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## Microbiological Assessment of Commercial Yogurt Sold in Ota Metropolis, Ogun State, Nigeria.

To cite this article: O. S. Taiwo *et al* 2018 *IOP Conf. Ser.: Earth Environ. Sci.* **210** 012019

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## Microbiological Assessment of Commercial Yogurt Sold in Ota Metropolis, Ogun State, Nigeria.

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### ABSTRACT

Ten authorized Yogurt products purchased within Ota metropolis, Ogun State of Nigeria were subjected to pH, Total Bacterial Count (TBC) and Total Fungal Count (TFC) analysis. pH values were in the range of 4.05 to 5.50, the TBC and TFC values ranged between  $1.0 \times 10^3$  -  $5.0 \times 10^5$  cfu/ml and  $1.0 \times 10^3$  -  $5.0 \times 10^5$  cfu/ml respectively. Eleven bacterial isolates were detected in the yogurt samples. *Lactobacillus spp.* and *Bacillus spp.* constituted 16% of the total microbial load, *Corynebacterium spp.*, *Klebsiella spp.*, *Staphylococcus spp.*, and *Pseudomonas spp.* constituted 8% while *Proteus spp.*, *Micrococcus spp.*, *Shigella spp.*, *Listeria spp.*, and *Streptococcus spp.* constituted 4%. Fungal isolates obtained were *Mucor spp.* (22%), *Geotrichum spp.* (17%), *Montospora spp.* (11%), while *Aspergillus spp.*, *Rhizopus spp.*, and *Fusarium spp.* constituted 6%. The antimicrobial susceptibility test showed that the isolates exhibited susceptible to Ciprofloxacin and Ofloxacin and resisted Nitrofurantoin, Augumentin, Cefixime, Ceufuroxime, Gentamicin and Ceftazidime. The isolates were plasmid encoded, with size range of 20,000-40,000 Kilo base pairs. Result show no significant difference within the bacteria isolates ( $P > 0.05$ ), while the fungi isolates showed significant difference ( $P < 0.05$ ). Significant difference also occurred between the bacteria and the fungi isolate ( $P < 0.05$ ).

Key words: Assessment, Bacteria, Fungi, Isolates and Plasmid.

### 1.0 INTRODUCTION

Man has been consuming fermented milk since the beginning of civilization (Mckinley, 2005). Yoghurt, a fermented dairy product popularly consumed around the world, can be achieved by the fermentation lactic acid in milk by the activity of a starter culture of *Lactobacillus delbruckii sub-specie bulgaris* and *Streptococcus thermophiles*. The two genera play important roles in the manufacture of yogurt from milk product through acidification and synthesis of aromatic



compounds (Serra *et al.*, 2009). The lactic acid fermentation of milk is also a means of prolonging the shelf life of the nutrients in milk (Hui, 1992; Oyeleke, 2009).

Yogurts are regarded as ready to drink foods commonly taken for energy production and for health, all over the world, especially in Nigeria (Alli *et al.*, 2010). It can also be consumed as a drink beverage to quench thirst (Alfa- Lawal, 1984). This product blend is rich in protein and improves healthy living (Cueva and Aryana, 2008). It is a balanced food which contains virtually almost all the nutrients present in milk and in more absorbable form. There is a wide range of flavors available to spice it (Anther, 1986; Oyeleke, 2009). Due to its nutritional, organoleptic and probiotic qualities, it is a popular drink that is in high demand (De *et al.*, 2014).

Industrial processes have improved the quality, storage, transportation and commercialization of the product. Microbiological parameters especially, general coliforms, *Escherichia coli* and *Enterococcus* bacteria counts are commonly used to ascertain these conditions (Tamine and Robison, 2007). The qualification of acidity and temperature are also observed in order to evaluate the preservation of yogurt status; statistics shows a variation from 0.6 to 1.5g of lactic acid per 100g of product and the temperature of preservation in dairy industries and markets must not be higher than 10°C (Rodrigues *et al.*, 2010).

Yogurt being a product of fermented milk from fresh milk can be easily contaminated. Yeast and mould are considered as primarily contaminants in Nigeria (Suriyarachchi and Fleet, 1981; Oyeleke, 2009). Fungi are capable of growing and reproducing in acidic environment with appreciable oxygen (De *et al.*, 2014). Some species of the genus *Aspergillus* have been implicated in the production of secondary metabolites known as aflatoxins which are toxic and carcinogenic (Issazadeh *et al.*, 2012). Contamination by fungi or moulds can result in changing or modifying the taste, colour, texture and organoleptic characteristics of the yogurt. Thereby, humans can be at risk taking or drinking it.

Ota is characterized by low level of environmental sanitation, poor waste disposal and lack of bacteriologically free water (potable). The aim of this study is to ascertain the microbial load or quality of some commercially vended yogurts retailed in Ota metropolis.

