MICROBIAL CONTAMINATION AND ANTIBIOTIC RESISTANCE IN SELECTED YOGHURT BRANDS IN ELELE, RIVERS STATE, NIGERIA

[1]Anagboso, O.Martin, [1]Ohaebuka, J. Chioma, [1]Mbah, I. Edith, [2]Osuala, J. Oluchi, [3]Daokoru-Olukole, G. Chidinma, [4]Onu, N. Euslar, [3]Agedah C. Ebisomu, [5]Nwankwo, A. Grace, [5]Okafor, O. Freda, [3]Silas-Olu, Daniel & [6]Okonko O. Iheanyi.

[1]Department of Microbiology, Madonna University Nigeria, Elele, Rivers State, Nigeria.
[2] Department of Pharmaceutical Microbiology & Biotechnology, Madonna University Nigeria, Elele, Rivers State, Nigeria.
[3] Department of Microbiology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.
[4]Department of Medical Microbiology, Faculty of Basic Sciences Clinical Medicine, Alex Ekwueme Federal University Ndufu-Alike, Ikwo Ebonyi State Nigeria.
[5]Department of Public Health, Madonna University Nigeria, Elele, Rivers State, Nigeria.
[6]Virus & Genomics Research Unit, Department of Microbiology, University of Port Harcourt, Port Harcourt, Nigeria.
: iheanyi.okonko@uniport.edu.ng; + (234) 7069697309

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Abstract:
Yoghurt, a widely consumed fermented dairy product, offers numerous nutritional benefits but is susceptible to microbial contamination, posing potential public health risks. This study evaluated the microbial contamination of ambient and frozen samples of twelve brands of yoghurt sold in Elele, Rivers State, Nigeria. The results indicated that the aerobic bacteria count of the various samples ranged from 1.2 x 10^3 CFU/ml to 3.5 x 10^6 CFU/ml. The results also revealed higher bacterial loads in yoghurt stored at room temperature, with *Bacillus* sp. (40%) being the most prevalent contaminant, followed by *Lactobacillus bulgaricus* (30%) and *Salmonella* sp. (35%), the latter of which is particularly concerning due to its potential to cause foodborne illnesses. Additionally, antibiotic susceptibility testing demonstrated that the isolated bacteria exhibited significant multi-drug resistance, especially *Salmonella* sp., which was resistant to eight antibiotics tested. These findings highlight the importance of proper storage conditions, strict hygiene practices, and enhanced safety measures during yoghurt production to minimise health risks associated with contaminated yoghurt products.

Keywords: **Antibiotics, Susceptibility, Testing, Microbial Contamination, Yoghurt, Nigeria**

1. INTRODUCTION

YOGHURT is one of the most famous fermented dairy products, and it is widely and generally consumed around the globe due to its energy content and health advantages [1]. Popular fermented dairy products like yoghurt are made by culturing full, low-fat, or skim milk or rehydrating powdered dry milk using live bacterial cultures, such as *Streptococcus thermophilus* and *Lactobacillus acidophilus* [2]. These cultures ferment the milk, resulting in a thick, custard-like product with a sour taste, often sweetened for consumption. Yoghurt can be made from various types of milk, including cow, goat, ewe, or buffalo milk, and is highly valued for its rich nutritional content, including proteins, vitamins, and minerals [3]. It also contains probiotics, which are beneficial for gut health and are tolerated better than milk by lactose-intolerant individuals.

Lactic acid bacteria *Lactobacillus delbruckii* spp. *Bulgaricus* and *Streptococcus thermophilus* contained in the starter culture helps ferment milk to produce yoghurt [1]. The role of these two bacterial genera in the production process can be put together as the acidification of the milk and the production of aromatic compounds [1].

The bacterial cultures used in yoghurt production play a vital role in fermentation and contribute to its flavour. For instance, *L. bulgaricus* produces amino acids that support the growth of *S. thermophilus*, which contributes to yoghurt's sourness, while *L. bulgaricus* provides its characteristic aroma. Yoghurt is also considered healthier than milk due to its higher protein, carbohydrate, and vitamin content and lower cholesterol levels [4].

