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# Microbial Analysis and Antibiogram of Reusable Plastic Bottles Used for Packaging Food Products in the Ikot Ekpene Metropolis.

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*Abstract: The reuse of plastic bottles for packaging food products has become increasingly common in many developing countries due to economic and environmental considerations. However, inadequate cleaning and poor handling practices may promote microbial contamination and pose significant public health risks. This study investigated the microbial contamination and antibiotic susceptibility patterns of microorganisms isolated from reusable plastic bottles used for packaging food products within Ikot Ekpene metropolis, Akwa Ibom State, Nigeria. Thirty (30) reusable plastic bottles were randomly collected and subjected to microbiological analysis using standard culture, biochemical, and microscopic techniques. Antibiotic susceptibility testing of bacterial isolates was performed using the Kirby–Bauer disc diffusion method. The results showed that 25 (83.3%) of the samples exhibited bacterial contamination, with total bacterial counts ranging from 3.0 × $10^5$ to 5.9 × $10^5$ CFU/mL, while 22 (73.3%) samples showed fungal contamination with counts ranging from 0.1 × $10^5$ to 2.0 × $10^5$ CFU/mL. Six bacterial species were identified, namely Escherichia coli, Staphylococcus aureus, Bacillus spp., Klebsiella spp., Pseudomonas aeruginosa, and Streptococcus spp. Four fungal genera were also isolated, including Aspergillus spp., Penicillium spp., Cladosporium spp., and Candida spp. Antibiotic susceptibility testing revealed that Escherichia coli and Streptococcus spp. were susceptible to all antibiotics tested, whereas Klebsiella spp., Pseudomonas aeruginosa, and Staphylococcus aureus exhibited resistance to one or more antibiotics. Ciprofloxacin, Augmentin, and Streptomycin demonstrated the broadest spectrum of activity against the bacterial isolates.The study revealed that reusable plastic bottles used for packaging food products in Ikot Ekpene metropolis harbour diverse bacterial and fungal contaminants, including potential pathogens and antibiotic-resistant organisms. The presence of these microorganisms underscores the need for improved sanitation practices, proper bottle disinfection, use of potable water during washing, and stricter regulatory monitoring to ensure food safety and protect public health.*

**Keywords:** *Reusable plastic bottles; microbial contamination; antibiogram; food safety; bacterial isolates.*

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## 1.0 Introduction

Global demand for bottled water has risen steadily in recent years, positioning it as the most rapidly expanding category within the non-alcoholic beverage industry (Doria, 2026). Nevertheless, the extensive use of single-use plastic bottles has been linked to greater environmental pollution and increased landfill accumulation. A reusable bottle refers to any bottle designed for multiple use cycles, whether refilled by the original manufacturer or by consumers.

Reusable plastic bottles are made from thicker, sturdier, and more rigid types of plastics (such as Tritan or HDPE). They do not degrade quickly and can withstand regular washing and handling without breaking or cracking.

Several industries produce water in plastic bottles of various volumes, which are discarded with other domestic waste, recycled for reuse, or thrown into drains, blocking water circulation and causing flooding (Tabeyang, 2018; Abrokwah et al., 2020). Beyond environmental concerns, the reuse of discarded plastic bottles for food and beverage packaging has emerged as a significant public health issue because these containers may harbour pathogenic microorganisms capable of causing foodborne diseases.

The growing consumer preference for reusable bottles is largely driven by concerns for environmental sustainability and health safety. As a form of reusable packaging, these bottles contribute to more sustainable practices. Within the waste hierarchy, reuse ranks above recycling and disposal. Multiple use cycles lower the amount of material needed per filling. In recent years, consumer demand for reusable drinking bottles for items such as water, coffee, soup, baby formula, and salad dressing has increased, largely in response to the economic and environmental drawbacks of single-use plastic bottles.

Approximately half of the population in developing countries relies on street-vended traditional foods (Rana & Ahirrao, 2016). Key factors driving this preference include affordability, flavor, and nutritional content. Additionally, these foods are often available near schools and workplaces, which is convenient given the extended travel times between home, school, or work (FAO, 2016).

Due to economic challenges, traditional foods like juices, fermented milk, and yoghurt are often packaged in recovered plastic bottles collected from garbage and drains (Ingram and Mala, 2010; Djoulde et al., 2013; Tabeyang, 2018; Abrokwah et al., 2020). These products are consumed by low- and middle-income individuals, including children and the elderly, without further treatment. The collection, handling, washing, filling, storage, and distribution processes associated with reused plastic bottles provide multiple opportunities for microbial contamination and cross-contamination of food products. Factors like ingredient quality, hygiene practices, packaging, and storage conditions contribute to poor microbiological quality (Gonzalez-Ravas et al., 2018; Abrokwah et al., 2020). Inadequate water, sanitation, and hygiene (WASH) practices contribute to a minimum of 9.1% of the global burden of disease (Prüss-Üstün et al., 2016). Additionally, an estimated 1.8 billion people globally rely on drinking water sources that are contaminated with faecal matter (Prüss-Üstün et al., 2008).

