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Research Article

Assessment of Bacteria Present in Yoghurt Sold on Awka Metropolis

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Abstract: Yogurt is a commonly consumed drink because of its energy constituent and health benefit. This study examined the bacteria present in yoghurt sold in Awka, a metropolis South east Nigeria. Undesirable microbes that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms, and lactic acid bacteria. The bacteriological quality of some yoghurt sold at different outlets in Awka metropolis in Anambra state was assessed. Five different brands of commercially prepared yoghurt were purchased and analyzed in the laboratory using standard laboratory methods. Four of the samples recorded growth in the range of 3.2×10^5 to 9.0×10^5 cfu/ml. After biochemical characterization, *Bacillus sp, Staphylococcus sp, Streptococcus sp, Klebsiella sp and Pseudomonas sp*, were the bacteria isolated. The result showed that many of the yogurts were contaminated by microorganisms that have public health implications. Thus, the yoghurts sold in Awka have poor bacteriological quality. Therefore, the attention of the appropriate government agencies is needed to ensure that adequate hygiene is maintained during preparation, processing, storage and distribution of yoghurts to produce high quality products that will not pose a health risk to the consumers.
Keywords: Bacteriological quality, Yoghurt, Dairy products, Contamination

INTRODUCTION

Yoghurt is a fermented milk product produced by bacteria fermentation of milk which is consumed throughout the world. The French called it 'la lait de la vieeternelle' - the milk of eternity as it was believed to have therapeutic powers and gave long life to those who consumed it. Yoghurt is high in protein and calcium, and may be easier for people with lactose intolerance to digest because of its active cultures. Yoghurt is made by the fermentation or the addition of healthy bacteria and live cultures to milk. It is a means of protein intake for an improved healthy living [1]. The bacteria used to make yogurt are known as yoghurt cultures. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and its characteristics [2]. *Lactobacillus delbrueckii* subsp. *bulgaricus* is commonly used alongside *Streptococcus thermophilus* [3] as a starter for making yoghurt. The two species work in synergy with *Lactobacillus delbrueckii* subsp. *Bulgaricus* producing amino acid from milk proteins which are then used by *Streptococcus thermophilus* [3]. Both species produce lactic acid which gives yoghurt its tart flavor and acts as a preservative. The resulting decrease in pH also partially coagulates the milk proteins, such as casein, resulting in yogurt thickness [4]. Some strains of *Lactobacillus delbrueckii* subsp. *Bulgaricus* also produce bacteriocins [5]. These yoghurt product blends are consumed worldwide and much more in Indian sub-continent [6]. The National Yogurt Association proposed a yogurt standard that: requires a minimum level of active cultures of 107 colony-forming units (CFU) per gram (g), requires an acidity of pH 4.6 or lower, requires a minimum level of total dairy ingredients of 51 percent, provides for pre-culture homogenization and pasteurization, permits the use of reconstituted milk and whey protein concentrate as standard dairy ingredients, provides for the use of any milk-derived ingredients as optional dairy ingredients, permits the use of safe and suitable sweeteners, emulsifiers, and preservatives, permits the optional use of any safe and suitable ingredients added for nutritional or functional purpose, and makes provisions for low fat and nonfat yogurts based on total fat content of the food per reference amount customarily consumed [7].

Fermented milk, like the fresh milk from which they are produced, is liable to contamination. Molds and yeast are the primary contaminants in yoghurt produced commercially in Nigeria.

Molds and yeasts growing in yoghurt utilize some of the acid and produce a corresponding decrease in the

acidity, which may favour the growth of putrefactive bacteria. So to ensure the proper quality of yoghurt there should be a complete check on the method yogurt is produced and sold in local markets and major streets. A practical approach towards the quality of yoghurt is to evaluate the different samples of yoghurt sold in local markets [8]. Some of the yoghurts sold by mobile vendors are linked to poor personal and handling hygiene and are deemed substandard [9].

Yoghurts as other dairy products are frequently contaminated by bacteria and this often led to food intoxication/poisoning. Evaluation of the bacterial quality of yogurt become apparent due to the high risk associated with consuming substandard or unhygienic yogurt containing pathogenic organisms; health complications associated with consumption of inadequately pasteurized milk products include serious infections that are hard to treat with antibiotics. This becomes clinically significant if organisms isolated from an assessed sample is resistant to conventional antibiotics. Thus, it can confer antibiotic resistance to the infected host while producing an alternative drug [10]. Early detection of food contaminants contribute greatly to safety of foods and thus to an improvement of public health [11]. The bacteriological quality of milk and dairy product is influenced by the initial flora of raw milk, the processing conditions, post-heat treatment contamination. Undesirable bacteria that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms and lactic acid bacteria.

In addition, various bacteria of public health concern such as *Salmonella* sp., *Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica*, pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *Staphyloccocus aureus* may also be found in milk and dairy products. For this reason, increased emphasis should be placed on the bacteriological examination of milk and dairy foods [12].

This study was carried out to determine the bacteria contaminants in yogurts sold in Awka metropolis which pose danger to public health.

