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Isolation and identification of *Escherichia coli (E. coli*) in home-made and commercially produced yoghurt sold in port Harcourt

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Abstract
This study investigated the presence of *Escherichia coli* (*E. coli*) in 20 brands of yoghurt sold in Port Harcourt, Nigeria. Using standard plate count and most probable number (MPN) techniques, the total viable bacterial counts and total coliform counts of the yoghurt samples were analyzed. Results showed that the total viable bacterial counts ranged from 1.1×10^2 to 8.0×10^5 cfu/ml, with no significant difference ($p\leq 0.05$) in microbial counts among the brands. The MPN index ranged from 2.2×10^1 to 1.6×10^3. *E. coli* was isolated from most yoghurt samples, with Mpraise yoghurt having the highest frequency of occurrence (20.4%). Our findings suggest poor bacteriological quality during production and handling, which may have public health implications. We recommend improving the production process and sanitary conditions to prevent contamination and ensure the safety of yoghurt consumers.

Keywords: Bacteria, Home-made/commercial production, *E. coli*, Port Harcourt, Yoghurt

1. Introduction
Yoghurt, a popular fermented dairy product in Nigeria and Africa (Osundahunsi *et al*., 2007), has numerous health benefits and nutritional value (Shukla and Leifson, 2002; Salisul *et al*., 2016) [19, 17]. It is enriched with probiotics, protein, vitamins, and minerals (Wakil and Onilude, 2011), and has the potential to prevent high blood pressure and aid digestion. The dairy industry contributes significantly to Nigeria's economy, creating employment opportunities and providing a source of revenue (Khan *et al*., 2008) [11]. Yoghurt production can be done on a small scale or large scale, using local raw materials such as milk and fruits (Sieuwerts *et al*., 2008) [20]. However, yoghurt production can be contaminated with harmful microorganisms like *E. coli*, which can cause food poisoning and environmental contamination (Michayova *et al*., 2007) [14]. Previous studies have revealed undesirable bacteria associated with dairy products, including Gram-negative psychrotrophs, coliforms, lactic acid bacteria, yeasts, and moulds (World Health Organization, 2018) [29]. According to Ugboma *et al*. (2023) [27], bacterial contamination of yoghurt cannot be overemphasized due to its physicochemical and sensory properties that encourage the growth of pathogenic bacteria. Mbaeyi-Nwaoha *et al*. (2012) [13] reported that the presence of coliforms in yoghurt brands is a serious public concern due to its health implications on consumers. This study aims to isolate and identify the presence of viable *E. coli* in homemade and commercially produced yoghurts sold in Port Harcourt, Nigeria, and evaluate the antimicrobial susceptibility pattern of the isolated *E. coli*. The findings will create awareness among yoghurt producers and handlers about the need to maintain adequate sanitary levels to prevent diseases of public health concern.

2. Materials and Methods
A. Collection of Yoghurt Sample
A total of 20 packaged yoghurt brands were randomly purchased from various sources in Port Harcourt, Rivers State, Nigeria, including supermarkets, stores, and hawkers. The yoghurts were properly labeled, designated by brand, and packaged in sterile cellophane nylons to prevent contamination. The samples were then stored in a freezer for analysis (Alderton, 2000) [1]. To minimize contamination, aseptic techniques were employed throughout the sampling and handling process, using sterile materials (Cheesbrough, 2006) [4].

B. Materials and Apparatus

The laboratory materials apparently used in this study like: all glass wares and metal materials including pipettes, conical flasks, test tubes, beakers, petri dishes, bent L-shape glass rods, forceps, spatulas, glass slide, wire loops, measuring cylinders were adequately washed with detergent, rinsed severally under running water, re-rinsed using sterile distilled water, separately wrapped in foil papers, autoclaved using autoclave at 121°C at 15 psi for 15 minutes and sterilized in hot air oven at 160°C for 1-2 hours (Cheesbrough, 2006) [4]. Other apparatus including: autoclave, incubator, microscope, weighing balance, refrigerator, hot air oven, staining rack, test tube rack, cotton wool, aluminum foil, Bunsen burner, masking tape, wash bottles and bowls were thoroughly cleaned using clean water, detergents and laboratory napkins (Cheesbrough, 2006) [4].