However, despite its nutritional benefits, yoghurt is susceptible to microbial contamination. During production and storage, improper conditions can lead

Corresponding Author: Prof. Okonko O. Iheanyi
Email: iheanyi.okonko@uniport.edu.ng

to the growth of harmful microbes such as coliforms, *Escherichia coli* (*E. coli*), *Enterococci*, and *Staphylococcus aureus*. These contaminants are often introduced through inadequate hygiene during production, storage, or distribution [5]. The presence of these organisms in yoghurt can result in foodborne illnesses, such as diarrhoea, and pose serious public health risks [6].

The most common spoilage organisms in yoghurt are yeasts and moulds, which thrive in the low pH environment of yoghurt. Their activity can reduce the acidity of yoghurt, leading to increased susceptibility to bacterial growth and spoilage. In areas like Rivers State, Nigeria, vendors' poor storage and sanitary practices further exacerbate the risk of contamination, making the consumption of yoghurt a potential health hazard. The study aims to evaluate the microbial contamination of selected yoghurt brands in Elele, Rivers State, Nigeria, to assess the potential health risks and recommend preventive measures to protect consumers.

2. MATERIALS AND METHODS

2.1. Samples collection

A total of twelve different brands of bottle-packaged yoghurts were purchased from hawkers and beverage stores in Elele, Rivers state. Two samples of each yoghurt were used and designated as A-F, giving a total of 12 yoghurt samples collected from the following locations: Boys hostel Minimart (A), Girls Hostel Minimart (B), Adekunle’s Store (C), Susan’s Supermarket (D), Kemi’s Store (E), By the roadside (F). The samples collected were taken to the laboratory and analysed within 6 hours of collection. The yoghurt purchased and tested were ambient and frozen yoghurt samples.

2.2. Sterilization of Materials

All materials (media and glass wares) used in this research work were autoclaved for 15 minutes at 121ºC and 15psi for sterilization. The workbenches were disinfected with 70% alcohol. Flaming was also used when needed. The general practice of aseptic techniques was maintained throughout the bench work.

2.3. Media Preparation

The preparation of MacConkey agar and De Man–Rogosa–Sharpe agar (MRS) agar followed the protocol specified by the manufacturer, which was used to isolate bacteria in yoghurt products.

2.4. Analysis of Samples

Each sample was placed in six tubes with 10-1 to 10-6 labels as part of a ten-fold serial dilution. Nine millilitres of the distilled water were poured into each test tube using a sterilised pipette. The test tubes were corked and autoclaved for 15 minutes at 121 degrees Celsius to guarantee that the distilled water was completely sterile. After letting the water cool, one millilitre of the sample was transferred using a sterile pipette into the first test tube, designated 10^{-1}. After stirring the test tube to ensure homogeneity, one millilitre was pipetted out and transferred into the second test tube, labelled 10^{-2}. One millilitre was eliminated from test tubes 10^{-6} after completing the same process in all the other test tubes. One millilitre (ml) of the test was extracted in the middle of the dilution and transferred onto sterile Petri plates with the proper labels. To allow the suspension to spread, 18 to 20 ml of molten nutrition agar, MacConkey agar, and De Man-Rogosa-Sharpe (MRS) agar that had previously been sterilised were added to the Petri plates. After the plates were hardened, they were incubated for 24 hours at 37°C.

Following incubation, counts and records were made of the representative colonies on nutrient agar. After counting the aerobic bacteria twice, an average was calculated. The isolates from the MacConkey agar and MRSA on the plates were subcultured on new nutrient agar to acquire pure cultures. For further examination, the pure cultures were moved into the nutrient agar slants [7].

2.5. Identification of Isolates

A battery of biochemical tests further identified the separated organisms after acquiring pure colonies and recording important traits. The colony shape and pigmentation, citrate utilization, Gram staining, indole synthesis, coagulase test, sugar fermentation, and catalase test were used to identify the isolates. Every step was taken following Chesbrough's instructions [8]. By utilising Bergey's handbook of systematic bacteriology, the isolates were identified.

