



BACTERIOLOGICAL ASSESSMENT OF SOME COMMERCIALY SOLD YOGHURT IN UTURU, ABIA STATE, NIGERIA: A PRELIMINARY STUDY

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ABSTRACT

A preliminary study was carried out to determine the bacteriological quality of commercial yoghurt sold in Uturu, Abia State, Nigeria. This involved isolation, characterization and antibiotics susceptibility of bacteria isolated from some commercially sold yoghurt in Uturu. Five different brands of yoghurt coded A to E were microbiologically analyzed using standard microbiological methods. The total viable count ranged from 0.9×10^2 cfu/ml – 2.0×10^3 cfu/ml, coliform count ranged from 0.4×10^2 cfu/ml – 1.0×10^3 cfu/ml, total staphylococcal count ranged from 1.1×10^1 to 1.8×10^2 . Bacterial isolated include *Staphylococcus* species, *Streptococcus* species, *Bacillus* species and *Lactobacillus* species. The antibiotics susceptibility pattern of the bacterial isolates proved that drovid had the highest zones of inhibition from 31.8 ± 1.2 mm to 36.1 ± 0.3 mm, followed by ofloxacin (29.5 ± 0.1 mm to 35.0 ± 0.8 mm). The presence of coliform and *Staphylococcus* in the yoghurt may be as a result of unclean water used in the production, contaminated milk and unhygienic condition of the handlers. It is recommended that yoghurt producers, sellers and handlers should ensure a high level of hygiene and avoid long exposure of yoghurt before selling to consumers.

Keywords: Bacteriological, Yoghurt, Uturu, Abia

INTRODUCTION

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 (Willey *et al.*, 2008). Yoghurt is a coagulated milk product that results from the fermentation of lactose in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Other lactic acid bacteria (LAB) are also frequently used to produce yoghurt with a unique characteristic (Adolfsson *et al.*, 2004). This product is widely regarded as nutritionally beneficial and safe, enjoying popularity for its appealing taste among a broad range of individuals.

Yoghurt is produced commercially by pasteurising the milk mixture, cooling to 45°C before being inoculated with known cultures of microorganisms referred to as starter culture. The starter culture may be mixed *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in a ratio of 1:1. They act on lactose and result in the production of lactic acid which increases the acidity of the yoghurt, thereby forming gel (Obukokwo *et al.*, 2017).

Yoghurt has been ranked as the most widely available fermented milk in the world today. Its mildly acid flavour, custard texture coupled with nutritive value made it to be among the commonly relished foods throughout the history of mankind (Taura *et al.*, 2005).

Yoghurt is among the foods or drinks that are packaged and sold in disposable polythene bags, paper packs and or in plastic bottles. Final packaging including the sealing of the content of such drinks was reported to be usually by hand or by simple mechanical sealing, machine in small and medium scale factories as opposed to the use of automated filler. Yoghurt should be stored at chilling temperature not higher than 5°C for a longer shelf life. On the contrary, they are usually held in storage compartments at about 10°C or left in open shelves in some supermarkets and are even sold by hawkers carrying the yoghurts in open cartons exposed to the heat of the sun. Yoghurts not properly refrigerated are prone to spoilage by yeast contaminants (Dublin-Green and Ibe,

2005). The changes in the physical, chemical and microbiological structure of yoghurt determine the storage and shelf life of the product. The quality of yoghurt in the market varies from one producer to another. A practical approach towards the quality of yoghurt is to evaluate the different samples of yoghurt sold in the market (Obiekezie *et al.*, 2012).

There are limited published studies on microbiological assessment of yogurts sold in Uturu, Abia State of Nigeria. Uturu is characterized by low level of environmental sanitation, lack of potable water and poor waste disposal. Contamination by total coliforms, other bacteria and yeast is possible. Therefore, this study was aimed at carrying out a bacteriological assessment of commercial yoghurt sold in Uturu, Abia State, Nigeria.

MATERIALS AND METHODS

Study Area

The study area is Uturu Community. The Uturu community is within Isukwuato Local Government, Abia State, Nigeria. The people are predominantly farmers mixed with civil servants. The community is a semi-urban encompassing one State University (Abia State University) and a Private University known as Gregory University Uturu.