C. Media and Reagents

Media and reagents including: MacConkey agar (MCA), Eosin methylene blue (EMB), Mueller-Hinton agar (MHA), nutrient agar (NA), nutrient broth, MacConkey broth, normal saline (diluents), Gram staining reagents, ethanol, oil immersion, sugars for biochemical test were made available for the microbial analyses (Holt, 1997) [10].

D. Preparation of Culture Media

All media used in this study including MacConkey agar (MCA), MacConkey broth (MB),
Eosin methylene blue (EMB) agar, Mueller- Hinton agar (MHA), Nutrient agar (NA),
Nutrient broth (NB) were prepared while maintaining high sanitary level according to their respective manufacturer's directions and specifications using standard microbiological procedures where, the appropriate amounts of the different media were weighed separately using electronic weighing balance suspended in different conical flasks containing1000ml of distilled water, sealed with cotton wool, wrapped with aluminum foil and sterilized by autoclaving at 121°C for 15 minutes (Cheesbrough, 2006) [4]. The medium was allowed to cool up to temperature of 45 °C, thereafter, aseptically dispensed on to a sterile Petri dishes accordingly, allowed to solidify to gel for about 10-15 minutes and were dried in hot air oven at 160 °C for 1 hour (Holt, 1997; Cheesbrough, 2006) [10, 4].

E. Microbiological Analysis of Yoghurt Samples

1. Preparation of Normal Saline (Diluent)

The entire working surfaces were also disinfected using clean cotton wool and 70% ethyl alcohol to minimize contaminants. Thereafter, preparation of normal saline was carried out by dissolving 8.5g of sodium chloride (NaCl) in 1000ml of distilled water. Mixture was vigorously shaken for proper homogenization (Alderton, 2000) [1]. After which, sterile pipette, 9ml was dispensed into different test tubes and autoclaved at 121°C at 15 psi for 15 minutes (Toder, 2004; Cheesbrough, 2006) [21, 4].

3. Serial dilution of the homogenate

Dilution of the purchased yogurt samples adopted the ten-fold serial dilution technique. One milliliter (1ml) of each yogurt sample was introduced into 9ml of sterile pipette. This was shaken for even distribution of the sample. The first test tube (10^{-1}) dilution was further diluted from test tube to test tube up till 10^{-5} dilution factor (Cheesbrough, 2006; Mbaeyi-Nwaoha, and Egbuche, 2012) [4, 13].

4. Isolation and enumeration of *E. coli* from purchased yoghurt

The spread plate technique was used to isolate *E. coli* from yoghurt samples. Aliquots (0.1ml) of each dilution were placed in sterile petri dishes containing MacConkey agar (MCA) and Eosin methylene blue (EMB) agar, in duplicates. The plates were incubated at 37°C for 24-48 hours, and the presence of discrete colonies was screened. The number of bacteria was estimated as colony-forming units per milliliter (CFU/ml). Suspected *E. coli* colonies, which appeared pinkish on MacConkey agar plates and greenish with a metallic sheen on Eosin methylene blue agar plates, were isolated from the yoghurt samples (Cheesbrough, 2006; Arora and Arora, 2012; Makwin *et al.*, 2014) [4, 2, 12]. The results were recorded for each dilution.

5. Sub-culturing and purification of bacterial isolate

Discrete colonies were isolated and re-inoculated into freshly prepared appropriate medium in order to obtain pure cultures of bacteria isolates. Sterile inoculating loops were separately used to make streaks in several patterns of each of the colonies in the medium and incubation was done at 37 °C for 24 hours (Cheesbrough, 2006) [4].

