*IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)*
*e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 16, Issue 5 Ser. I (Sep. –Oct. 2021), PP 34-39*
*www.Iosrjournals.Org*

# Antibiogram Of Some Pathogenic Bacteria Species Isolatedfrom Branded Yoghurt Drink Sold In Rumuolumeni, Rivers State, Nigeria.

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***Abstract***
*Thisstudyinvestigated the antibiogramprofile of some pathogenic bacteria species isolated from branded yoghurt drink sold in Rumuolumeni, Rivers State, Nigeria. Samples of two brands of yoghurt, Hollandia and Freshyo were bought from different supermarkets in Rumuolumeni and analyzed for the presence of pathogenic bacteria using standard microbiological methods. Pour plate technique was employed for the culture and isolation of bacteria from these yoghurt. The isolates were identified using colonial morphology, gram staining and biochemical tests.The antibiotic susceptibility of each isolate was determined by the modified Kirby Bauer diffusion method. The bacteria count for Holandia yoghurt ranged betwen $2.5x10^{-3}$ to $4.0x10^{-3}$ cfu/ml, while that of Freshyo was from $2.5\ x10^{-3}$ to $3.5\ x10^{-3}$cfu/ml.The pathogenic bacteria species isolated includeStaphylococcus aureus and Escherichia coli with frequency occurrence of 50% and 12.5% respectively. The percentage antibiotic resistance of Staphylococcus aureuswas very high for Augmentin (87.5%),Sparfloxacin (87.5%), Chloramphenicol (75.4%), Amoxacillin (75.4%) andSeptrin (75%). TheE. coli also showed high resistance to Erythromycin (90.2%), septrin (80.2%) andSparfloxacin (75.3%).The indiscriminate and frequent use of antibiotics may have been responsible for the development of high antibiotic resistance by these pathogenic bacteria. The public health implication is that consumers of these yoghurt products are at risk of being infected with antibiotic resistant strains of pathogenic S. aureus and E. coli.*

Date of Submission: 18-09-2021 Date of Acceptance: 03-10-2021

## I. Introduction

According to Lindmark*et al.,* (2013) and Anjum*et al*., (2019), close to fifty percenttonnage of milk produced is either eaten fresh or pasteurized, while one sixth is consumed as yoghurt or curd and the remaining utilized in the production of other milk based products like ice cream and butter.Javaid*et al.,* (2019) noted that yoghurtor milk based products contain vital nutritional ingredients such as fat, proteins, carbohydrates, minerals, vitamins which play significant roles in human nutrition. Saeed*et al*., (2019)stated that the availability of these organic nutrientsin yoghurt hasmade it a good culture medium for the growth of different species of pathogenic microorganisms. The proliferation of these microorganisms reduces the stability, quality and shelf-life of yoghurt. To prevent growth of microorganisms,check thespread of diseases andextend the storage quality of yoghurt for human consumption, milk is usually pasteurized (Edema&Akinge, 2017).Unfortunately, Stabel*et al.*, (2014)have reported that certain human pathogenic bacteria species, such as *Mycobacterium paratuberculosis, Bacillus cereus, Clostridium* spp, *Listeria monocytogenesand Salmonella spp*can survive the conventional heat applied during milk pasteurization.Several workers have isolated and identified numerous species of bacteria including *Staphylococcus aureus, Escherichia coli, Salmonella* spp, *Pseudomonas* spp, *Enterobacter*spp, *Klebsiella*spp, *Proteus* sppand *Yersinia* sppfrom unprocessed milk (De Buyser*et al.,* 2013 Ayebo*et al.,* 2016).

Many workers in Nigeria, have reported that milk products are contaminated with several bacterial pathogens such as *Staphylococcus* spp, *E. coli, Klebsiella*spp, *Enterobacter*sppand *Salmonella* spp(Yabaya*et al.,* 2012; Adeleke*et al*., 2013; Uzeh*et al.,* 2016; Umoh*et al.,* 2017; Okpalugo*et al.,*2018;).Hence, milk can act as a medium for the transmission of bacterial diseases such as, *Salmonellosis,E. coli infections, Cholera, Brucellosis, Streptococcal* infections and *Listeriosis*. A good number of these organisms have also been confirmed to be pathogenic and toxicogenic, and implicated in gasteroenteritis due to milk (Maguire *et al.,* 2012: Bergdoll, 2019). S*taphylococcus aureus is* by far the most frequent pathogen implicated with outbreaks of milk-borne infections (85.5% of the outbreaks), followed by *Salmonella* (10.1%) (De Buyser*et al.,* 2014). *Staphylococcus aureus*causes food borne intoxication through the production of enterotoxins, which can be transferred into yoghurt through the cow udder or skin of humans.