MATERIALS AND METHODS

Collection of Samples

Five different brands of commercially prepared yogurts packaged in plastic containers were purchased from street vendors, hawkers and beverage stores in different locations in Awka, capital of Anambra State. The yogurts were refrigerated before the commencement of the analysis. Information on the labels of the yogurt was recorded. The culture medium used in carrying out this analysis is: nutrient agar with antifungal agent (Nystatin) incorporated to inhibit fungal growth.

Bacteriological Analysis

The yogurt samples which have assumed room temperature were shaken vigorously to suspend microbial content. The laboratory work area and the containers of the yogurt were swabbed thoroughly with 70% ethanol before opening to avoid contaminating the sample. Each sample was serially diluted using sterile distilled water as diluents. 9ml of distilled water was measured into several test tubes and sterilized by autoclaving along with 250ml of nutrient agar. After sterilization, the sample was diluted by measuring 1ml of the sample from the container into the first test tube containing 9ml of sterile distilled water using a sterile syringe. The tube was properly mixed and using a different sterile syringe, 1ml from the first test tube was introduced into the second test tube containing 9ml of sterile distilled water, this is continued following the same procedure till the last dilution. Using the spread plate method 0.1ml each of the diluent was poured on the nutrient agar plates and spread using sterile glass slide. The plates were incubated at 37°c for the 24hr.

After incubation the representative colonies on the plates were sub cultured on fresh nutrients agar to obtain pure cultures of the isolates. The pure cultures were then transferred into nutrient agar slants for biochemical identification. The various biochemical tests carried out to identify the isolated organisms [18].

RESULTS

Table 1 show the total viable counts of the samples which was obtained after serial dilution of the samples and incubation at 37ºC for 24hours. Sample A has no viable growth after 24hours, samples B, C, D and E has colony counts of 75, 32, 87 and 90 respectively, with sample E having the highest count.

Table 1: Total viable count of the organisms

Samples	Dilution factor	Colonies per plate	Bacterial count (CFU per ml)
A	10^{-3}	NG	NG
B	10^{-3}	75	7.5×10^5
C	10^{-3}	32	3.2×10^5
D	10^{-3}	87	8.7×10^5
E	10^{-3}	90	9.0×10^5

Key: NG = No Growth

The table below is a scheme of the appearance of the organisms isolated from the yoghurt cultures on the cultural media.

Table 2: Colonial characteristics of the bacterial isolates from the yoghurt samples

Organisms	Shape	Elevation	Margin	Pigment
Bacillus sp	irregular	flat	lobate	milky
Staphylococcus aureus	circular	convex	entire	yellow
Streptococcus sp	circular	raised	entire	milky
Klebsiella sp	circular	raised	entire	pink
Pseudomonas sp	circular	raised	undulate	blue-green

Table 3: Biochemical Characteristics of the Bacterial Isolates

Sample	Gram reaction	Form	Catalase	Coagulase	Indole	Methyl-red	Voges-Proskauer	Oxidase	Citrate	Motility	Urease	Spore	Glucose Fermenta tion	Lactose Fermentation	Suspected Organism
1	+	Rod	+	-	-	+	+	+	+	+	+	+	A	A	*Bacillus sp*
2	+	Coccus in clusters	+	+	+	-	-	-	+	-	-	ND	A/G	A/G	*Staphylococcus aureus*
3	+	Coccus in chains	-	-	-	+	-	-	-	-	-	ND	A	A/G	*Streptococcus sp*
4	-	Rod	-	-	+	-	-	-	+	-	-	ND	A	A	*Klebsiella sp*
5	-	Rod	+	-	-	+	+	+	+	+	-	ND	A	A	*Pseudomonas sp*

Key: + = Positive result**; -** = Negative result**;** A = Acid production, A/G= Acid and gas production**,** ND= Not determined

Table 4 is a frequency distribution table that shows the number of times an organism occurred in different samples which is represented in the graph below. *Staphylococcus sp* has the highest occurrence with *Bacillus sp* and *Pseudomonas sp* occurring less.

Table 4: Total Bacterial Isolates from the Yoghurt Samples

Organisms	A	B	C	D	E	Frequency of occurrence	%
Bacillus sp	−	−	+	−	−	1	20
Staphylococcus sp	−	+	+	+	+	4	80
Streptococcus sp	−	+	+	−	+	3	60
Klebsiella sp	−	−	+	−	+	2	40
Pseudomonas sp	−	+	−	−	−	1	20

