The increased awareness of the benefits of constant yoghurt consumption has increased the rate of yoghurt consumption in Calabar and Nigeria as a whole, leading to the establishment of many small-scale factories for yoghurt production. Many unqualified people whose products lack certification by the appropriate agencies have also moved in for the sole purpose of making money. The purpose of this study was to assess the bacteriological quality of yoghurt sold in Calabar Municipality,to determine whether or not they are free from harmful bacteria, to identify the pathogens and determine the microbial load therein.*
Method:*Ten different brands of sachet packaged yoghurt were obtained fromvarious sales outlets in Calabar Municipality. Two samples of each brand were used and properly labeled giving a total of 20 yoghurt samples. Representative portions of the yoghurt samples were placed into sterile universal containers.Serial dilution was done and samples were cultured using the spread plate method on Cysteine Lactose Electrolyte Deficient, Chocolate and Blood Agar platesfor colony counts. Standard bacteriological and biochemical tests were used to identify isolates.*
Results:*The pH values of the yoghurt samples ranged between 2.35 and 3.26. One out of the 10 brands had no visible bacterial growth while the other brands had growth. The bacteria isolated from the yoghurt included Klebsiella sp., Streptococcus sp., Bacillus sp., Escherichia coli and Staphylococcus aureus. S. aureus had the highest frequency of 70% followed byKlebsiella sp. with a frequency of 60%, Bacillus sp. 50%, Escherichia coli 40% and Streptococci 20%.*
Conclusion: *Considering the frequencies of occurrence above, it is evident that these products are potential health hazards. Hence, the agencies responsible for ensuring food safety should put in more effort in ensuring that good manufacturing practices (GMP) are employed and ininspecting commercially sold yoghurt before they get to the consumers.*
Key words: *Bacteriological quality,good manufacturing practices,yoghurt*

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I. Introduction

Milk is nutrient-laden and contains high concentrations of protein, fat, vitamins and minerals. Milk fermentation is a classic method widely employed for milk preservation and conversion to value added products (Adolfsson*et al.*, 2008). Yoghurt is a fermented dairy product obtained from the lactic acid fermentation of milk. It is one of the most popular fermented milk products in the world and produced commercially at home.It has a characteristic acidic taste and pH ranging from 3.7-4.2 with viable and abundant fermenting microorganisms (Willey *etal*., 2008). Yoghurt has been described as a balanced food, containing almost all the nutrients present in milk but in a more digestible form(Ojokoh, 2009). It is a good source of iodine, calcium, phosphorus, zinc, riboflavin, vitamin B5 and vitamin B12. It is also nutritionally rich in protein, molybdenum and pantothenic acid (Okpalugo*et al.*, 2008).

In its commercial production, non-fat or low fat milk is pasteurized, cooled to 43°C and inoculated with known cultures of microorganisms referred to as starter cultures(Anthar, 2010). The starter cultures may be a pure culture of a particular species of *Lactobacillus* or a mixed culture of *Streptococcus thermophilus* and

Lactobacillus bulgaricus in a 1: 1 ratio. The coccus which is the *Streptococcus thermophilus* grows faster than the rod which is the *Lactobacillus bulgaricus* and is primarily responsible for acid production while the rod adds flavor and aroma(Adolfsson*et al.*, 2008). The growth of these microorganisms causes the transformation of milk sugar, lactose into lactic acid. This process gives yoghurt its texture(Gray, 2007). The associative growth of the two organisms results in acid production at a rate greater than that produced by them individually.

While the microorganisms fermenting milk confer on it certain health benefits such as immunity boost, protection against ulcers and prevention of yeast infection, inadequately pasteurized milk may contain microorganisms of special importance to man, leading toemergence of substandard and contaminated yoghurt in the market (Boor and Murphy, 2002). The presence or absence of these microorganisms in milk may reflect success or failure of good manufacturing practice (GMP) or cause infection when consumed together with food. This is of economic significance in Africa where the HIV/AIDS and cancer scourges have left the populace, who consume milk products, immune suppressed and prone to bacterial and fungal infections (Boor, 2007).