Oliver *et al.,* (2015) revealed that the bacterial contamination of yoghurt and other milk products can emanate from within or outside the cow udder, and surface of the equipment used for handling and storage of the unprocessed milk.The prevailing health status of the cow, procedures used for milking, quality of water and personal hygiene conditions of the workers can influence the level of microbial contamination of raw milk The holding temperature and length of time of storage before testing and processing of the yoghurt can increase proliferation of bacterial contaminants

The presence of enterotoxigenic and antimicrobial resistant strains of bacteria pathogens haveincreasinglybecome a common occurrence in milk (Normanno*et al.,* 2017). Contamination of milk with antibiotic resistant bacteria constitutes a major threat to public health, because the antibiotic resistant genes can be transferred to other pathogenic bacteria,which compromises the treatment of severe bacterial infections.The lack of effective controls on antibiotic usage in human health and in animal production systems enhances the risk of exposure to antibiotic resistant milk borne pathogens.

Notwithstanding the dangers identified with antibiotic resistant bacteria species isolated from milk based products, many individuals still relish and consume yoghurt. This study was designed to determine the prevalence of anti-biotic resistant bacteria species isolated from yoghurt sold inRumuolumeni Town, Rivers State.

## II. Materials And Methods

### 2.1 Study Area

The study was carried out atIwofe in Rumuolumeni, Obio/Akpor Local Government Area, Rivers State.

### 2.2 Sample Collection

Two samples of different brands of yoghurt (Hollandia and Freshyo) were bought from Iwofemarket in Rumuolumeni, Port Harcourt and placed in a sterile polythene bag and taken to the Biology Laboratory of Ignatius Ajuru University of Education for microbiological analysis.

### 2.3 Microbiological Analysis

Each of the Hollandria and Freshyo packs was surface sterilised with 95% ethanol and mixed properly by swirling gently. 10ml of each sample was aseptically transferred into 90ml of sterile peptone water and homogenized. A tenfold serial dilutions of the samples were carried out in duplicatesupto $10^4$.O.lmlaliquot of the diluted sample was used to inoculate freshly prepared culture media by the pour plate method. Nutrient agar for viable count, Desoxycholate agar (DCA) for *Salmonella* and *Shigellasp,*Mannitol salt agar (MSA) for *S. aueus,*MaConkey agar for coliform, Eosin methylene blue agar for *E. coli,*and Sabouraud dextrose agar (SDA) for fungiwere used for isolation and enumeration. Following inoculation and incubation, the resulting growth colonies were counted.

### 2.4 Purification, characterization and identification of isolates

After enumeration of colonies on each medium, sub-cultureof the isolates was carried out on nutrient agar platesand incubated for 24- hours at 37°C for bacteria growth to obtain pure cultures, which were stocked in nutrient agar slants and stored in the refrigerator at $4^{0C}$ for biochemicalanalysis. The biochemical tests carried out for the identification of the isolates include; citrate utilization, indole production, methyl red, vogues-p test, oxidase, catalase, coagulase, sugar fermentation; triple sugar test (TSI), motility, and starch utilization.Characterization and identification of bacteria isolates wasdone according to the outline ofBergey's Manual of Determinative Bacteriology(Bergey& Holt, 1994).

### 2.5 Antibiotic Sensitivity Test

The antibiogramprofile of theisolated test organismswas monitored using standard microbiological protocol asadopted by Obi and Ike, (2015). The standard antibiotics discs used includes: septrin (30 ug), erythromycin ( E 10ug), gentamycin (GM, 30 ug, E. Merck, 6100, Darmstadt, Germany, amoxycillin (Amo 30 ug, Smiihkline Beecham Pharm GMSH SOGQ, Munchen 40, Germany), ciproftoxacin (30ug),

Augmentin(30ug), Chloramphenicol (30), Sparfloxacin(30), Pefloxacin(30ug). The test was carried out with overnight culture of each isolate, which was standardised to $10^6$cfu/ml and used to inoculate melted Mueller Hinton agar (MHA) at 45 $^C$C and aseptically poured into sterilized petri dish plates in duplicates. The media were allowed to cool and solidify. The standard antibiotic dics were then gently placed at reasonable equidistance, on all the inoculated media and left to stand for1 hr. All the culture media were incubated at 37 °C for 18 hr. Measurement of the diameter of zone of inhibition produced by each antibiotic discwas donewith meter rule. The result each measurement was interpreted as or Resistant (R), Susceptible (S),Intermediate(I) to the respective antibiotic agentused, depending on the length of zone of inhibition produced as compared to standard length of zone of inhibition stated by NCCLS, (2006).