6. Identification and characterization of bacterial isolate (*E. coli*)

The bacterial isolates were identified using Bergey's manual of determinative bacteriology, based on their Gram stain reaction, microscopy examination, and biochemical tests. The microscopy examination included characteristics such as size, color, edge, elevation, shape, and texture, as well as cellular and colonial morphology. The biochemical tests performed included oxidase, catalase, indole, citrate, motility, urease, methyl red, and Voges Proskauer tests (Holt *et al.*, 1997) [10]. After identification, the isolates were preserved and maintained as stock cultures on agar slants in McCartney bottles and stored in the refrigerator at 4°C for future use (Cheesbrough, 2006) [4].

7. Sugar fermentation test

This test aimed to determine the fermentation range of various sugars (glucose, lactose, maltose, and mannitol) by isolated microorganisms. Bacteria typically ferment sugars to produce acid and products, often accompanied by gas evolution (CO2 and H2). To conduct the test:

- Peptone water was prepared according to the manufacturer's instructions.
- 1 g of sugar and 0.2% indicator (phenyl red) were added to 1000 ml of peptone water.
- Durham tubes were inserted into the tubes and sterilized at 121 °C for 15 minutes.
- The tubes were inoculated with test organisms and incubated at 37 °C for 24 hours.
- Acid production was detected by a color change from red to yellow in the medium.
- Gas production was observed by the displacement of air in the inverted Durham's tube.

This test was performed according to Cheesbrough (2006) [4].

F. Enumeration and Isolation of Coliform Bacteria

The most probable number (MPN) technique was used to enumerate coliform bacteria. The procedure involved:

- Using double strength (DS) and single strength (SS) MacConkey broth for 10 ml, 0.1 ml, and 1 ml yoghurt samples.
- **A three-step test**

1. **Presumptive test:** Detecting gas production in test tubes.
2. **Confirmatory test:** Streaking positive tubes onto MacConkey agar plates using a sterile inoculating loop.
3. **Completed test:** Incubating plates at 37 °C for 24 hours.

This process followed established protocols (Cheesbrough, 2006; Heaton and Jones, 2008; Darma *et al*., 2016) [4, 6].

1. Presumptive test

In the presumptive test, serial dilutions of the yoghurt samples were prepared using peptone water

- 1ml of yoghurt sample + 9 ml peptone water (1:10 dilution, labeled 10-1).
- 1ml from 10-1 dilution + 9 ml peptone water (1:100 dilution, labeled 1-2).
- 1ml from 1-2 dilution + 9 ml peptone water (1:1000 dilution, labeled 10-3).

From each dilution, 1ml was transferred into MacConkey broth (9ml) with inverted Durham's tubes. The tubes were incubated at 37°C for 24 hours and observed for gas production, which was recorded accordingly (Cheesbrough, 2006; Oyeleke and Manga, 2008) [4, 16].

2. Confirmation Test

A loopful of inoculum from the positive tube (gas produced) was inoculated onto a sterile Eosin methylene blue agar plate and incubated at 37°C for 24 hours to observe colony characteristics (Cheesbrough, 2006; Oyeleke and Manga, 2008) [4, 16].

3. Completed Test

Colonies from the 24-hour culture were inoculated into MacConkey broth with inverted Durham's tubes and incubated at 37°C for 24 hours. Gas and acid production indicated the presence of coliform bacteria (*E. coli*), confirming the completed test (Cheesbrough, 2006; Oyeleke and Manga, 2008) [4, 16].

