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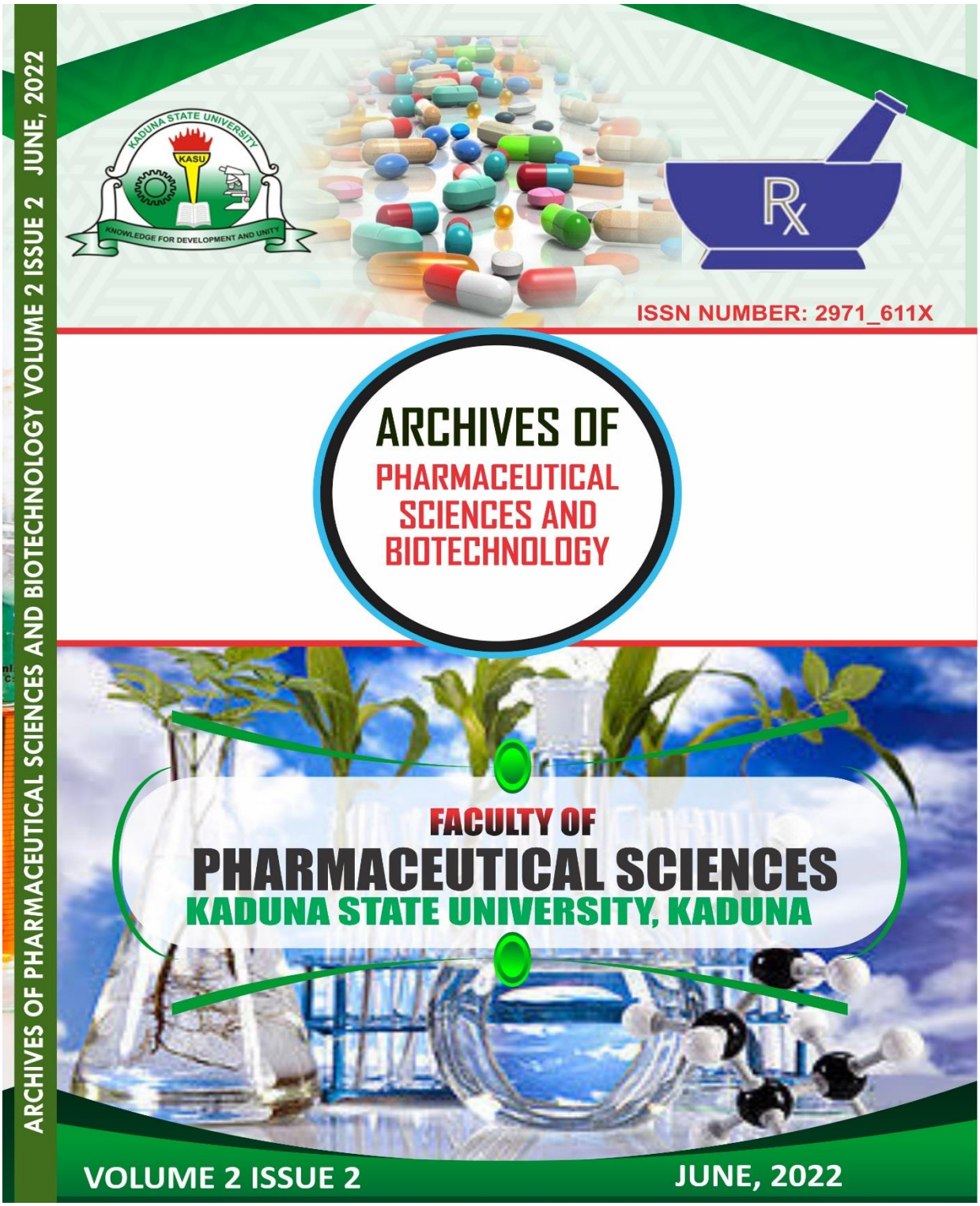

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# MICROBIAL EVALUATIONS OF BRANDS OF YOGHURT SOLD AT NUHU BAMALLI, POLYTECHNIC, ZARIA

**[1*]Salihu, M.S., [2]Musa, A. and [3]Bata M.M.**

[1]Department of Pharmaceutical Microbiology and Biotechnology, Kaduna State University
[2]Department of Science Laboratory Technology, Nuhu Bamalli Polytechnic, Zaria
[3]Department of Microbiology, Federal University Birnin Kebbi

**Corresponding author's email address: ubabasani84@gmail.com**

## ABSTRACT

**Background**: Microbial evaluations of yoghurt play an important role in the detection, elimination and prevention of unwanted microorganisms, which may become contaminant if found in the product. The presence of pathogenic bacteria in yoghurt serves as a risk factor for the health of the consumers, such as food borne illness, food poisoning and gastrointestinal infections.