## III. Results

The results of microbiological evaluation of Hollandia and Freshyoyoghurt are presented in the tables and figures below:

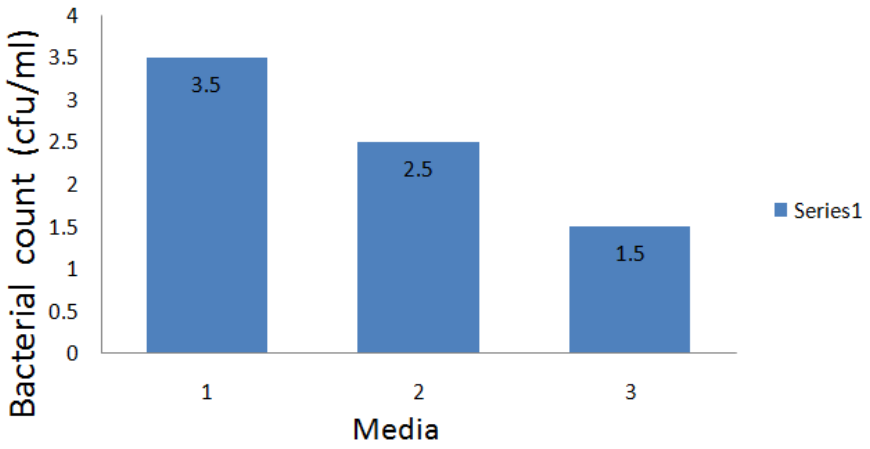


Fig. 1: Bacterial count of Hollandia on Nutrient Agar, Eosin Methylene Blue Agar, Manitol Salt Agar from Iwofe

The figure above shows the bacterial count of Hollandia on the different culture media used such as nutrient Agar (3.5 cfu/ml), Eosin Methylene Blue Agar (2.5 cfu/ml) and Manitol Salt Agar (1.5cfu/ml) from Iwofe. Bacterial growth occurred more on the nutrient agar medium than in the other two media.

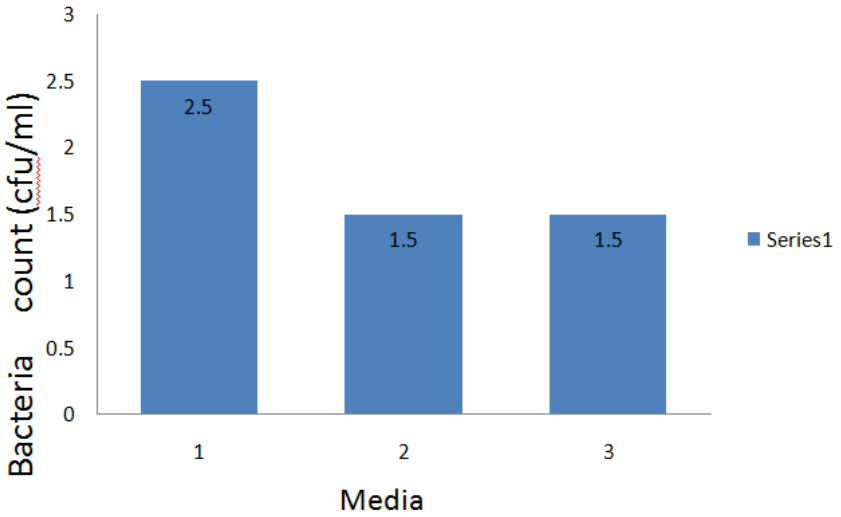


*Fig. 2:* Bacterial count of Freshyo on Nutrient Agar, Eosin Methylene Blue Agar, Manitol Salt Agar from Nkpor

This figure shows the bacterial count of freshyo on nutrient Agar, Eosin Methylene Blue Agar and Manitol Salt Agar from Nkpor. The total colony count was highest in the nutrient agar medium(2.5cfu/ml) followed by mannitol salt agar and Eosin Methylene Blue agar respectively.