G. Antimicrobial Activity Test

Antimicrobial susceptibility testing was performed using the Kirby Bauer method (disc diffusion method) according to National Committee for Clinical Laboratory Standards guidelines. The identified bacterial isolates were tested against various antibiotics (concentrations in brackets): OFX (10 µg), PEF (10 µg), AU (30 µg), CPX (10 µg), CN (10 µg), S (30 µg), CEP (10 µg), NA (30 µg), SXT (30 µg), and PN (30 µg). The test organisms were grown on sterile nutrient broth, and the bacterial suspensions were streaked onto Mueller-Hinton agar plates. Antibiotic discs were placed on the plates, incubated at 37 °C for 18-24 hours, and the zones of inhibition were measured in millimeters (Hajna and Perry, 1999; Vogt and Dippold, 2005; Oyeleke and Manga, 2008) [8, 22, 16]. The results were interpreted according to the Becton Dickinson microbiology company's guidelines (Vogt and Dippold, 2005; Oyeleke and Manga, 2008) [22, 16].

Results

A. The Results of Microbiological Assessment of Yoghurt Samples

At the end of the present study, the results showed that total viable bacterial count (TVBC) from the samples ranged from 1.1×10^2 –8.0×10^5 cfu/ml and total coliform bacterial count (TCBC) had 1.0×10^2 to 3.1×10^4 cfu/ml, as shown in Table 4.1. The highest TVBC was found in kylin yoghurt (8.0×10^5), followed by azee yoghurt (7.5×10^5 cfu/ml) while, the lowest TVBC was found in Hollandia yoghurt (1.1×10^2 cfu/ml) and F.A.M VITA (1.1×10^2 cfu/ml). The highest TCBC was found in Mpraise yoghurt (3.0×10^4 cfu/ml) while, the lowest TCBC was found in Nutri yo and FAM vita yoghurts. However, there was no TCBC recorded in Jossy, Fresh yo, Farm fresh, Fage and Soy yoghurt samples.

Table 1: Microbiological assessment of yoghurt samples purchased from some selected districts in Port Harcourt metropolis

Yoghurt name	TVBC (CFU/ml)	TCBC (CFU/ml)
Peak	5.0×10^4	2.1×10^4
Kamy	1.2×10^3	2.0×10^4
Hollandia	1.1×10^2	1.5×10^2
Jossy	1.4×10^2	-
Yogo	2.0×10^4	2.1×10^4
Fresh yo	2.0×10^3	-
Nutri yo	4.5×10^4	1.0×10^2
Top rank	4.2×10^4	2.6×10^3
Mpraise	2.4×10^4	3.1×10^4
Victory	2.2×10^4	1.5×10^3
Kylin	8.0×10^5	2.6×10^4
F.A.M VITA	1.1×10^2	1.0×10^2
Habib	1.8×10^3	2.0×10^3
Nayalli	3.7×10^4	3.0×10^4
Azee	7.5×10^5	2.8×10^4
Greek	7.0×10^5	1.4×10^4
Farm fresh	6.0×10^3	-
Fage	2.0×10^3	-
Soy	4.5×10^5	-
Fam	5.0×10^5	2.3×10^4

B. The Results of Bacteria Isolates from Yoghurt Samples

The results of the bacteria isolated from the various yoghurt samples from the present study are presented in table 2. Similar bacteria species including *Staphylococcus aureus*, *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Corynebacterium* sp. was isolated from most of the yoghurt samples. However, *E. coli* was observed in all the yoghurt samples except in in Jossy, Fresh yo, Farm fresh, Fage and Soy yoghurt samples (Table 2).