**Aim**: Microbial Evaluations of Brands of Yoghurt Sold at Nuhu Bamalli, Polytechnic, Zaria, was aimed to evaluate the level of contaminants, investigate the presence of Coliforms and other associated pathogenic microorganisms.

**Methods:** A total of 10 samples, 2 samples from each brand were analyzed for the presence ofcoliforms and other pathogenic microorganisms, total bacterial count and Coliform count.

**Results**: Some samples contain both Gram positive and negative bacteria while others contain only Gram negative. Bacteria isolated from these yoghurt samples include *Staphylococcus aureus*, *Escherichia coli* and *Bacillus spp*. Total bacterial count of the bacterial isolates range from 1.2 $\times 10^3$ to 3.2 $\times 10^4$ CFU/ml while the coliform counts ranged from 2.1 $\times 10^2$ to 3.0 $\times 10^2$ per 100ml. None of the samples contain yeast or mould.

**Conclusion**: Yoghurts sold in the Institution contain significant pathogenic bacteria contaminants which might be from both fecal and personnel.

**Recommendation**: Personnel in charge of production should maintain a high level of hygiene to reduce contamination to the lowest level.

**Keywords**: Microbial evaluation, Yoghurt, pathogens, Nuhu Bamalli Polytechnic

## INTRODUCTION

Yoghurt is a fermented milk product of creamy texture that can be prepared from milk most often made from cow milk. It contains protein, calcium and vitamins, in high proportion. It is popularly patronized all over the world including Nigeria. Yoghurt is produced from pasteurized, none or low fat milk at 450$^o$C by the help of either *Lactobacillus bulgaricus* and *Streptococcus thermophilus* that are resistant to pasteurization temperature (8). Lactobacillus breaks down lactose presence in milk to lactic acid. The lactic acid is responsible for the lowering of PH, preservation and denaturing of milk protein, thereby giving a characteristic taste of the yoghurt (6).

Due to nutritive value of yoghurt, it provides a favourable condition for the growth of microorganism which leads to spoilage of the product (9). The presence of these microorganisms in yoghurt may be attributed to negligence of the various monitoring agencies in Nigeria to supervise the hygienic practices of the various yoghurt companies that have been registered under them.

Yoghurts prepared under good manufacturing practices (GMP) should contain not more than 10 yeast cells and should have a shelf life of 3-4 weeks stored

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at a temperature of 5ºC. moreover, an initial bacterial counts of >100 cfu/ml in yoghurt tend to spoil quickly. The presence of moulds during production and distribution is connected to technological problems, economic losses and health aspects. It is mainly caused by the decomposition of products, deterioration of organoleptic properties and health risk due to the potential production of mycotoxins or allergic conidia, ascospores, and mycelia fragments (5). Coliforms are routinely used as indicator of the quality of the milk and milk products as some members of coliforms are responsible for the development of objectionable taints in milk and its products rendering them of inferior quality or even unmarketable (1).
Therefore, the aims and objectives of this study is to evaluate different brands of Yoghurt sold in Nuhu Bamalli Polytechnic, Zaria for the presence of pathogens and the level of contamination

## MATERIALS AND METHOD

### Sample Size

**Sample size was calculated base on the relationship below**

$$[Z^2 * P^{(1-p)} / e2 / 1 + [Z^2 * p(1-p)] / e^2 * N]$$

Where,
*N* = population size
*z* = z-score
*e* = margin of error
*p* = standard of deviation (7)

### Collection of Samples

Ten samples from 5 different brands of yoghurt were purchased at main campus and anex, Nuhu Bamalli Polytechnic, Zaria. The samples were named as sample A1,A2, B1,B2, C1,C2, D1,D2 and E1, E2. All the samples were analysed within four hours of collection

### Sample Processing

### Media Preparation

All media were prepared according to manufacturer's instruction. The media used include Nutrient agar, peptone water and citrate agar.