2.6. Testing for Antibiotic Susceptibility

The disc diffusion technique as reported by Bauer et al. [9] was used to examine the isolates' antibacterial susceptibility. The Muller-Hinton agar (Difco Laboratories GmbH, Augsburg, Germany) was made and transferred onto sterile petri plates. The agar medium was left to harden on a level bench at room temperature. Next, a few colonies of the recently cultured isolates were smeared onto the well-dried agar plate surfaces. After that, sterile forceps were used to gently and firmly position many antibiotic discs containing (Septrin, Ciprofloxacin, Erythromycin, Pefloxacin, Gentamycin, Ampiclox, Amoxicillin, Rocephin, Streptomycin, and Zinnacef) on the agar plates. The plates were then incubated for 18–24 hours at 35–37 degrees Celsius. The observed inhibition zones were then measured and recorded to the closest millimetre. Isolates were categorised as resistant, intermediate, or sensitive based on the Clinical and Laboratory Standard Institute classification and by WHO guidelines [8]. If an isolate exhibited resistance to three or more of the tested antibiotics, it was deemed to be multi-drug resistant.

3. RESULTS

Table 1 below shows the aerobic bacteria count of the various samples. The counts ranged from $1.2x10^3$CFU/ml to $3.5x10^6$ CFU/ml. The sample with the lowest aerobic bacteria count is the frozen fresh Yo (plain) with a count of $1.3x10^6$ CFU/ml. The samples with the heaviest isolates are the Frozen fresh Yo strawberry and ambient Farm fresh with a count of $3.5x10^6$CFU/ml.

Table 2 shows the isolates identified in the samples following the results and reactions from the biochemical tests carried out. Results showed that *Bacillus* sp was isolated from samples including room temperature Freshyo plain, frozen Freshyo plain, and frozen Freshyo strawberry. *Lactobacillus sp* was isolated from Room temperature Freshyo strawberry, Yugo sweetened, Hollandia plain, and frozen Hollandia strawberry. *Escherichia coli* was isolated from frozen Yugo sweetened and room-temperature Farm fresh yoghurt. *Salmonella* sp was isolated from frozen temperature farm fresh yoghurt.

TABLE 1
TOTAL AEROBIC BACTERIA COUNT GOTTEN FROM AMBIENT AND FROZEN TEMPERATURES OF DIFFERENT YOGHURT PURCHASED FROM ELELE, RIVERS STATE, NIGERIA

Brand of Yoghurt	Ambient Yoghurt		Frozen yogurt	
	Dilution factors/colonies		Dilution factors/colonies	
	10^3CFU/ml	10^6CFU/ml	10^3CFU/ml	10^6CFU/ml
FreshYo (plain)	5.4	1.6	2.3	1.3
FreshYo (Strawberry)	5.6	3.0	4.3	3.5
Yugo (Sweetened)	4.2	3.2	3.6	3.2
Hollandia (plain)	2.9	2.2	2.0	2.4
Hollandia (Strawberry)	1.4	1.8	1.2	2.1
Farm Fresh	3.1	3.5	2.5	NG

Key: NG: No growth, CFU: Colony forming units

Table 3 displays the frequency of presence of bacteria obtained from several brands of yoghurt. According to the data, 45.0% of the isolates were found from the yoghurt brands that were collected at freezing temperature, and 55.0% of the isolates were from other brands that were stored at room temperature. The isolates of bacteria are different; *Bacillus* sp. (40.0%, n = 16) is the most common, followed by *Salmonella* sp. (35.0%, n = 14), and the least isolated bacterium is Lactobacillus bulgaricus (25.0%, n = 10). The most common bacteria in room temperature (RMT) samples were *Bacillus* sp. (62.5%, n = 10), *Salmonella* sp. (57.1%, n = 8), and *Lactobacillus bulgaricus* (40.0%, n = 4)-the least isolated bacterium. However, in freezing temperature (FT) samples, *Lactobacillus bulgaricus* (60.0%, n = 10) was the most prominent, followed by *Salmonella* sp. (42.9%, n = 6), with *Bacillus* sp. (37.5%, n = 6) as the least isolated bacteria (Table 3).