Yogurts is the fermentation of dairy product that is obtained by lactic acid fermentation of milk through the activity of a starter culture of two bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii sub-specie bulgaris*. These microorganisms play important roles in the production of yogurt, from milk product through acidification and synthesis of aromatic compounds (Serra *et al.*, 2009). This lactic acid fermentation of milk also serves as a means of prolonging the shelf of the nutrients in milk (Oyeleke *et al.* , 2009, Hui, 1992).

Yogurts are regarded as ready to drink foods usually consumed as drink to quench thirst (Alfa-Lawal, 1984). This product is rich in protein and improves healthy living (Cueva and Aryana,

2008). Yogurt is a balanced food drink which contains virtually almost all the nutrients present in milk and in more absorbable form and can be produced from whole or skimmed milk and there is a wide range of flavors available to spice it (Anther, 1986; Oyeleke *et al.*, 2009). Due to the increasing demand for yogurt drinks, automated equipment is required to facilitate industrial production, in order to meet with both quality and safety quality products (Salinas, 1986).

The improved industrial process has improved the storage, transportation and commercialization of the product (Tamine and Robinson, 2007). Yogurts like any other milk product are liable to contamination of bacteria, yeast and moulds, which are primarily source of contaminants in commercially produced yogurt in Nigeria (Oyeleke, 2009; Suriyarachchi and Fleet, 1981). Ota is characterized by low level of environmental sanitation, poor waste disposal and lack of bacteriologically free water (potable).

In 2000, World Health Organization (WHO) recognized food and drink safety as an essential public health function. Food safety can be defined as the opposite of food risk that is “the probabilities of not being adversely affected from ingesting a particular food or drink” (Henson and Traill, 1993). Lack of adequate awareness on the origin and severity of foodborne diseases, that made food handling practices less motivated to change. Un-hygienic food drinks can affect the health of individuals and this can be prevented by practicing food safety hygiene (Schafer *et al.*, 1993). It’s important to have clear knowledge about prevention of foodborne diseases, though gain of such knowledge might not lead to total change in people’s behavior and that is why approach is very essential (Henson and Traill, 1993; Gotsch, 2012). Attitude, knowledge and practice formed by manner with impressions that comes as regards economic, social, cultural aspect of life (Scharff, 2010).

Most persons lack knowledge of basic rules pertaining to food hygiene and consequently, report shows that approach and ability of consumers generally demographic and socio-economic differs in their background, such as age, gender and academic status (Sockett, 1995; Wilcock *et al.*, 2004). Men show more risky approach than women and the prevalence of risky behavior also increased with increasing socio-economic status (Altekruse *et al.*, 1999). Research revealed in the United States (U.S.) by (Unklesbay *et al.*, 1998) showed that students entered into a curriculum which consist food safety data possesses greater food safety ability contrast to others. Lifestyle changes has shown considerable influence on user’s in the U.S.; results show that consumers lack adequate knowledge on the microorganisms causing food poisoning, type of foods associated with these microorganisms, and the need of avoidance of cross contamination (Williamson *et al.*, 1992).

There are three important aspects to be considered in the incidence of food drink pathogens as regards the personnel handling food drink: Approach, practice and attitude (Scharif and Al-Malki, 2009). “Persons that prepare and sell food drink are known as Food drink handlers (WHO, 1989). Ability and approach are essential; these indicate differences within food safety

ability and self-reported methods (Woodburn and Raab, 1997). Results show that most of the outbreaks resulted from improper food drink handling practices (Ehiri and Morris, 1996). Different studies showed that considerable number of illnesses is caused by improper handling practices (Flint *et al.*, 2005). Observation shows that most peddler exhibit poor handling method, and so food drinks are exposed to hazardous situations such as cross contaminations and undesirable temperature (Ekanem, 1998).

The pathogens can be ingested through the nose, skin and faeces especially through hands of the handlers (WHO, 1989). Research revealed that *Escherichia coli*, *Salmonella*, *Campylobacter* and other bacteria remain viable under finger tips and other surfaces for some days (Howes *et al.*, 1996).

Hawking of food drinks in the street is a source of diet of most people towns (Suneetha *et al.*, 2011). About 2.5 billion people worldwide rely on vended food drinks every day, and as such most nations have observed changes in their socio-economic level in about ten years ago; the changes led to significant growth in the majority of vended street food drinks (WHO, 1996). With increase in population and urbanization in developing countries, it is expected that vended food drinks will be on the increase (WHO, 1996).

Street-hawked food drink has many benefits and these include:

1. It provides source of income for majority of people
2. It is not expensive, convenient, and nutritive drink for both rural and urban area
3. It also provides business low capital and allows for self –employment (WHO, 1996).  
Beside the fact that, hawking of these drinks can result to problem of waste disposal in the city, the major concern is how safe they are. These are possible cause of severe drink poisoning outbreaks in most nations around the world, via microbiological threat (WHO, 1996).