Table1: Bacteria counts of Hollandia and Freshyo on Nutrient Agar

| **Location** | **Iwofe** | **Nkpor** |
|---|---|---|
| Samples (Duplicates) | Hollandia | Freshyo |
| 1 | $4.0 \times 10^{-3}$ | $3.0 \times 10^{-3}$ |
| 2 | $3.0 \times 10^{-3}$ | $2.0 \times 10^{-3}$ |
| Mean ( $\bar{X}$ ) | $3.5 \times 10^{-3}$ | $2.5 \times 10^{-3}$ |

**Table2:** Bacteria counts of Hollandia and Freshyo on MacConkeyAgar

| **Location** | **Iwofe** | **Nkpor** |
|---|---|---|
| Samples | Hollandia | Freshyo |
| 1 | $2.0 \times 10^{-3}$ | $1.0 \times 10^{-3}$ |
| 2 | $3.0 \times 10^{-3}$ | $2.0 \times 10^{-3}$ |
| Mean ( $\bar{X}$ ) | $2.5 \times 10^{-3}$ | $1.5 \times 10^{-3}$ |

**Table3:** Bacteria counts of Hollandia and Freshyo on Manitol Salt Agar (MSA)

| **Location** | **Iwofe** | **Nkpor** |
|---|---|---|
| Sample | Hollandia | Freshyo |
| Plate 1 | $1.0 \times 10^{-3}$ | $1.0 \times 10^{-3}$ |
| Plate 2 | $2.0 \times 10^{-3}$ | $1.0 \times 10^{-3}$ |
| Mean ( $\bar{X}$ ) | $1.5 \times 10^{-3}$ | $1.0 \times 10^{-3}$ |

**Table 1:** Identification of isolates using cultural characteristics and biochemical tests.

| Characters | *Escherichia coli* | *Staphylococcus auerus* |
|---|---|---|
| Colony character | pink, small or large raised convex colonies. | distinctly white color and yellow in manitol salt agar |
| Morphological character | rod | round |
| Gram staining | -ve | +ve |
| **Biochemical Test** | | |
| Motility | motile | non motile |
| Catalase | +ve | -ve |
| Oxidase | -ve | -ve |
| Citrate | -ve | +ve |
| Coagulase | -ve | +ve |
| Indole | +ve | -ve |
| Urease | -ve | +ve |
| Methyl red | +ve | -ve |
| Vogues proskauer | -ve | +ve |

| | | |
|---|---|---|
| Sugar fermentation | +ve | +ve |

**Table 2:** Percentage Resistance of Isolates

| **Antibiotics** | **Concentration (µg)** | ***Escherichia coli*** | **Staphylococcus aureus** |
|---|---|---|---|
| Septrin | 30 | 78.2 | 74.0 |
| Chloramphenicol | 30 | 9.8 | 73.4 |
| Sparfloxacin | 10 | 75.1 | 85.0 |
| Ciprofloxacin | 30 | 5.1 | 25.0 |
| Amoxicillin | 30 | 15.2 | 75.2 |
| Augmentin | 30 | 10.1 | 87.3 |
| Gentamycin | 30 | 10.0 | 12.4 |
| Pefloxacin | 30 | 20.1 | 25.2 |
| Erythromycin | 10 | 90.1 | 12.4 |
| Streptomycin | 30 | 10.3 | 37.4 |

Table 3: Antibiotic sensitivity profile of *Escherichia coli* isolates (%)

| Sensitive | Am | Cip | Sp | Au | S |
|---|---|---|---|---|---|
| **R** | | | | | |
| **I** | 15.2(I) | 5.1(I) | 10.1(I) | | |
| **S** | | 75.2(S) | | 80.1(S) | |

**Table 4:** Antibiotic sensitivity profile of *Staphylococcus aureus* isolate (%)

| Sensitive | Am | Cip | Sp | Au | S |
|---|---|---|---|---|---|
| **R** | | 25.1(R) | | | |
| **I** | | | | | |
| **S** | 75.2(S) | 85.3(S) | 87.4(S) | 75.1(S) | |