From the result in Table 4, out of the ten antibiotics exposed to *Salmonella* sp., it was susceptible to only two of them (Ciprofloxacin and Gentamycin) and resistant to the remaining eight antibiotics. The *Lactobacillus bulgaricus* was susceptible to three (Ciprofloxacin, Gentamycin and Ceporex) out of the ten antibiotics tested, had an intermediate effect on four antibiotics, and was resistant to three antibiotics. *Bacillus* sp. was susceptible to three antibiotics (Gentamycin, Norfloxacin and Chloramphenicol) out of the ten antibiotics it was exposed to; intermediate to three antibiotics and resistant to four antibiotics it was exposed to.

4. DISCUSSION

Milk and dairy products are one of the potential categories of resources for providing functional food products due to their content in various essential components [10]. Every mammal species has a unique milk composition regarding major and minor constituents such as proteins, polyunsaturated fatty acids (FAs), vitamins, and minerals [10-12].

This study revealed that there was a high bacterial load on the different brands of yoghurt products that were stored at various temperatures. As such, the yoghurt products that were kept at room temperature had the highest bacterial counts of 5.6×10^3 CFU/ml, while the yoghurt kept at freezer temperature recorded the lowest bacterial load across the samples with a value of 1.2×10^3 CFU/ml. This investigation concurs with Awah et al.'s findings [13], who reported the same bacterial counts on a microbial assessment of yoghurts sold in Amawbia, Nigeria. The increase in bacterial load in yoghurt at both storage temperatures is an indication they encourage bacterial proliferation due to the presence of nutrients available within the yoghurt medium, which as such causes yoghurt spoilage, which in turn results in food poisoning when we consume it. Also, from this study, it was established that the bacterial load from the yoghurt kept under the freezing state or condition had a lesser bacterial population compared to that of the room temperature, indicating that it is appropriate to store the yoghurt product under such conditions because it aids in slowing down their metabolic activities or processes, thereby inhibiting their reaction, thus preserving the shelf life of the yoghurt products.

More importantly, when the condition is conducive to bacterial organisms, they tend to develop rapidly and proliferate, hence endangering the health and safety of the consumers. Furthermore, in this present study, the yoghurt products that had the highest bacterial load were found to be Fresh YO (strawberry) and Farm fresh, which indicates the suitability of nutrients that encourage the growth and proliferation of bacteria. Also, this may be due to poor management of production materials, inactive monitoring processes, and inability to strictly adhere to manufacturing standard procedures and HACCP protocols.

When yoghurt or its products are kept at room temperature, bacterial organisms invade because they are regarded as ubiquitous. On the other hand, yoghurt products have a normal bacterial organism commonly referred to as yoghurt starter. Still, these starters are usually used in the production processes in a minimal number. Still, when they pass the required number, it becomes an issue for the yoghurt products as they intoxicate humans when consumed. When yoghurts are refrigerated or frozen, it helps to check the growth of bacterial organisms by reducing the population and preventing the product from being good.

In this study, 40 bacterial isolates comprising three (3) different classes of bacterial genera were isolated. This study showed that bacteria known as *Salmonella* sp., *Bacillus* sp., and *Lactobacillus bulgaricus* were isolated and identified from different brands of yoghurt sold in Elele, Rivers State, Nigeria. This result agrees with the previous work done by Dike-Ndudim et al. [2].

Some analysed samples included *Bacillus sp*., a rod-shaped, motile, Gram-positive bacterium. Dust, dirt, and raw food items contain heat-resistant spores that are produced by it [1]. With 16 (40.0%), Bacillus was the most common bacterial contamination, consistent with earlier research by Agu et al. [14]. This can be the result of spores dispersing easily across the surroundings. Furthermore, the spores of this organism are resistant to harsh climatic conditions, which may account for their spread and contamination of dairy products [14]. Food poisoning agents, such as *Bacillus* sp, may be present in high concentrations due to improper handling, processing, and packaging [15]. Their presence in samples of yoghurt suggests contamination after pasteurisation [1].