### Serial Dilution of Yoghurt Samples

Nine millilitres of peptone water was dispensed in each of the 4 test-tubes provided labelled A to D. one millilitre of yoghurt was dispensed in test-tube labelled A, this test-tube was shake gently. One millilitre of the solution from test-tube A was transferred to test-tube B and from test-tube B to C and finally from test-tube C to D making a dilution factor of $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ respectively. This process was repeated for all the samples (3).

### Inoculation and Cultivation of Serially diluted samples (Determination of Total Viable Bacterial Count)

One millilitre of the variable dilutions factor was transferred unto freshly, solidified and sterile nutrient agar. A bent and sterile glass rod was used in spreading the diluted samples properly on the surface of the nutrient agar plates. The plates were incubated at 37ºC for 24 hours. The colonies were counted using colony counter and the total viable bacterial count in CFU/ml was calculated using the relation below for plates that their colonies were above 30 and less than or equal to 300 (3).

Total Bacterial Count (CFU/ml) = No. of colonies divide by Dilution factor × amount inoculated.

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**Determination of Coliforms Count**

**Presumptive Test:** A three sets of test tubes were arranged and each set containing 5 tubes. 10ml of sterile and double strength of lactose broth will be dispensed in the first 2 sets of test tubes containing Durham tubes in an inverted position and another 10ml of single strength lactose broth was dispensed in the third set of test tubes containing Durham tubes in an inverted position. Using a sterile pipette, 10ml, 1ml and 0.1ml of yoghurt sample was dispensed in to first, second and third sets of test tubes respectively. All the test tubes were incubated at 37$^{o}$C for 24 hours. After 24 hours the tubes were observed for color change and gas production and the results were recorded (3).

**Confirmatory Test:** A loopful from tubes with color change and gas production was inoculated on two separate freshly and dried eosin methylene blue agar, one was incubated at 37$^{o}$C and the other at 44.5$^{o}$C for 24 hours. After 24 hours, greenish metallic sheen colonies were observed and recorded (3).

**Completed Test:** Greenish with metallic sheen colonies from eosin methylene blue agar plate was inoculated on lactose broth fermentation tubes with an inverted Durham tubes in them and nutrient agar slant. All the tubes were incubated for 24 hours at 37$^{o}$C. After 24 hours, lactose broth fermentation tubes was observed for gas production while cultures from agar slant was Gram stained and observed microscopically for Gram negative and non-spore forming bacilli (3).

**Isolation and Identification of Bacteria**

**Cultural Identification:** Microorganisms was isolated based on colonial morphology i.e. color, shape, size, elevation, surface, etc

**Biochemical tes**t: Biochemical reactions were carried out on the isolated organisms and this include: indole, methyl red, Voges Proskauer, citrate utilization

**Indole:** A tube containing tryptophan broth was inoculated with an overnight culture of the isolate, this was incubated at 37$^{o}$C for 24 hours. After 24 hours, about 0.5 ml of Kovac's reagent was added to the broth culture. This was left for about 10 minutes, a pink color ring was observed at the surface for organisms that are indole positive (3).

**Methyl Red and Voges Proskauer Test**: A pure culture of the investigated isolate was inoculated in to MR-VP broth. This was then incubated at 35$^{o}$C for 4 days. After incubation period, the broth culture solution was aseptically divided into two places, methyl red was added to one and was left for few minutes while Barrit's reagent was added to the solution and was shaked for several minutes. A red color was observed for organisms that MR and VP positive (3).

**Citrate Utilization Test:** An overnight culture was streaked on green citrate agar slant. This was incubated at 37$^{o}$C for 5 days. After incubation period, a color change from green to blue along the slant was observed for organisms that utilize citrate (3).

**Gram Staining**

Isolates was Gram stained by preparation of smear, heat fixing and staining the heat fixed slides

**Preparation of the smear:** With the aid of a sterile wire loop, a loopful of the isolate was placed on the slide. It was spread by means of circular motion of the inoculating loop to

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about one centimetre in diameter. This was allowed to air dried.