Table 2: Bacteria isolated from yoghurt samples

Yoghurt brand name	Types of Bacteria isolated
Peak	*E. coli, Staphylococcus aureus*
Kamy	*Bacillus sp, Staphylococcus* sp, *E. coli*
Hollandia	*Bacillus cereus, E. coli,*
Jossy	*Pseudomonas* sp, *Staphylococcus* sp
Yogo	*E. coli, Bacillus* sp
Fresh yo	*Pseudomonas aeruginosa, Bacillus* sp
Nutri yo	*Corynebacterium sp, E. coli, Staphylococcus, Bacillus* sp
Top rank	*Staphylococcus* sp, *E. coli*
Mpraise	*Bacillus* sp, *E. coli, Staphylococcus* sp
Victory	*E. coli, Pseudomonas* sp, *Corynebacterium sp*
Kylin	*E. coli, Staphylococcus* sp, *Bacillus* sp, *Pseudomonas* sp
F.A.M VITA	*Bacillus* sp, *Staphylococcus* sp, *E. coli,* Pseudomonas sp
Habib	*Staphylococcus* sp, *E. coli, Bacillus* sp, *Pseudomonas* sp
Nayalli	*E. coli, Pseudomonas* sp, *Bacillus* sp
Azee	*E. coli, Bacillus* sp, *Staphylococcus* sp, *Pseudomonas aeruginosa*
Greek	*Staphylococcus* sp, *Pseudomonas* sp *E. coli*
Farm fresh	*Bacillus* sp
Fage	*Staphylococcus*
Soy	*Pseudomonas* sp, *Staphylococcus sp*
Fam yogo	*E.coli, Bacillus* sp, *Staphylococcus aureus*

C. The Results of Enumeration of Coliform Bacteria (*E. coli*.)

Results showing *E. coli* count from the various yoghurt samples are presented in Table 3. The highest count of *E. coli* was found in Mpraise yoghurt (30), followed by Kylin yoghurt (25) while, the lowest count was found in Hollandia and Nutri yoghurt (2). Nevertheless, *E. coli* was not detected in some yoghurt samples (Table 3). Also, the frequency of occurrence of *E. coli* from the various yoghurt samples confirms the number of times *E. coli* isolates occurred in each yoghurt sample as represented in the graph in Figure 1. Below. From the graph, it was observed that, Mpraise yoghurt had the highest occurrence of *E. coli* whereas, Kylin yoghurt and Hollandia yoghurt had the lowest occurrence of *E. coli*. Furthermore, Table 4. Shows most probable number (MPN) index of the fifteen (15) bacterial isolates that had the presence of *E. coli* in the yoghurt samples. All the samples showed positive results by evolution of acid and gas production (Table 4).

Table 3: Number of *E. coli* isolates from respective yoghurt samples

Yoghurt samples	Number of Isolates
Peak	6
Kamy	5
Hollandia	2
Jossy	0
Yogo	7
Fresh yo	0
Nutri yo	2
Top rank	9
Mpraise	30
Victory	9
Kylin	25
F.A.M VITA	3
Habib	8
Nayalli	12
Azee	4
Greek	10
Farm fresh	0
Fage	0
Soy	0
Fam yogo	15
Total number of isolates	147

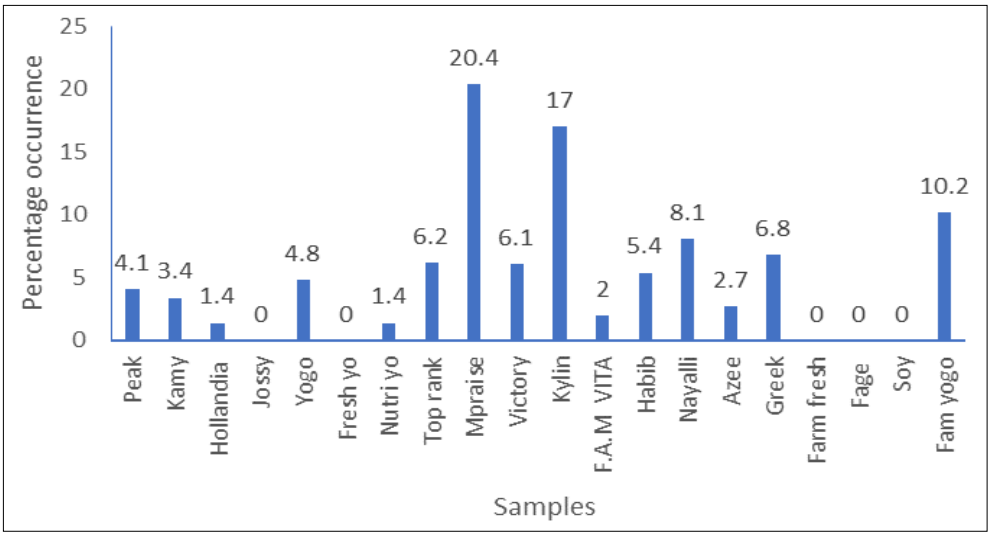

Fig 1: Percentage/frequency occurrence of *E. coli* isolated from yoghurt samples