A lactic acid bacterium (LAB) utilised in making yoghurt is *Lactobacillus delbrueckii* subsp. *bulgaricus* [10]. Since *Lactobacillus bulgaricus* is the starter culture that gives milk its flavour and fragrance and ferments it, its presence is ideal for creating yoghurt [13]. The assertions about *Lactobacillus sp*.'s functions as an essential species in the fermentation of milk to produce yoghurt are supported by the presence of these bacteria in the yoghurt samples [1]. Yoghurt is largely made by *Lactobacillus bulgaricus*, and the finished product has a sour flavour and resembles custard-like food that is often sweetened [1]. *S. thermophilus* is induced to create formic acid after *L. bulgaricus* has finished producing amino acids. The survival and development of *L. bulgaricus* depend on this [1].

In this present study, *Salmonella* sp. is the second most occurring bacteria genera with a value of 14(35.0%). *Salmonella* in the yoghurt suggests that such products, when consumed, have the efficacy of causing disease conditions in humans such as typhoid fever and salmonellosis. Thus, their occurrence in yoghurt may also be due to inadequate heat processing and post-pasteurization contamination from the environment and handlers.

In the cross-contamination of yoghurt products, the factory staff, distributors, retailers, vendors, and storekeepers contribute to the contamination process. When they are not properly stored with good storage facilities or equipment, bacterial organisms can be encouraged to strive. Once again, a large number of locally owned, home-based food and beverage manufacturers fill bottles, polythene bags, and packs haphazardly without adhering to any cleanliness regulations during the yoghurt drink's preparation and packing, which therefore raises the number of bacteria [16].

From the results obtained, it is evident that the yogurt samples are contaminated with varying bacterial organisms, which are regarded as pathogenic and are of public health concern. Proper hygiene should be carried out for these bacterial pathogens to be eradicated. When the yoghurt products are refrigerated at 5ºC, it will help to keep and maintain the quality of these yoghurts. By extension, acid production by lactic acid bacteria in yoghurt will be prevented [17]. The antibiotic susceptibility result in this recent study revealed that *Salmonella* sp., and *Lactobacillus bulgaricus*, were resistant to nalidixic acid, spectrin, and ampicillin. Meanwhile, they were susceptible to ciprofloxacin and gentamycin, respectively.

The antibiotic susceptibility result showed that all the isolates were multidrug resistant with MAR > 0.2. *Salmonella* sp. showed high-level resistance as it was resistant to eight of the ten antibiotics it tested on. In a real sense, antibiotics are supposed to combat bacteria, not the bacteria refusing to be subject to the action of antibiotics. Still, when such cases occur it brings to mind that there is a problem which the people must be enlightened and properly guided to avoid reoccurring diseases emanating from such bacteria genera.

TABLE 2
BIOCHEMICAL CHARACTERIZATION OF BACTERIAL ISOLATES OBTAINED FROM DIFFERENT BRANDS OF YOGHURT PURCHASED AT ELELE, RIVERS STATE, NIGERIA

Isolate codes	Colony Characteristics	Cell shape	Gram stain	Catalase	Indole	Coagulase	Citrate	Sugar fermentation			Probable organisms
								G	L	S	
SRT1	Creamy, circular, smooth slightly raised colonies	Rod	+	+	_	_	_	A	A	A	*Bacillus* sp.
SFT1	Creamy, circular, smooth slightly raised colonies	Rod	+	+	-	-	-	A	A	A	*Bacillus* sp.
SFT2	Creamy, circular, smooth slightly raised colonies	Rod	+	+	_	_	_	A	A	A	*Bacillus* sp.
SRT2	White round slightly Raised colonies	Rod	+	-	-	-	-	A	A	A	*Lactobacillus bulgaricus*
SRT3	White round slightly Raised colonies	Rod	+	-	-	-	-	A	A	A	*Lactobacillus bulgaricus*
SFT3	Milkish	Rod	-	+	+	-	-	A	A	A	*Escherichia coli*
SRT4	White round slightly Raised colonies	Rod	+	-	-	-	-	A	A	A	*Lactobacillus bulgaricus*
SFT5	White round slightly Raised colonies	Rod	+	-	-	-	-	A	A	A	*Lactobacillus bulgaricus*
SRT6	Milkish	Rod	-	+	+	-	-	A	A	A	*Escherichia coli*
SFT6	Pink	Rod	-	+	-	-	+	A	A	A	*Salmonella* sp.