Key: + Present, − Absent

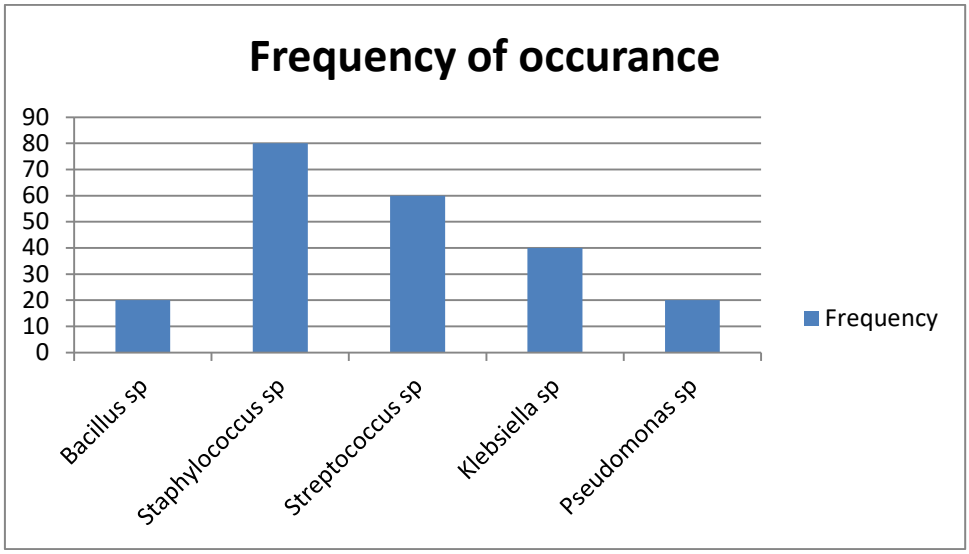

Fig. 1: Frequency of occurence

DISCUSSION

The production, expiry dates and NAFDAC registration number of the samples were noted. The bacterial viable count recorded a highest count of 9.0 x 10^5cfu/ml in sample d and lowest of 3.2 x 10^5cfu/ml in sample c with no growth recorded in sample A. The bacteria isolates isolated include: *Bacillus sp, Staphylococcus sp, Streptococcus sp, Klebsiella sp, and Pseudomonas sp*. High aerobic bacterial load in yoghurt was attributed to inadequate hygienic measures in production or inadequate processing recontamination. The five samples recorded counts that ranged from 3.2 x 10^5 to 9.0 x 10^5which is an indication of contamination of the product either during packaging or at the preparatory stage or during handling.

The occurrence of Streptococci is in line with the works of Bramley et al., [13] who showed that organisms that contaminate the surface teat and udders of the cow include Staphlococci, Streptococci, spore formers, coliforms and gram negative bacteria which can survive pasteurization temperature and Streptococci which can grow under refrigeration.

Staphylococcal count recorded the highest count of 9.2 x 10. Although the staphylococcal count does not exceed the hazardous level of 106 [14], but the presence should be discouraged because it could increase if poorly stored [15]. Also, Park C *et al*. [16] reported the frequent contamination of dairy products by *Staphylococcus aureus.* The possible source of this organism may be from the nasal passage, skin and other mammals. Talking, coughing and talking produce droplets which settle on the yogurt during production, transportation, storage and retailing. *Staphylococcus aureus* is resistant to heat, drying and radiation. The presence of *Staphylococcus aureus* in yogurt may cause staphylococcal food poisoning which is a major type of food intoxification caused by ingestion of improperly stored or cooked food in which *S. aureus* has grown [17].

The presence of coliform indicated contamination and the poor level of hygiene after processing. Coliforms are not supposed to be present in yogurt because of high temperature short time pasteurization and effective cleaning and good hygienic procedures [9], the presence of coliforms from this poses great danger to the health of the consumers and suggest neglect on the part of the processors or the yogurt vendors. The tolerable limit for coliform presence in yogurt is less than 10 cfu/mL but a higher count of 4000

is of serious concern. This contamination might be from contaminated water source or equipment used or probably as reported by [8] due to contamination at storage and display/sale outlet.

Coliforms are considered as normal flora of the intestinal tract of human and animals and their presence indicates direct faecal contamination. They have been used as indicator organisms for bacteriological quality of milk and its products [14]. *Klebsiella sp*, is a coliform, it is susceptible to pasteurization but its presence in post pasteurized yogurt may be as a result of faulty heat process or water used in manufacture, unhygienic hawking habits, handlers with poor sanitary practices and unhygienic storage environment. The level of presence of coliform and indicator organisms has been described as index of food hygiene. *Klebsiella sp*, has been related to bacterial pneumonia cases more severe than those produced by *Streptococcus pneumonia* and urinary tract infection.

In most foods, the total bacterial count is often an indication for the sanitary quality, safety and utility of foods. It may reflect the conditions under which the product is manufactured such as contamination of raw materials and ingredients, the effectiveness of processing and the sanitary conditions of equipment and utensils at the processing plants [14].

CONCLUSION

From the available result, it can be concluded that most of the yogurt on sale within Awka metropolis do not present adequate bacteriological quality. This suggests the need for

- Strict hygienic measures to be applied during production, processing and distribution of yogurts and its products to avoid contamination with unwanted materials and microorganisms.
- Periodical factory inspection must be done by regulators in the industry such as NAFDAC to checkmate the problem of poor hygiene and to apply sanctions where necessary.
- The manufacturers should make it a duty upon themselves to educate their staff on clean and hygienic practices considering the high level of coliform contamination.

NAFDAC registered samples are commonly products of high standard but in this case these products are not safe for people to consume. So there needs to be a HACCP (Hazard Analysis Critical Control Points) program for transportation, packaging and storing yogurt in Nigeria.

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