Sample Collection

The samples selected for this research work were five in number from different brand, four samples were sold in plastic bottled containers and one was sold in sachet form. All the yogurts sampled were registered by NAFDAC.

Yoghurts samples were procured from different vendors within Uturu community, Isukwuato Local Government, Abia State. The samples were labeled as A-E. After proper labeling, samples were brought to Advanced Microbiology Laboratory, Gregory University, Uturu in ice packed cooler for microbiological analysis.

Enumeration of Total Viable Count, Total Coliform Counts, Faecal Coliform Count and Staphylococcal Count

All the media (Eosine methylene blue agar, MacConkey agar, Mueller-Hinton agar, Nutrient agar, Nutrient Broth and Peptone water) used were prepared according to the manufacturer's specification; heated in water bath till the agar powder melted, and the medium was sterilized in an autoclave at 121°C for 15 minutes and was kept in the incubator overnight for sterility test, and kept in refrigerator for further use.

The determination of microbial load in the yoghurt samples were carried out. Nutrient Agar was used for bacterial count, MacConkey Agar for coliform count, EMB for Faecal coliform count and mannitol Salt Agar for Staphylococcal count. Diluents were prepared aseptically by pipetting 9ml of distilled water into 5 tubes and sterilized at 121°C for 15 minutes in an autoclave. 1ml of the labelled yoghurt sample was introduced into the cooled sterile water and serial dilution was done to 10^{-5} dilution factor. 1ml of the diluted yoghurt sample was mixed with MacConkey medium, EMB and Mannitol Salt agar medium respectively. The agar plates were allowed to solidify and incubated at 37°C for 24hrs. The colonies that grew were counted and the values expressed as colony forming units (cfu/ml). Pure cultures of isolates were obtained by repeated sub culturing onto fresh media and the cultures were maintained on agar slants for further identification (Oyeleke, 2009). The plates were gently swirled clockwise and anticlockwise for even distribution of the organisms, allowed to solidify and incubated inverted at 37°C for 24 hours. It was removed and observed for colonies (Willey *et al.*, 2008), observation recorded and result expressed as colony forming units (cfu) (Oluwafemi and Da Silva, 2006).

Identification and Characterization of Bacterial Isolates

The different discrete colonies of the bacteria isolates were identified using a combination of morphological, biochemical and microscopic examinations of colony morphology, cell shape and size, which provided initial clues for identification. The microbiological identification procedures included: Gram staining method, motility test, colour / pigmentation on culture plates, smell. The biochemical tests included tests for: Oxidase, Citrate utilization, Catalase, Coagulase, Urease, Indole, Methyl red test (MR), and Vogesprokauer (VP) and sugar fermentation tests were performed to further classify the isolates (Ekeleme *et al.*, 2024)

Determination of activity of different antimicrobial agents against the isolates obtained from yogurt samples

Isolates from the yogurt samples were tested for susceptibility to standard conventional antibiotics like, cefazidime (30µg), cefuroxime (30µg), gentamicin (10µg), cefixime (5µg), ofloxacin (5µg), augumentin (30µg), ciprofloxacin (5µg), and nitrofurantoin (300µg), manufactured by Abtek Biological Ltd, England. A loop full growth of each of the 18hr isolate on nutrient agar was suspended in sterile distilled water, and was serially diluted in steps of ratio 1:10 to give turbidity equivalent to 0.5 MacFarland standard that is a density of

1×10^8 cells/ml before inoculation. Nutrient agar was inoculated with 0.5ml suspension of each isolate adjusted to 1×10^8 cell/ml, with the aid of sterile forceps sensitivity disc containing antibiotics were placed on the surface of each nutrient agar plate evenly seeded with test isolates and was incubated for 24 hours at 37°C. The zones of inhibition produced were measured to the nearest millimeter using a calibrated meter ruler, after then, it was compared against the Clinical and Laboratory Standard Institute (CLSI, 2006).

DataAnalyses

Data generated from the study was analyzed using SPSS version 21. Results were presented in tables and charts as appropriate.