Keys: SRT = Sample Room temperature, SFT = Sample freeze temperature, G = Glucose, L = Lactose, S = Surcose, A = Acid

TABLE 3
FREQUENCY OF OCCURRENCE OF BACTERIAL ISOLATES IN DIFFERENT BRANDS OF YOGHURT

Bacterial Isolates	Frequency (%)	Room Temp. (%)							Freeze Tempt (%)						
		RMT (%)	FYP	FYS	YS	HP	HS	FF	FT (%)	FYP	FYS	YS	HP	HS	FF
***Bacillus* sp.**	16(40.0)	10 (62.5)	2	1	2	1	1	3	6(37.5)	1	2	1	0	0	2
***Salmonella* sp.**	14(35.0)	8(57.1)	1	2	1	1	0	3	6(42.9)	2	1	1	0	1	1
Lactobacillus bulgaricus	10(25.0)	4(40.0)	1	0	1	2	0	0	6(60.0)	1	2	1	1	1	0
Total	40(100.0)	22(55.0)							18(45.0)						

Key: RMT= Room Temperature, FYP =Fresh YO (Plain), FYS= Fresh YO(Strawberry), YS= Yugo (Stweetened), HP= Hollandia (Plain), HS=Hollandia (Strawberry), FF= Farm fresh, FT= Freeze Temperature.

TABLE 4
ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES OBTAINED FROM DIFFERENT BRANDS OF YOGHURT PURCHASED FROM ELELE, RIVERS STATE, NIGERIA

Bacteria isolates	ANTIBIOTICS (mm)																
	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN	NOR	AML	ERY	RIF	CHL	APX	LEV
***Salmonella* sp.**	12 (R)	10 (R)	21 (S)	8 (R)	23 (S)	9 (R)	2 (R)	0 (R)	5 (R)	1 (R)	ND	ND	ND	ND	ND	ND	ND
Lactobacillus bulgaricus	15 (I)	20 (I)	22 (S)	14 (R)	23 (S)	17 (I)	21 (S)	19 (I)	6 (R)	5 (R)	ND	ND	ND	ND	ND	ND	ND
***Bacillus* sp.**	ND	ND	18 (I)	ND	21 (S)	19 (I)	ND	ND	ND	ND	20 (S)	15 (R)	19 (I)	7 (R)	21 (S)	2.0 (R)	4.0 (R)

Keys: R=Resistance 1-10, I= Intermediate 10-14mm, S= Sensitive 15 above (EUCAT, 2011). OFX= Tarivid, PEF = Reflacine, CPX = Ciprofloxacin, AU = Augmentin, CN = Gentamycin S = Streptomycin, CEP = Ceporex, NA = Nalidixic Acid, SXT = Septrin PN = Ampillicin. NOR = Norfloxacin, AML = Amoxil, ERY = Erythromycin, RIF = Rifampicin, CHL = Chloramphenicol, APX = Ampiclox, LEV = Levofloxacin. Clinical and Laboratory Standard Institute (CLSI) Guidelines 2021: 31st Edition: Sensitive (S)= $\geq$ 20.0, Intermediate (I)=15-19, Resistant (R)= $\leq$ 14.

5. CONCLUSION

This study highlights significant microbial contamination in various yoghurt brands available in Elele, Rivers State, Nigeria, with notable differences between ambient and frozen storage conditions. Yoghurt samples stored at room temperature exhibited higher bacterial loads than those kept frozen, suggesting that refrigeration effectively slows bacterial growth. Noteworthy contaminants included *Bacillus sp.* and *Lactobacillus bulgaricus,* with *Salmonella* sp. raising public health concerns due to its potential to cause serious illness. Additionally, antibiotic susceptibility testing revealed alarming levels of multi-drug resistance, particularly in *Salmonella sp*., emphasizing the need for improved hygienic practices during yoghurt production and storage. Enhanced food safety measures and stringent adherence to proper storage conditions are critical to ensuring consumer health and reducing the risk of foodborne diseases associated with yoghurt consumption.

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