Research revealed that WHO (WHO, 1996), most nations have reported many cases of food drink; here is the submission of the research:

1. Vending facilities were different, from fixed stalls to mobile carts.
2. Limited infrastructures lead to restricted access to drinking water (47%), sewage (15%) and refrigeration (43%).
3. The abuse of temperature and time aided factors leading to drink and food borne diseases
4. Majority of the countries have reported insufficient inspection of the handlers; that is to say, insufficient application of Hazard Analysis Critical Control Point (HACCP).

### *1.1 Statement of the problem*

The way and manner vendors handle and transport the finished product from the manufacturers or production site to their various outlets for sales to the consumers poses a challenge and can result in post-production contamination. They do not put into consideration temperature and the way it's been handled if the package is dumped in an un-hygienic truck or vehicle, it gets contaminated. These result in risk in public health.

Generally, the challenge of yogurt production in Nigeria is characterized by inadequate housing facility, lack of bacteriologically free water (potable); poor waste management, lack of adequate environmental sanitation in areas of high population density and people with low income. The Agencies in charge of enforcing good environmental sanitation laws are not usually available to caution or prosecute offenders. This regulatory negligence results in the nonchalant attitude of these industries in disposing of their waste adequately.

### *1.2 Aims and objectives*

The purpose of this research is to assess the quality of selected yogurt commercially produced and sold in Ota Metropolis, Ogun State. Specific objectives are:

1. To isolate and characterize microorganisms (fungi and bacteria) that may be present in the yogurt samples
2. To ascertain whether microbial isolates are pathogenic
3. To ascertain the presence of starter cultures in the products

## **2.0 MATERIALS AND METHODS**

### *2.1 Materials*

Materials used include; Glass wares such as test tubes, beakers, conical flasks, Petri-dishes, McCartney bottles, stirring glass rod, measuring cylinder, etc. Media such as Sabouraud Dextrose Agar (SDA), Nutrient Broth (NB), Nutrient Agar (NA), Plate Count Agar (PCA), MacConkey Agar (MA), Agarose 0.7%, Sulphide Indole Motility Agar (SIM agar), Biochemical test reagents, Gram's staining kit, Lacto phenol cotton blue stain, TENS buffer etc..

The equipment used include; Light binocular microscope (Lieca), Transilluminator Benchtop UV (BioDoc-It Imaging system), Electrophoretic tank and casting tray, Microwave (Currys

essentials), Micro pipette and tips of different sizes, Autoclave (Surgifriend Medicals, England), Oven (Genlab), Weighing balance (Symmetry Cole-Parmer), Incubator (Uniscop, England), Bunsen burner with gas supply, wire loop, inoculating needles etc.

All glass containers and wares were washed with detergent, rinsed with distilled water, drained and dried after which they were sterilized in hot air oven at 160<sup>0</sup>C for 1 hour.

## *2.2 Media preparation*

Each medium was accurately weighed and dissolved in appropriate quantity of distilled water, according to the manufacturer's specification; heated in water bath till the agar powder melted, and the medium was sterilized in an autoclave and was kept in the incubator overnight for sterility test, and kept in refrigerator for further use.

## *2.3 Collection of samples*

The samples selected for this research work were ten in numbers, eight were the ones in plastic bottled containers and two were in sachet form. All the yogurts tested were registered by NAFDAC.

Yoghurts samples were procured from different vendors within Ota metropolis, Ota, Ogun State. The samples were labeled as A1-J1. After proper labeling, samples were brought to Microbiology Laboratory in ice packed cooler for microbiological analysis.

## *2.4 Determination of pH of collected samples*

The pH values of the yogurt samples were measured three times, at 12 hourly intervals and the average was taken, using pH meter (Adwa ad 1040 pH/Mv.), after calibrating with buffer solution pH 7.0, the results is indicated in table 1.

## *2.5 Microbiological analysis*

### *2.5.1 Enumeration of bacteria*

One millimeter (1ml) of each Yoghurt sample was homogenized in 9.0 ml of sterile distilled water and this one was used as stock solution. Then dilutions were made up to 10<sup>-6</sup> from the stock solution using dilution method as described by

One milliliter (1.0ml) of each dilution was seeded on Nutrient agar (NA), Plate count agar (PCA) and Mac Conkey agar (MA) plates using pour plate method and the plates were then

incubated at 37<sup>0</sup>C for 24hours. Total colonies on the surface of the plates were counted and expressed as log<sub>10</sub> colony forming unit per milliliter (log<sub>10</sub> cfu/ml) of the yoghurt sample.

### 2.5.2 Fungal count

One milliliter (1.0ml) of each sample dilution (section 3.5.1) was seeded on Sabouraud Dextrose Agar (SDA) plates using pour plate method and the plates were incubated at 25+/- 2<sup>0</sup>C (room temperature). The colonies were counted and expressed as log<sub>10</sub> colony forming unit per milliliter (log<sub>10</sub> cfu/ml) of the yoghurt sample.