Table 4: Most probable number (MPN) index

Samples	Combination of positives	MPN index/100ml
1.	4.4.0	3.4×10^1
2.	4.2.1	2.2×10^1
3.	4.3.0	2.7×10^1
4.	4.3.1	3.3×10^1
5.	4.4.0	3.4×10^1
6.	5.3.2	1.4×10^2
7.	5.5.4	1.6×10^3
8.	5.5.2	5.0×10^2
9.	5.5.2	5.0×10^2
10.	5.5.3	9.0×10^2
11.	5.1.2	6.0×10^1
12.	5.0.0	2.3×10^1
13.	5.4.2	2.2×10^2
14.	5.5.5	1.6×10^3
15.	5.3.1	1.1×10^2

D. The Results of Antimicrobial Activity Test

Table 5. shows the antimicrobial susceptibility profile of *Escherichia coli* isolated from yogurt samples for all the fifteen (15) isolates positive to *E. coli* which were subjected to antibiotic susceptibility test against ten (10) different antibiotics. *E. coli* isolated from yoghurt samples were resistant to Ampicillin, but sensitive to Tarivid, Reflacine, Ciprofloxacin, Augmenitin, Gentamycin, Streptomycin, Ceporex and Nalidixic acid and intermediate to Ciprofloxacin and Septrin.

Table 5: Antimicrobial susceptibility profile of *Escherichia coli* isolated from yogurt samples sold in Port Harcourt metropolis (n= 15)

Antibiotics	Susceptible [n (%)]	Intermediate [n (%)]	Resistant [n (%)]
Tarivid	15 (100)	0	0
Reflacine	15 (100)	0	0
Ciprofloxacin	4 (26.7)	11 (73.3)	0
Augmentin	15 (100)	0	0
Gentamycin	15 (100)	0	0
Streptomycin	15 (100)	0	0
Ceporex	15 (100)	0	0
Nalidixic acid	15 (100)	0	0
Septrin	0	15 (100)	0
Ampicillin	0	0	15 (100)

Source: Clinical and Laboratory Standard Institute (CLSI) 2012

Discussion

The research found that the total viable bacterial count in yoghurt samples ranged from 1.1 × 10^2 to 8.0 × 10^5 CFU/ml, with Kylin yoghurt having the highest count and Hollandia yoghurt having the lowest. This suggests contamination during preparation, production, packaging, or handling, contradicting previous studies that reported lower bacterial counts and proper sanitary standards in yoghurt production. The elevated bacterial count in some samples exceeds the acceptable safety limits set by the International Commission on Microbiological Specifications for Foods (ICMSF), indicating a potential health risk for consumers.

The studies found that various bacterial species, including Staphylococcus aureus, Bacillus sp., Pseudomonas aeroginosa, and Corynebacterium sp., were present in most yoghurt samples. However, *E. coli* was detected in all samples except for five brands (Jossy, Fresh yo, Farm fresh, Fage, and Soy yoghurt). Similar bacteria species were also found in street-vended yoghurt samples in Port Harcourt, Nigeria (Obire *et al*., 2014) [23]. The presence of these pathogens in yoghurt can have negative health implications, and Staphylococcus aureus is particularly known to cause food poisoning through food intoxication.