**Heat Fixaton of the Smeared Slide:** This was carried out by passing the smeared slide over the flame about 3 times.

**Staining the Heat Fixed Slide:** The heat fixed slide was placed on the staining tray. Crystal violet was gently flooded on the slide and was left for 1 minute. This was gently rinsed with water. Gram's iodine was flooded and left for 1 minute; this was also rinsed with water. Acetone was flooded and was allowed to stand for 5 seconds and rinsed with water. The slide was then counter stained with safranine for about 1 minute, this was also rinsed with water. The slide was allowed to air dried. The air dried stained slide was observed using the microscope under oil immersion.

**Isolation and Identification of yeasts and moulds**

Ten millilitre of Sample of yoghurt was aseptically dispensed, mixed in a container containing 10ml of 0.1% sterile peptone solution. This solution was then innoculated on a freshly prepared plate count agar (PCA), incubated at 28$^{o}$C for 48hours.

## RESULTS AND DISCUSSION

**Total Viable Bacterial Count**

The total viable bacterial count of the bacterial isolates isolated from different samples range from 1.2 $\times 10^3$ to 3.0 $\times 10^4$ CFU/ml. The summary of the result was shown in table 1

**Table 1: Staphylococcal Count of the Bacterial Isolates**

| Sample code | Total Bacterial Count (CFU/ml) |
|---|---|
| SA1 | $3.0 \times 10^4$ |
| SA2 | $2.1 \times 10^4$ |
| SB1 | $1.5 \times 10^4$ |
| SB2 | $1.2 \times 10^4$ |
| SC1 | $1.4 \times 10^3$ |
| SC2 | $1.8 \times 10^3$ |
| SD1 | $2.2 \times 10^4$ |
| SD2 | $1.2 \times 10^4$ |
| SE1 | $1.6 \times 10^3$ |
| SE2 | $1.2 \times 10^3$ |

**Total Coliform Count of the bacterial isolates**

The coliform count of the bacterial isolates of all the samples range from 2.1 $\times 10^2$ to 3.0 $\times 10^2$ per 100ml. The summary of the result was shown in table 2

**Table 2: Total Coliform count of the bacterial isolates**

| Sample code | Total Coliform count per 100ml |
|---|---|
| SA1 | $2.5 \times 10^2$ |
| SA2 | $2.3 \times 10^2$ |
| SB1 | $2.2 \times 10^2$ |
| SB2 | $2.0 \times 10^2$ |
| SC1 | $3.0 \times 10^2$ |
| SC2 | $2.8 \times 10^2$ |
| SD1 | $1.8 \times 10^2$ |
| SD2 | $2.0 \times 10^2$ |
| SE1 | $2.5 \times 10^2$ |
| SE2 | $2.4 \times 10^2$ |

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**Gram Reaction of the Bacterial Isolates**

The Gram reaction of the bacterial isolates revealed both purple, pink, short rods and cocci in clusters. The summary of the result is shown in Table 3.

**Table 3: Morphological Characteristics of stained colonies**

| Sample Code | Colony morphology | Type of bacteria |
|---|---|---|
| SA1 | Both pink and short rods colonies and cocci in clusters | Both Gram negative and Gram positive bacteria present |
| SA2 | Both pink and short rods colonies and cocci in clusters | Both Gram negative and Gram positive bacteria present |
| SB1 | Both pink and short rods colonies and cocci in clusters | Both Gram negative and Gram positive bacteria present |
| SB2 | Both pink and short rods colonies and cocci in clusters | Both Gram negative and Gram positive bacteria present |
| SC1 | pink and short rods colonies | Gram negative bacteria present |
| SC2 | pink and short rods colonies | Gram negative bacteria present |
| SD1 | Both pink and short rods colonies and cocci in clusters | Both Gram negative and Gram positive bacteria present |
| SD2 | Both pink and short rods colonies and cocci in clusters | Both Gram negative and Gram positive bacteria present |
| SE1 | Both pink and short rods colonies and cocci in clusters | Both Gram negative and Gram positive bacteria present |
| SE2 | Both pink and short rods colonies and cocci in clusters | |