### 2.6 Isolation of organisms

For this purpose, dilutions were made up to 10<sup>-6</sup> for all the collected samples. One ml of 10<sup>-1</sup>, 10<sup>-3</sup> and 10<sup>-5</sup> dilution was inoculated on NA, SDA, PCA and MA. The colonies were isolated and sub-cultured onto appropriate media for identification purpose.

### 2.7 Identification and characterization of bacterial isolates

Bacterial isolates were characterized by morphological, biochemical characteristics and were identified by using Bergey's Manual of Systematic Bacteriology (Holt *et al.*, 1994). 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 Voges prokauer (VP).

#### 2.7.1 Gram staining method

A loop full drop of distilled water was dropped on a clean grease free glass slide, the inoculating loop was sterilized by dry heat i.e. flaming it until it was red hot. The loop was allowed to cool and a little portion of the isolate was picked and smeared in the drop of distilled water on the slide, making the smear a thin one and was allowed to air dry. Afterwards the smear was heat fixed with gentle heat by passing it over flame.

The smear was flooded with 1% Gential violet for 60 seconds and was flooded with water, and was again flooded with Lugols Iodine for 60 seconds and washed off with water. The slide was decolourized with 75% alcohol and was washed off almost immediately in other to avoid over decolourization, the slide was counter stained with Safranin for 60 seconds, flood with tap water, and smeared glass slides were air- dried. The slides were viewed the light microscope, using the x 100 objective lens (Oil immersion lens).



The bacteria cells that appeared purple were the Gram-positive either cocci or rod, while those that appeared pink were the Gram-negative either rod or cocci.

### *2.7.2 Motility test (hanging drop method)*

The test organisms were inoculated into peptone water and incubated over-night at 37<sup>0</sup>C. From the over-night culture, a wet mount was made by placing a drop of the broth on a grease free slide, and then it was covered with Vaseline bound cover slip and was viewed under the microscope using x40 objective lens for movement.

### *2.7.3 Biochemical tests*

#### **I. Catalase test**

A little part of the test organism was emulsified using a sterile inoculating loop and placed on a clean glass slide, with the aid of a dropper or Pasteur pipette, 1 drop of 3% H<sub>2</sub>O<sub>2</sub> was applied onto the organism on a clean slide, and observed for immediate effervescence (bubble formation), which indicates a positive reaction while if there was no bubble formation it's indicative of negative reaction.

#### **II. Coagulase test**

A suspension of the test organism was made on a clean glass slide a drop of fresh human plasma was added and mixed, and then it was observed for clumping, which indicates a positive result.

#### **III. Oxidase test**

Oxidase disc was soaked with about four inoculating loops of deionized water using a loop, a large mass of pure bacteria was transferred on to the disc aseptically, and the disc was left for 2 minutes and was observed. Change on colour from dark-blue to maroon or to almost black indicates positive reaction while no colour change indicates a negative result.

#### **IV. Indole test**

Pure bacterial culture was grown in sterile tryptone broth over-night, 1.0ml of Chloroform was added to the broth and shaken gently. After this, 5 drops of P-Dimethylaminbenzaldehyde (KOVAC'S reagent), was added to the broth culture. Red colour on the surface layer of the broth is indicative of positive result while negative result appeared yellow on the surface of the broth.

#### **V. Citrate test**

The medium Simmon's citrate agar, was prepared according to the specification of the manufacturer's and 15ml was dispensed into McCartney bottles and then autoclaved at 121<sup>0</sup>C for 15 minutes. The slants were inoculated with the test organisms and were incubated at 37<sup>0</sup>C for 4 days, leaving one un-inoculated medium which served as the control. Colour change of the medium from green to blue indicates a positive result, while a negative reaction is indicated with no colour change of the medium.

#### **VI. Urease test**

A slant of Christensen's urea agar was inoculated with test organism, leaving one un-inoculated slant which served as the control. The slants were incubated at 37<sup>0</sup>C for 5 days observing daily for colour change, for positive case the colour changed from yellow to pink, while the negative ones remain unchanged in colour.

#### **VII. Methyl red (MR) test**

The test organism was inoculated into test tubes containing 5.0ml of sterile MR VP broth aseptically. The tubes were incubated at 37<sup>0</sup>C for 48 hours, then 5drops of methyl red indicator were added to each test tube and the medium was observed for colour change. Red colour indicated positive result, while yellow colour indicated negative result.

#### **VIII. Voges-prokauer (VP) test**

The test organism was inoculated into test tube containing 5.0ml of sterile MR-VP broth, after 48hours of incubation, 5 drops of Alpha- Naphthol ( Barrit's A) and Pottassium hydroxide (Barrit's B) was added. A dark red colour appears within 20-30minutes which indicates a positive result.