Health complications associated with consumption of inadequately pasteurized milk products include serious infections that are difficult to treat(Urassa*et al.*,2010). This situation becomes clinically significant when the organisms isolated from such infections are resistant to conventional antibiotics, thus, conferring antibiotic resistance to the infecting organisms while providing no alternative drug (Gould, 2001). It is important therefore to evaluate the bacteriological quality and hence the microbial load of some commercially sold yoghurt in Calabar Municipality.

II. Materials And Methods

Sample collection

Ten different brands of sachet packaged yoghurt were obtained from hawkers and beverage storesin Calabar Municipality. Two samples of each brand were used and the brands were designated A1, A2; B1, B2; C1, C2; D1, D2; E1, E2; F1, F2; G1, G2; H1, H2; I1, I2; J1, J2 respectively giving a total of 20 yoghurt samples. The samples were brought to the laboratory and analyzed within 2 hours of collection.

Sample analysis

The National Agency for Food and Drug Administration and Control (NAFDAC) registration numbers, production and expiry dates of the samples were noted.Representative portions of the yoghurt samples were placed in sterile universal containers. Using a sterilized wire loop,a loopful of each sample was picked and inoculated on CLED, Chocolate and Blood Agar plates respectively.Serial dilutions of the yoghurt samples were made with distilled water and these were cultured for colony counts andincubated at 37^{o}C for 18 to 24 hours. After incubation, isolates were identified based on cultural, morphological and biochemical characteristics following standard microbiological methods (Ochei and Kolhatkar, 2007; Cheesbrough, 2010), includingGram staining, urease test, indole test, oxidase test, coagulase test, catalase test,citrate utilization test, Voges-Proskeur test, methyl red test andsugar fermentation.

III. Results

The bacteriological analysis of commercially sold yoghurts in Calabar Municipality was carried out. Table 1 shows the pH of the different yoghurt samples analyzed. Table 2 shows the total bacterial and coliform counts of the samples while Table 3 indicates the distribution of the bacterial isolates from the yoghurt samples. Four samples had no NAFDAC registration number nor production and expiry dates written on them.The bacteria isolated included*Klebsiella sp., Streptococcus sp., Escherichia coli, Bacillus sp. and Staphylococcus aureus.*

TABLE 1
pH values of the different yoghurt brands

Yoghurt sample	pH value
A	2.35
B	3.11
C	3.18
D	3.10
E	3.01
F	2.56
G	2.58
H	3.11
I	2.95
J	3.26

TABLE 2
Total bacterial and coliform count

Yoghurt Sample	Bacterial count	Coliform count
A	5.0×10^{-3}	1.0×10^{-3}
B	0.0×10^{-3}	0.0×10^{-3}
C	3.0×10^{-3}	1.0×10^{-3}
D	7.6×10^{-3}	2.3×10^{-3}
E	2.2×10^{-3}	1.0×10^{-3}
F	9.5×10^{-3}	2.5×10^{-3}
G	4.0×10^{-3}	2.1×10^{-3}
H	6.0×10^{-3}	4.4×10^{-3}
I	3.1×10^{-3}	1.1×10^{-3}
J	2.1×10^{-3}	6.0×10^{-3}

TABLE 3
Distribution of bacterial isolates from the yoghurt samples

Bacterial isolates	Samples										Frequency of Occurrence	% of Occurrence
	A	B	C	D	E	F	G	H	I	J		
Staphylococcus aureus	+	_	_	+	+	+	+	+	+	_	7	70
Streptococcus sp.	_	_	+	_	_	_	_	+	_	_	2	20
Klebsiella sp.	_	_	+	+	+	+	+	+	_	_	6	60
Bacillus sp.	+	_	+	_	_	+	_	_	+	+	5	50
Escherichia coli	_	_	_	+	+	+	+	_	_	_	4	40