**Biochemical test of the isolates**

The biochemical tests carried out include indole, methyl red, Voges Proskauer, citrate, triple sugar iron, coagulase, catalase oxidase and motility. Base on the outcome of the biochemical reaction, there are presence of *Escherichia coli, Staphylococcus aureus* and *Bacillus spp*. The summary of the result is shown in Table 4

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**Table 4: Biochemical Characterization of the Bacterial Isolates**

| Sample code | Biochemical Test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | MR | VP | C | TSI($H_2S$) | M | CAT | COG | OX | Organism |
| SA1 | + | + | - | - | - | + | - | - | - | *Escherichia coli* |
| | - | - | - | - | - | - | + | + | - | *Staphylococcus aureus* |
| | - | - | + | + | - | + | - | + | + | *Bacillus spp* |
| SA2 | + | + | - | - | - | + | - | - | - | *Escherichia coli* |
| | - | - | - | - | - | - | + | + | - | *Staphylococcus aureus* |
| SB1 | - | - | - | - | - | - | + | + | - | *Staphylococcus aureus* |
| | + | + | - | - | - | + | - | - | - | *Escherichia coli* |
| SB2 | + | + | - | - | - | + | - | - | - | *Escherichia coli* |
| SC1 | - | - | - | - | - | - | + | + | - | *Staphylococcus aureus* |
| SC2 | + | + | - | - | - | + | - | - | - | *Escherichia coli* |
| SD1 | + | + | - | - | - | + | - | - | - | *Escherichia coli* |
| | - | - | - | - | - | - | + | + | - | *Staphylococcus aureus* |
| SD2 | + | + | - | - | - | + | - | - | - | *Escherichia coli* |
| SE1 | + | + | - | - | - | + | - | - | - | *Escherichia coli* |
| SE2 | + | + | - | - | - | + | - | - | - | *Escherichia coli* |

**Keys*:*** I- indole, MR- methyl red, TSI- triple sugar iron, M- motility, CAT- catalase, COG- coagulase, OX- oxidase

## DISCUSSION

The presence of these bacteria in yoghurt is an indication of contamination and this may be poor manufacturing processes as described by Yabaya and Idris (1) that due to the negligence of the various monitoring agencies in Nigeria to supervise the hygienic practices of the various yoghurt companies that have been registered under them, many of them have relaxed in their sanitary routine leading to contamination of the products.

Stewart and Humphrey (9) also explained that microbial evaluations of yoghurt play an important role in the detection, elimination and prevention of unwanted microorganisms which may become contaminant if to be found in the product. Therefore, the presence of coliform bacteria in these samples is an indication of faecal contamination during production.

The results of this research work is in congruent with other researchers carried out both abroad and in Nigeria. João Luís Ferrão and Gininha Micaela Pitrosse (4), conducted a similar research work in Chimoio, Mozambique and revealled the presence of *Staphylococcus aureus* with a count of $0.72x10^2$ CFU / ml from yoghurt.

Abdul Matin *et al*., (2), revealled the presence of both mould, yeast, and bacteria including coliform bacteria, a research carried out in Bangladesh. Total viable bacterial count and coliform count of $1.72\times10^7$ to $5.04\times10^8$ CFU/ml and $1.02\times10^2$ to $4.51\times10^2$ CFU/ml respectively.

Taiwo *et al*. [10], also conducted a research on yoghurt in Ota, Ogun State revealled the presence of both bacteria and fungi. These organisms include *Lactobacillus spp, Bacillus spp, Corynebacterium spp., Klebsiela spp., Staphylococcus spp, Pseudomon spp., Proteus spp., Micrococcus spp., Shigella spp., Listeria spp. anStreptococcus spp. Mucor spp.,*

*Geotrichum spp., Montospora spp., Aspergillus spp., Rhizopus spp.,*and *Fusanrium spp.*

## CONCLUSION

Base on the outcome of this research, yoghurt sold in Nuhu Bamalli polytechnic, Zaria, is grossly contaminated with different species of bacteria including coliform. The research also revealed the presence of total bacterial count of the bacterial isolates range from $1.2 \times 10^3$ to $3.2 \times 10^4$ CFU/ml while the coliform counts range from $2.1 \times 10^2$ to $3.0 \times 10^2$ per 100ml while none of the sample contains yeast or mould.

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