#### *2.8 Identification of fungal isolates*

A speck of growth from Sabouraud Dextrose Agar (SDA) plate was cut and placed on a grease free glass slide, containing a drop of Lacto phenol cotton blue stain as mounted and then it was covered with a clean cover slip. The mycelium was then examined under the light microscope with x40 objective lens

#### *2.9 Determination of activity of different antimicrobial agents against the isolates obtained from yogurt samples:*

Isolates from the yogurt samples were tested for sensitivity against standard conventional antibiotic discs like, Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5µg), Ofloxacin (5µg), Augmentin (30µg), Ciprofloxacin (5µg), and Nitrofurantoin (300µg),

manufactured by Abtek Biological Ltd, England. A loop full growth of each 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  $1 \times 10^8$  cells/ml before inoculation. Nutrient agar was inoculated with 0.5ml suspension of each isolate adjusted to  $1 \times 10^8$  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  $37^{\circ}\text{C}$  (Benson, 2005).

### *2.10 Determination of plasmid profile of some isolates from yogurt samples:*

Plasmid profiling of the four gram-negative isolates were carried out, by following the TENS-Mini Prep method Zhou *et al.*, 1990.

Plasmid DNA isolation (TENS prep)

1. Overnight culture of 1.5 ml was dispensed into a microfuge tube
  2. The deposit was spun for 1 min at 10rpm to pellet the cells
  3. The supernatant was discarded, and 150ul of media was left in the tube
  4. It was vortexed to re- suspend the cells
  5. TENS buffer of 300 $\mu\text{l}$  was added to the vortexed cells
  6. The tube was inverted 3-4 times gently, in order to lyse the cells completely (the liquid turned from turbid to clear)
  7. Sodium acetate 3M (pH 5.6) of 150ul was added and invert 3-4 times gently, in order to allow for a white precipitate formation, and then the white precipitates were spurned for 5mins to pellet.
  8. The clear supernatant was pipetted to a clean tube
  9. Ethanol (95%) of 900  $\mu\text{l}$  was added and tubes were inverted to mix
  10. The DNA pellets were recovered by spinning at maximum speed for 2mins.
  11. The solution was discarded and then Ethanol (70%) of 500 $\mu\text{l}$  was added to wash the pellet by vortexing and was spurned for 1min.
  13. Ethanol (70%) was discarded and the DNA pellets were dried to pellet
  14. DNA was dissolved in 50 $\mu\text{l}$  10mM Tris (pH 8) (Can be supplemented with RNase)
- TENS buffer: Tris-Hydrogen Chloride (HCL) 10 mM (pH 8.0) EDTA 1 mM Sodium hydroxide (NaOH) 0.1 N Sodium Dodecyl sulfate (SDS) 0.5% (w/v).

The results are shown in plate 1.

### *2.11 Method of Statistical Analysis*

The method of statistical analysis used for the study was the analysis of variance using the Statistical Package of Social Sciences SPSS16 version.

### 3.0 RESULTS

#### 3.1 Determination of pH

The pH values of yogurt samples were in the range of 4.05-5.50. The results are expressed in Table 1.

#### 3.2 Enumeration of bacterial, fungal isolates and the pH of the yogurts

It has been shown that for sample A2, the values of TBC, TFC and pH were  $1 \times 10^3$  cfu/ml,  $1 \times 10^5$  cfu/ml and 4.05 respectively, there was no significant growth for all the ( $10^{-1}$ ,  $10^{-3}$ ,  $10^{-5}$ ), that were plated on SDA plates. Bacteria growth was observed on PCA plate for dilutions  $10^{-1}$  ( $2 \times 10^2$  cfu/ml) and  $10^{-3}$  ( $2 \times 10^3$  cfu/ml), each plate had two colonies on, there was growth of creamy shining colonies for all the dilutions on NA. Bacterial growth was only present for dilution  $10^{-1}$  on MCA plate, as indicated in table 1.

Sample B1, values of TBC, TFC and pH were  $1 \times 10^1$  cfu/ml,  $2 \times 10^2$  cfu/ml and 5.50 respectively, significant growth was only observed on dilution  $10^{-1}$  ( $1 \times 10^1$  cfu/ml) for SDA plate and it appears brownish in colour. Significant growth was observed on NA plate which covers the entire plate, however there was no significant growth for all the dilutions, on PCA and MA plates respectively, and it can be seen in table 1.

Sample C1, table 1 shows values for TBC, TFC and pH were  $1 \times 10^1$  cfu/ml,  $2 \times 10^2$  cfu/ml and 4.18 respectively, there was no significant growth on all the dilutions for SDA plates, and one colony was observed each on the MA and NA plate for  $10^{-1}$  and  $10^{-5}$  dilutions. It was also observed that on MA plate growth was only on  $10^{-1}$  dilution, and these can be seen in table 1.