**N/B: R - Resistance**
**I - Intermediate**
**S - Sensitive.**

## IV. Discussion

The results from the microbiological analysis showed that the two different brands of yoghurt used in the study were contaminated with bacteria. The bacterial load of the two different yoghurt drinks sold in Rumuolumeni was relatively high, with the highest count of (7.0 X 10-$^{7}$cfu/ml) for yoghurt bought from Iwofe, while the lowest count of (5.0 X 10-$^{7}$cfu/ml) was recorded at Nkpor village. The Hollandiayoghurt had the highest bacterial count of 4.0 X 10-$^{3}$cfu/ml at Nkpor village, while the lowest count of 3.0 X 10-$^{4}$ cfu/ml was obtained atIwofe.The highest bacterial count for Freshyo(3.0 X 10-$^{3}$cfu/ml), was recorded in Iwofe with the lowest count of2.0 X 10-$^{4}$cfu/ml recorded at Nkpor village. Yoghurts are nutrient rich foods that can support the growth of all kinds of microorganisms. Similar observation was made by (Murinda*et al*., 2014; Oliver *et al.,* 2015) where they noted that raw or processed milkisa well known medium for the growth ofbacteria. Yoghurt is an important nutritional component for human diet and plays a prominent role in human nutrition. Some of the substances present in yoghurt provide both energy and other materialsneeded for growth and maintenance of health body.

Two genera of bacteria (*Escherichia coli* and *Staphylococcus aureus)* were isolated and identified from the two different yoghurt drinks. Debuyser*et al*., (2013) and Ayebo*et al*., (2016) have in different studies of microbiological evaluation of yoghurt drinks isolated and identified similar species of bacteria.

*Escherichia coli*is the most predominant bacteria isolated with frequency occurrence of 50%, followed by *Staphylococcusaureus* which hada frequency of 20%. The presence of *Escherichia coli* an indicator offeacal pollution in the two yoghurts sold in Rumuolumenipotends a serious risk to the health of the public, especially the consumers of these product. *Staphylococcus aureus* is a normal flora of the human skin and may colonize the nasopharyngeal region. The presence of this species of bacteria in two of the yoghurt may have come from the human handlers. However, the absence of *Staphylococcus aureus* on hollandia yoghurt may suggest that this species probably did not survive on the hollandia due to its fatty nature. Several stains of *Salmonella* and *Staphylococcus* species are known to be pathogenic to humans and their presence in the two brand of yoghurt, though at relatively small percentages, further revealed the health risk associated with the consumption of

yoghurts sold in Rumuolumeni. Maguire *et al*., (2012) andDebuyser*et al*., (2014) made similar observation that some of these organisms have been found to be pathogenic and toxigenic in milk-borne gastroenteritis.

*E. coli* was the most predominant bacterial isolate with percentage frequencies of 80% and 60% for hollandia and freshyo respectively. This is followed by *Staphylococcusaureus* which had percentage frequencies of 20%, 30% for hollandia and freshyo respectively.

The result of the theantibiogramshowed that *E.coli* was highly resistant toErythromycin (90.2%), (80.2%), Septrin and Sarfloxacin (75.3%).Similarly, *Staphylococcusaureus* was highly resistant to Sarfloxacin (87.5%), and Augmentin (87.5%).Chloramphenicol (75.4%),Amoxacillin (75.4%), Septrin (75%).The sensitivity pattern of the bacterial species isolated is comparable to the report of some workers(Inyang 2009, Udo*et al.,* 2001).

The prevalence of the resistant strain of *Escherichia coli*, *Staphylococcus aureus* in the two yoghurt products is a reflection of the use and misuse of antibiotics in the society. This is not surprising because, there is indiscriminate use of antibiotics by the Nigerian of which Rumuolumeni is a part.The Public health implication of this investigation is that antimicrobial resistant strain of pathogenic bacteria may colonize the human population through consumption of contaminated yoghurts sold in Rumuolumeni, and according to (Normanno*et al*., 2017) this can lead to failures of chemotherapy among the individual consumers of these yoghurt drinks.

## V. **Conclusion**

The confirmation of the presence of resistant strains of *Escherichiacoli* and *Staphylococcusaureus* in Hollandia and Freshyo sold in Rumuolumeni, suggest that consumption of any of these products has potential health risk to the consumers. The consumers of these products may experience chemotherapeutic failures of commonly used clinical antibiotics due to resistance. The handlers or the producers of these yoghurts should be careful during the milking process to avoid dirt andeven air as source of microbes in milk and proper sterilization should be carried out.

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