Ugboma *et al*. (2023) [27] found similar bacteria species in homemade and commercial yoghurt in Port Harcourt, Nigeria, indicating yoghurt as a reservoir of bacterial diversity. This diversity may be due to poor sanitary and handling practices during production and distribution, potentially stemming from improperly cleaned human body parts (hands, skin, hair, and nose) and production materials (clothing). While small amounts of S. aureus are expected in human-handled products, large amounts suggest faulty sanitation or production practices, potentially leading to enterotoxin presence (Darma *et al*., 2014) [5].

The analysis revealed that Nayalli yoghurt had the highest coliform bacterial count, while Nutri yo and F.A.M VITA yoghurt had the lowest. Fifteen out of twenty yoghurt brands (A, B, C, D, E, F, H, I, J, K, L, M, N, O, P, and T) were contaminated with *E. coli*, indicating faecal contamination and poor hygiene after processing. The presence of *E. coli* in these yoghurt brands is a concern, as it should be eliminated during pasteurization. The remaining 5 brands were free from *E. coli*, indicating they are safe for human consumption. Overall, the findings suggest that some yoghurt brands may have compromised quality and sanitation standards, potentially posing a risk to consumers.

The presence of *E. coli* in yoghurt brands is a serious health concern, as it can have negative implications for consumers. According to NAFDAC standards, *E. coli* should not be detectable in any 100ml yoghurt sample (Mbaeyi-Nwaoha *et al*., 2012) [13]. The frequency of *E. coli* occurrence in yoghurt samples was highest in Mpraise yoghurt (20.4%), followed by Kylin yoghurt (17.0%), and lowest in Hollandia yoghurt (1.4%). The high frequency in Mpraise yoghurt may be due to processor neglect or poor hygiene practices. This highlights the need for improved quality control and sanitation measures in yoghurt production to ensure consumer safety.

Darma *et al*. (2016) [6] found that 4 out of 10 yoghurt brands in Kano Metropolis were unsanitary due to high coliform counts, including *E. coli* and S. aureus. Two brands with low coliform counts still exceeded NAFDAC limits, indicating heavy contamination. This poses a serious health risk to consumers, as these bacteria can cause severe infections like food poisoning and septicemia (Arora *et al*., 2012) [2]. However, Salisul *et al*. (2016) [17] found no *E. coli* in 18 yoghurt samples from Sokoto State, with bacterial loads within safe limits for human consumption. The conflicting results suggest varying levels of sanitation and quality control in yoghurt production across different regions. The study used the Kirby-Bauer disk diffusion test to investigate the antibiotic susceptibility of *E. coli* isolated from yoghurt samples. The results showed that the isolated *E. coli* exhibited resistance to various antimicrobial agents, indicating a potential public health concern. The study highlights the importance of proper hygiene and sanitation in yoghurt production to prevent contamination with harmful pathogens like *E. coli*. The findings also underscore the need for effective antibiotic stewardship to combat the growing threat of antibiotic resistance. The Kirby-Bauer method provides a rapid and effective means of determining antibiotic susceptibility, helping to guide treatment decisions and prevent treatment failures. Overall, the study emphasizes the need for vigilance in ensuring the microbiological quality of yoghurt and other food products to protect consumer health.

Conclusion and Recommendation

The study's findings suggest that 15 out of 20 yoghurt brands in Port Harcourt are contaminated with *E. coli*, posing a significant risk to public health. To address this, the study recommends:

1. Adoption of good agricultural practices and proper monitoring and quality control.
2. Education of yoghurt producers and staff on clean and hygienic practices.
3. Improved product quality and durability through refrigeration at 5°C after production.
4. Regular factory inspections by regulators like NAFDAC to ensure good manufacturing practices and apply sanctions when necessary.
5. Implementation of HACCP programs for transportation, packaging, and storage to ensure safety and quality.

These recommendations aim to prevent foodborne infections and intoxications, ensure environmental and food safety, and protect public health.

Conflict of Interest

Not available

Financial Support

Not available

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