Sample D1, the TBC, TFC and pH values were nil,  $1 \times 10^2$  cfu/ml and 5.41 respectively, there was no significant growth on all cultured plates (SDA, PCA and, MA) for all the dilutions, but for NA plate, growth of whitish colony on  $10^{-1}$ , and on  $10^{-3}$  dilution, a creamy colony was observed, as shown in table 1 and 2.

Sample E1, table 1 shows that the TBC, TFC and pH values were, nil,  $1 \times 10^2$  cfu/ml and 4.07 respectively, it was also observed that there was no growth on SDA and PCA plates for all the dilutions, while growth was only observed on MA  $10^{-5}$  and for all the dilutions on NA plates as shown in table 1.

Sample F2, from table 1 and 2, it shows values for TBC, TFC and pH as  $2 \times 10^3$  cfu/ml,  $1 \times 10^2$  cfu/ml and 4.07 respectively, it was observed that there was no significant growth on SDA and MA plates for all the dilutions, while growth were observed on PCA and NA plates for dilutions respectively.

Sample G1, table 1 and 2, shows that there were no growth observed on all the dilutions on SDA, PCA and MA, and values for TBC, TFC and pH were Nil, Nil and 4.12 respectively, growth of whitish colony was observed on NA plate, for all the dilutions and were found to be fungi growth.

Sample H1, the values of TBC, TFC and pH were nil, nil and 5.08 respectively, there was no growth observed on PCA plate for all the dilutions and it was also observed that there were partial growth on SDA plate for dilution  $10^{-5}$  which appear fluffy white colony suspected to be fungi. There was also growth on MA and NA plates, for all the dilutions and were found to be bacteria and fungi, as shown in table 1 and 2.

Sample I1, table 1 shows that the TBC, TFC and pH values were  $6 \times 10^3$  cfu/ml, nil and 5.38 respectively, it was observed, that there was growth on PCA which has the highest TBC values and there was significant growth on NA plates for all the dilutions. For SDA and MA, growth was observed on  $10^{-1}$  and  $10^{-3}$  dilutions, as seen in table 1.

Sample J1, it was observed that there no significant growth on all the plates, and the values for TBC, TFC and pH were found to be nil, nil and 4.84 respectively, as shown in table 1.

### 3.3 Isolation and identification of organisms

#### 3.3.1 Bacterial isolates

Twenty five bacterial colonies were isolated from samples and were labeled as 1-25. It was observed that *Lactobacillus* and *Streptococcus* sp. were isolated from yogurt samples, which confirms them as organism used as a starter culture, while *Pseudomonas*, *Staphylococcus*, *klebsiella*, *Shigella*, *Bacillus*, *Proteus specie* etc. Were also implicated in some of the yogurt drink samples as contaminants as seen in table 3. *Bacillus* and *Lactobacillus specie* had the highest percentage occurrences of 16% each, followed by *Corynebacterium Klebsiella*, *Staphylococcus* and *Pseudomonas sp.* were all having 8% each and for *Streptococcus*, *Shigella*, *Proteus*, *Micrococcus*, *Saccharomyces*, *Listeria sp.* and *Yersinia sp.* Were all having 4% each being the lowest value.

Consequently, table 3 shows that eleven bacteria and one yeast, were isolated and were identified from the collected samples.

All the isolated fungal sp. were labeled as Table 2 showed, cultural characteristic and total fungi growth on SDA for each yogurt drink samples from sample A all through to sample J, which yielded no significant growth.

It was observed that six fungi were isolated and identified using cultural, morphological characteristics. *Mucor sp.* was found to have the highest occurrences with 22%, followed by

*Geotrichum sp.* Having 17%, *Montospora sp.* having 11% while *Aspergillus*, *Rhizopus* and *Fusarium* have 6% respectively as shown in table2.

Antibiotic sensitivity test were carried out on some selected bacterial isolates to ascertain their safety if consumed in the yogurt samples, this is shown in table4. Result of the antibiotic screening showed that all the selected organisms had multiple resistances to the antibiotic disc used for the testing.

The Agarose Gel- Electrophoresis test:

It was observed that the four Gram-negative organisms has more than one plasmid and these plasmids were heavier than the Deoxyribonucleic acid (DNA) marker 1kilobase pair (kbp) used for the plasmid profiling. The four Isolates showed different plasmid sizes ranging from 20,000-40,000kb as seen in plate 1.