KEY
+ = Present
- = Absent

IV. Discussion

Dairy products such as yoghurt contain probiotic cultures e.g. Lactobacilli and are currently among the best known examples of "functional foods" (Oyeleke, 2009).The pH values of the different brands of yoghurt analyzed in this study (Table 1) shows that they were all acidic with sample A having the highest acidity level of 2.35 and sample J with a weak acidity level of 3.26. This is not abnormal as it is one of the attributes of yoghurt generally. The acidic nature of yoghurt is due to the lactic acid produced by the bacteria involved in its fermentation, which is a vital step in the production of yoghurt. This confers on the yoghurt its characteristic flavor, taste and texture (Mardigan*et al.*, 2009). Table 2 shows the total bacteria and coliform counts of the samples which were obtained after a serial dilution of the samples and incubation at 37^0C for 18 – 24hours. Sample B had no bacterial and coliform growth while A to J had visible growth. The morphological characteristics of the bacterial isolates in the different yoghurt samples and their biochemical characteristics were observed. Table 3 shows the distribution of the bacterial isolates from the different yoghurt samples.

Sample F had the highest bacterial count while sample C recorded the lowest count. The high aerobic bacterial load observed in yoghurt samples was attributed to inadequate hygienic measures during production. The samples recorded counts that ranged from 2.1×10^{-3}cfu/ml to 9.5×10^{-3}cfu/ml for total viable count and 1.0×10^{-3} cfu/ml to 4.4×10^{-3}cfu/ml for total coliform count. This is an indication of contamination of the products either during production or packaging. The occurrence of Streptococci agrees with the work of Poramloy*et al*. (2010), who reported that organisms that contaminate the surfaces of teats and udders of the cow included Staphylococci, Streptococci, spore formers, coliforms and Gram negative bacteria which can survive pasteurization temperature and Streptococciwhich can grow under refrigeration.

The frequent contamination of dairy products by *Staphylococcus aureus* is as a result of the possible source of the organism which may be from the nasal passage, skin, buccal cavity of production personnel while talking or coughing. This produces droplets that settle on the yoghurt during production. *Staphylococcus aureus* is resistant to heat, drying and radiation (Arnott*et al.*, 2010).

The presence of coliforms in the samples indicates contamination and the poor level of hygiene after processing(Campbell, 2007). Coliforms are not supposed to be present in yoghurt because of high temperature, short pasteurization time and effective clearing and good hygienic procedures (Kawo*et al.*, 2006). The presence of coliforms in yoghurts poses great danger to the health of the consumers and suggests neglect of good manufacturing procedures. The tolerable limit for coliform presence in yoghurt is less than 10cfu/ml but a higher count of 4000cfu/ml is of serious concern. This contamination may be from contaminated water source or

equipment used or probably as reported by Karagul-Yucer*et al.* (2002), due to contamination at storage and display at sales outlet. Coliforms are considered as normal flora of the intestinal tract of humans and animals and their presence indicates direct fecal contamination(Bramley *et al.*, 2011). They have been used as indicator organisms for bacteriological quality of milk and its products.

Klebsiella sp. is a coliform susceptible to pasteurization, but its presence in post pasteurized yoghurt may be as a result of faulty heat process or water used in manufacturing, unhygienic hawking habits, handlers with poor sanitary practices and unhygienic storage environment. *Klebsiella sp.* has been implicated in more bacterial pneumonia cases than *Streptococcus pneumoniae*(Willey *et al*., 2008).

The total bacterial count is often an indication for the sanitary condition, safety and utility of most foods. It may reflect the condition under which the product was manufactured such as contamination of raw materials, the effectiveness of processing and the sanitary conditions of the equipment and utensils at the processing plant (Park *et al.*, 2012).

V. Conclusion

In conclusion, the evaluated milk products clearly pose some yet undefined risks. This is of clinical significance in immunosuppressed people who may consume these products. These groups of people should be conscious when consuming yoghurts as they may ingest pathogenic isolates. The concentration of bacteria isolated from commercially sold yoghurt in Calabar differs from one product to the other, and lack of standardization makes it difficult to ascertain the quality of their products. Since this study assessed commercially sold yoghurt in Calabar Municipality only, it is important that the food monitoring agencies provide standards for producers in order to reduce the risk that their products may pose to consumers andensure that they adhere to Good Manufacturing Practice (GMP) before their products get to the consumers.

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