RESULTS AND DISCUSSION

Enumeration of bacterial isolates from samples of commercially sold yoghurts in Uturu

The result for the enumeration of bacterial isolates from commercially sold yoghurt in Uturu is shown in Table 1. For sample coded A, the values of total viable count, total coliform count and total staphylococcal count were 2.0×10^3 cfu/ml, 1.0×10^3 cfu/ml and 1.0×10^2 respectively. No bacteria growth was observed on Eosin methylene blue for total faecal coliform count for sample dilutions of 10^{-6} .

For Sample coded B, values of total viable count, total coliform count and total staphylococcal count were 1.0×10^2 cfu/ml, 0.6×10^2 cfu/ml and 1.0×10^1 cfu/ml, respectively. The plate for total faecal coliform had no bacteria growth as seen on in Table 1.

Sample coded C had the lowest microbial load among other samples, values for total viable count, total coliform count and total staphylococcal were 0.9×10^2 cfu/ml, 0.4×10^2 cfu/ml and 0.2×10^1 cfu/ml respectively; there was no growth for EMB (Table 1).

The sample coded D had 1.0×10^2 cfu/ml for total viable count 0.7×10^2 cfu/ml for total coliform count and 0.5×10^1 cfu/ml for total staphylococcal count respectively. No faecal coliform was reported on the seeded plates.

Sample E had no record of faecal coliform count. 2.0×10^3 cfu/ml, 1.2×10^2 cfu/ml and 1.8×10^2 cfu/ml for total viable count, total coliform count and total staphylococcal count respectively (Table 1).

Identities of Bacteria Isolated from commercially sold Yoghurt in Uturu

The conventional biochemical method of identification was employed to identify the bacteria from the yoghurt samples sold in Uturu, Abia State. The bacteria isolated and identified were *Staphylococcus* species, *Lactobacillus* species, *Bacillus* species and *Streptococcus* species.

Antibiotics Susceptibility Pattern of Bacterial Isolates

The antibiotics susceptibility pattern of the bacterial isolates showed thatdrovid produced the highest zones of inhibition from 31.8 ± 1.2 mm to 36.1 ± 0.3 mm, followed by ofloxacin (29.5 ± 0.1 mm to 35.0 ± 0.8 mm) as shown in Table 2.

Table 1: Microbial load (cfu/ml) of yogurt samples sold in Uturu, Abia State

Samples	TVC	TCC	TFCC	TSC
A	2.0×10^3	1.0×10^3	NBG	1.0×10^2
B	1.0×10^2	0.6×10^2	NBG	1.0×10^1
C	0.9×10^2	0.4×10^2	NBG	0.2×10^2
D	1.0×10^2	0.7×10^2	NBG	0.5×10^2
E	2.0×10^3	1.2×10^2	NBG	1.8×10^2

Keys: TVC = Total viable count, TSC = Total Staphylococcal count, TCC = Total coliform count, TFCC = Total faecal coliform count, NBG = No bacteria growth, CFU/ml = Colony Forming Unit per Mil

Table 2: Antibiotics susceptibility zones of inhibition produced against the bacteria isolates from yogurt samples sold in Uturu, Abia State (mm)

	Ciprofloxacin (5µg)	Norflaxacin (10µg)	Gentamycin (10µg)	Lincocin (20µg)	Streptomycin (10µg)	Rifampicin (20µg)	Chloramphenicol (30µg)	Ampiclox (20µg)	Floxapen (20µg)	Penicillin (10µg)	Drovid (10µg)	Augmentin (10µg)	Oflaxacin (10µg)
<i>Lactobacillus sp.</i>	28.5±0.2	30.9±0.1	17.9±0.1	19.0±0.1	14.8±0.3	21.0±0.9	18.0±0.2	16.0±0.1	18.1±0.2	12.0±0.2	33.1±0.6	29.1±0.4	29.5±0.1
<i>Streptococcus spp</i>	32.4±0.5	23.0±0.5	18.7±0.6	15.0±0.7	17.0±0.9	18.6±0.6	14.0±1.0	15.0±0.1	20.9±0.4	13.0±1.0	36.1±0.3	25.5±0.1	33.4±0.9
<i>Staphylococcus spp.</i>	31.0±0.1	21.1±1.1	19.4±0.5	16.3±0.9	18.0±0.6	14.6±0.1	14.0±1.0	16.0±1.0	17.8±0.5	14.0±1.0	34.1±0.2	21.4±0.5	32.0±1.0
<i>Bacillus spp.</i>	26.0±0.3	33.7±1.8	6.0±0.5	17.6±1.9	13.6±1.1	20.0±1.0	18.0±0.1	12.0±0.6	15.0±0.7	15.8±0.1	31.8±1.2	20.9±1.3	33.7±1.8