**Table 1:** Total Bacterial Count (TBC), Total Fungal Count (TFC) and pH of the Yogurt samples.

S/N	SAMPLE CODE	TBC (cfu/ml)	TFC (cfu/ml)	pH
1	A2	$2 \times 10^3$	$1 \times 10^5$	4.05
2	B1	$1 \times 10^1$	$2 \times 10^2$	5.50
3	C1	$1 \times 10^2$	$2 \times 10^2$	4.18
4	D1	-	$1 \times 10^2$	5.41
5	E1	-	$1 \times 10^2$	4.81
6	F2	$2 \times 10^3$	$1 \times 10^2$	4.07
7	G1	-	-	4.12
8	H1	-	-	5.08
9	I1	$6 \times 10^3$	-	5.38
10	J1	-	-	4.84

**Table 2:** Gram's reaction and biochemical characteristics of isolates

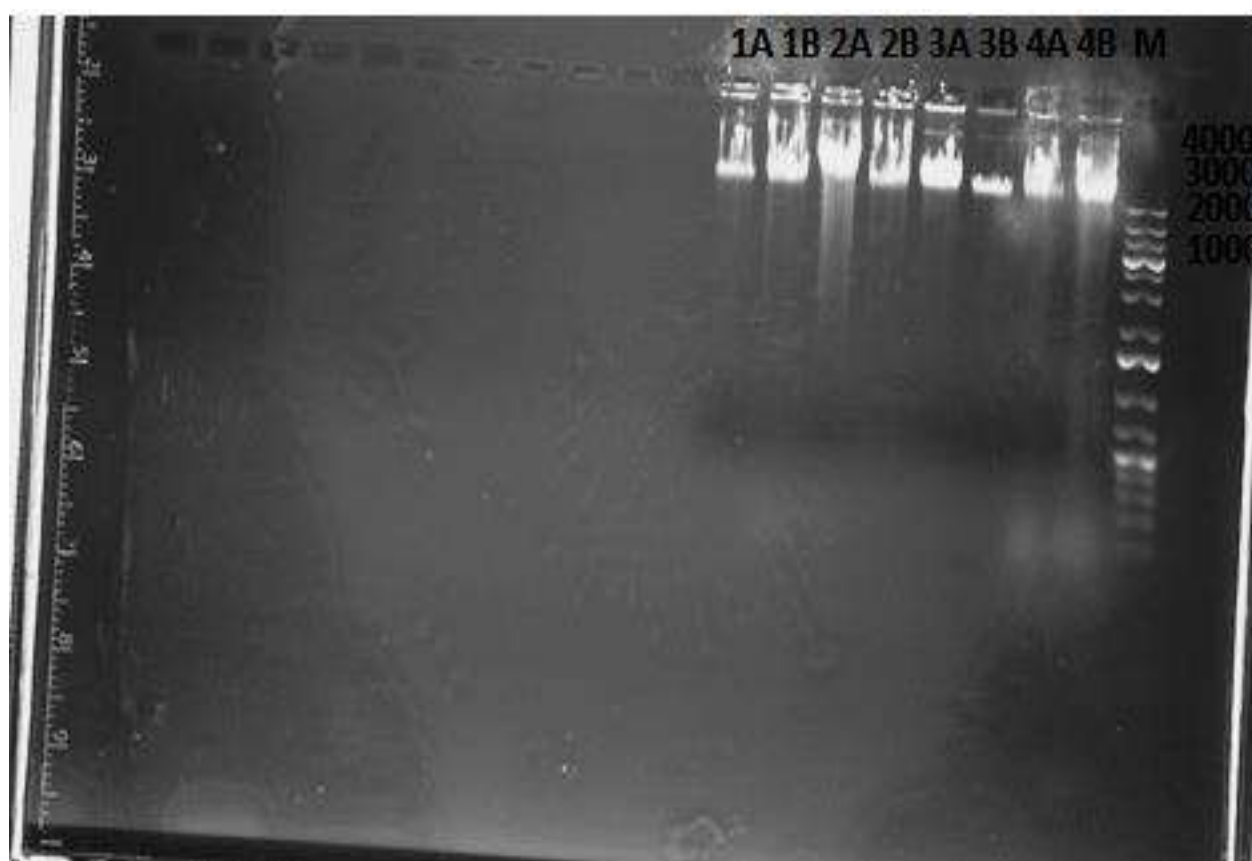
SN	SC	GR	ST	CA	CO	CT	UR	IN	OX	MO	MR	VP	LA	SU	MA	GL	FR	HS	PO
1	1 & 2	- Short rods	+	+	-	-	-	-	-	-	+	-	++	--	+-	+-	+-	-	<i>Pseudomonas</i> sp.
2	3,11,12 & 20	+ Bacilli	-	+	+	+	-	-	+	-	-	-	-	-	-	+-	+-	-	<i>Bacillus</i> sp.
3	4	+ Cocci (yellow)	+	-	+	+	-	-	-	-	+	-	-	-	+-	+-	+-	-	<i>Streptococcus</i> sp.
4	5	- Short rods	-	+	-	-	-	-	-	-	-	-	-	-	+-	-	+-	-	<i>Shigella</i> sp.
5	6,16,17,24 & 25	+ long rods	-	-	+	+	+	-	+	-	+	-	-	-	++	+-	+-	-	<i>Lactobacillus</i> sp.
6	7	+ Short rods	-	+	-	-	+	-	-	-	-	-	-	-	-	+-	+-	-	<i>Proteus</i> sp.
7	8	Cocci (Yellow)	+	+	+	+	+	-	+	-	-	-	-	-	+-	+-	+-	-	<i>Micrococcus</i> sp.
8	9,22 & 23	+ Short rods, (B)	+	+	-	+	+	-	-	-	-	-	-	-	-	-	+-	-	<i>Corynebacterium</i> sp.
9	10	Budding yeast	-	-	-	-	-	-	-	-	+	-	++	-	-	+-	+-	-	<i>Saccharomyces</i> sp.
10	13 & 15	+ Cocci (Cluster)	+	+	-	-	-	-	-	-	+	-	-	+-	+-	+-	+-	-	<i>Staphylococcus</i> sp.
11	14 & 21	- Short rods	-	-	-	+	-	-	-	-	+	-	+-	+-	+-	+-	+-	-	<i>Klebsiella</i> sp.
12	18	+ Short rods	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+-	-	<i>Listeria</i> sp.
13	19	- Long rods	-	+	+	-	-	-	-	-	-	-	-	-	-	+-	+-	-	<i>Yersinia</i> sp.