Interpretative standard: Clinical and Laboratory Standard Institute (CLSI) (2006).

Discussion

Yoghurt is one of the most nutritious fermented dairy products, unique and sometimes preferred by most consumers. Yoghurt production is made from different ingredients which account for its susceptibility to microbial spoilage. In order to determine an effective safeguarding standard during and after yoghurt production, it is paramount to examine the microbial flora and microbial contaminants (Shoneye *et al.*, 2012).

Using traditionally available method such as morphological and biochemical of identification, the following microorganisms such as *Staphylococcus* species, *Lactobacillus* species, *Bacillus* species, and *Streptococcus* species were identified in the yogurt samples sold in Uturu community. However, no faecal coliform was isolated from the samples examined. The predominant bacteria in all yoghurt samples were *Lactobacillus* spp. and *Streptococcus* spp. The microbiology of yoghurt is limited essentially to these two organisms. The activities and metabolism of these organisms leads to production of yoghurt with its characteristics taste and flavour (Robinson and Tamine, 1986; Obiekezie *et al.*, 2012).

Bacillus species has been implicated in food-borne intoxication. *Staphylococcus* species causes diseases like mastitis, abortions and upper respiratory complications (Ogbulie, 2001). The presence of coliform in yoghurt might be due to lack of cleanliness or the use of contaminated milk or unclean water. Coliform and *Bacillus* species were contaminants and this was not surprising considering the low level of hygiene and development of Uturu. This finding is supported by the study of Ekeleme *et al.* (2020), who reported the presence of *Staphylococcus*, Coliform and *Bacillus* in the domestic water sources of the Uturu community. The presence of coliform could signify the presence of other microorganisms like *Erwinia* species. However, coliform has been widely used as indicator organism in food analysis (Ifeanyi *et al.*, 2013). *Staphylococcus* species could be transferred to the yoghurt from the skin of handlers. Food handling practices by vendors is also a major concern in contaminating what they market, since they do not undergo any formal training before embarking on yogurt drinks buying and selling (Nwamaka and Chike, 2010). The presence of these bacterial contaminants in commercial yoghurt has been reported in previous studies (Oyeleke *et al.*, 2009; Ifeanyi *et al.*, 2013; Afolabi *et al.*, 2017).

The susceptibility of the bacterial isolates showed that drovid produced the highest zones of inhibition from 31.8±1.2 mm to 36.1±0.3 mm, followed by ofloxacin (29.5±0.1 mm to 35.0±0.8 mm). The bacterial isolates had no resistance to penicillin especially *Staphylococcus* species. This could not mean that these bacterial isolates were not necessary pathogens as they could pose threat to human life if ingested. The uncontrolled use of penicillin over the years for different treatment did not result to resistance in these microorganisms. Rationally, the use of this antibiotic as the first line drug of choice during treatment particularly in developing countries leads to the development of multi drug resistant (MDR) organisms in pathogenic organisms as reported by Gugsat *et al.* (2022). Not minding, organisms such as *S. aureus* have been documented to show MDR particularly in hospitals (Raji and Jiya, 2019).

CONCLUSION

In conclusion, the presence of *Staphylococcus* species in yoghurt may be as a result of unclean water used in the production, contaminated milk or unhygienic condition of the handlers. Based on the analysis of the various yoghurt

samples, it could be recommended that yoghurt producers, sellers and handlers should avoid long exposure of yoghurt before selling to consumers. Quality Control (QC) measures with Good Manufacturing Practices (GMPs) should be encouraged. Similarly, standard organization for control of industrial products should ensure that strict measures are taken for compliance to standard.

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