**Key :** ST-Starch, CA-Catalase, CO-Coagulase, CT-Citrate, UR-Urease, IN- Indole, OX-Oxidase, MO-Motility, HS- Hydrogen Sulphide, MR- Methyl red, VP-Voges-prokauer, LA-Lactose, MA-Matose, SU-Sucrose, GL-Glucose, FR-fructose, - Negative, +-Positive, Yellow-(yellow pigmentation), Spore-(Center), TS-(terminal spore) and B-(Brown pigmentation), Sp- Specie, GR- Gram's reaction, S/N- Serial number, SC-Sample code and PO- Probable organism.

**Table 3:** Antibiotic sensitivity test on some selected isolates

S/N	ORGANISMS	ZONE OF INHIBITION IN (MM)							
		CA	CR	GE	CX	OF	AU	NI	CP
		Z	X	N	M	L	G	T	R
1	<i>Pseudomonas sp</i>	R	R	15	R	16	R	R	16
2	<i>Shigella sp</i>	R	R	12	R	13	R	R	14
3	<i>Corynebacterium sp</i>	R	R	13	R	19	R	R	26
4	<i>Listeria sp</i>	R	R	14	R	18	R	R	25

**Key:** CA- Ceftazidime, CR- Cefuroxime, GE-Gentamicin, CX-Cefixime, OF-Ofloxacin, AU-Augmentin, NI-Nitrofurantion, CP-Ciprofloxacin, MM- Millimeter, S/N- Serial number and Sp-Specie.

**Plate 1.** Agarose gel-Electrophoresis, showing plasmid encoded on the organisms.



M = Marker 1 kilobase (Kbp), 1A and 1B = 18- *Listeria sp.*, 2A and 2B= 9- *Corynebacterium sp.*, 3A and 3B= 5- *Shigella sp.*, and 4A , 4B=1- *Pseudomonas sp.*, 1000= 10,000, 2000=20,000, 3000=30,000 and 4000=40,000kbp respectively.

#### 4.0 DISCUSSIONS AND RECOMMENDATION

Studies on the microbiological assessment and quality on the yogurt samples, showed unacceptable levels of both bacteria and fungi; *Pseudomonas*, *Shigella*, *Proteus sp.* as bacteria present while fungi isolated include *Mucor*, *Aspergillus sp.* Etc, Some sp of *Aspergillus* have been implicated in the secretion of aflatoxins, which are carcinogenic to human when consumed, this indicates that most of the yogurt drinks were not good for consumption, according to the WHO specification if we need to correctly address food drinks safety (WHO, 2000). Food handling practices by vendors is also a major concern in contaminating what they market, since they don't under go any formal training before embarking on yogurt drinks buying and selling.

There should be regulation and the necessary regulatory body to implement a monitoring body to ensure and enforce the law in other to increase the hygiene level of yogurt vendors, thereby reducing the health risk of end consumers. Government should provide more infrastructures such as, public toilets, drinking water and ways to dispose waste.

Governments in many countries acknowledge a code of hygiene practice as an important tool in this aspect, and is called the code needs to lay emphasis on hygiene from the start of production of the yogurt to the point of sales, could be considered the principle of Codex document (WHO, 1996). In processing and preparation adequate measure should be taken to reduce or eliminate pathogens to an acceptable level, in order to prevent growth of pathogens, production of toxic chemicals and the introduction of physical pathogens; and to ensure that foods and drinks are not re-contaminated (WHO, 1996).

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