The reuse of recovered plastic bottles poses a serious public health risk because such containers are frequently exposed to environmental contaminants and microbial pathogens present in garbage dumps, drains, and other unsanitary environments. Biofilms can form inside bottles, making them hard to clean (Gonzalez-Rivas et al., 2018). Street traders selling these products are common in developing countries (Tambekar et al., 2009; Nawawee et al., 2019), driven by affordability and convenience.

Locally processed beverages like Zobo and kunu are often packaged in handpicked plastic bottles from trash cans and dumpsites, with questionable sterility (Kigigha et al., 2018).

In many urban and semi-urban communities in southern Nigeria, including Akwa Ibom State, the practice is common due to economic constraints, inadequate recycling infrastructure, and limited enforcement of food packaging regulations. Microbial species associated with contamination include bacteria like Bacillus, Staphylococcus, and E. coli, and fungi like Aspergillus and Penicillium (Izah et al., 2016; Kigigha et al., 2018). Inadequate recycling facilities and poor disinfection methods can lead to serious health hazards and foodborne diseases. Several studies have reported varying levels of microbial contamination in reused plastic containers and packaged beverages. However, differences in environmental conditions, sanitation practices, and handling procedures across geographical locations make it difficult to generalize findings from one region to another. Furthermore, the emergence of antimicrobial-resistant microorganisms in food-contact materials has become an increasing public health concern because such organisms may contribute to the transmission of resistant infections within communities. Although previous studies have documented microbial contamination of reused plastic bottles and locally packaged beverages in some parts of Nigeria and other developing countries, there is a paucity of information on the microbial quality and antibiotic resistance profiles of microorganisms associated with reusable plastic bottles used for packaging food products in Ikot Ekpene metropolis, Akwa Ibom State. Consequently, the public health risks associated with their continued use remain poorly understood.

Reusable bottles are therefore, food-contact surfaces requiring proper cleaning and sanitizing. Unfortunately, consumers may not be aware of the potential hazards related to reusable plastic bottles; thus, there is a possibility for complacency with regard to cleaning behaviors. It is recognized that water can be a source of disease outbreaks (Maunula et al., 2005; Wyn-Jones et al., 2001), However, limited information has been published on the cleanliness of reusable water bottles and consumer behaviors related to reusable water bottles. Therefore, this study aimed to determine the microbial contamination levels and antibiotic susceptibility patterns of croorganisms isolated from reusable plastic bottles used in packaging food products within Ikot Ekpene metropolis, Akwa Ibom State, Nigeria..

The findings of this study will provide baseline data on the microbiological safety of reusable plastic bottles used for food packaging in the study area. The results will be useful to public health authorities, food safety regulators, environmental agencies, food vendors, and consumers in developing strategies to improve hygiene practices, reduce foodborne illnesses, and limit the spread of antibiotic-resistant microorganisms.

## 2.0 Materials and Methods

### *2.1 Study Area*

This study was conducted in Ikot Ekpene Metropolis, located within Ikot Ekpene Local Government Area of Akwa Ibom State, Nigeria, in the South-South geopolitical zone. Ikot Ekpene lies between latitudes 5°09′ and 5°15′ N and longitudes 7°41′ and 7°45′ E. The area experiences a tropical humid climate characterized by two distinct seasons: a long rainy season extending from March to October and a short dry season from November to February. Annual rainfall averages approximately 2,200 mm, while temperatures range from 22°C to 35°C throughout the year. The high humidity and warm temperatures prevalent in the area provide favourable conditions for microbial growth and survival.

### *2.2 Sample Collection*

A total of thirty (30) reusable plastic bottles used for packaging food products were collected from different locations within Ikot Ekpene metropolis using a simple random sampling technique. Samples included bottles used for packaging locally processed beverages and other food products. During collection, sterile disposable gloves were worn to prevent external contamination. Each sample was aseptically placed in a sterile, sealable polyethylene bag, appropriately labeled, and ransported to the Microbiology Laboratory for immediate analysis.

### *2.3 Microbiological Analysis*

Microbiological examination of the reusable plastic bottles was carried out using standard microbiological procedures. The internal surfaces of the bottles were swabbed using sterile cotton swabs moistened with sterile nutrient broth. Approximately 15 cm² of the internal bottle surface was thoroughly swabbed. Each swab was transferred into 9 mL of sterile physiological saline and vigorously agitated for 1–3 minutes to dislodge attached microorganisms.

Serial ten-fold dilutions of the resulting suspension were prepared using sterile saline solution. Thereafter, 0.1 mL aliquots from appropriate dilutions were spread-plated onto different culture media. Nutrient Agar was used for total heterotrophic bacterial counts and incubated at 37°C for 24 hours using the $10^{-6}$ dilution. MacConkey Agar was used for the isolation of enteric bacteria and incubated at 37°C for 24–48 hours using the $10^{-3}$ dilution. Sabouraud Dextrose Agar supplemented with chloramphenicol was used for fungal isolation and incubated at room temperature (28 ± 2°C) for 5–7 days using the $10^{-3}$ dilution.

### *2.3.1 Enumeration of Microbial Counts*

Following incubation, visible colonies were counted using a digital colony counter. Plates containing 30–300 colonies were selected for enumeration. Microbial loads were expressed as colony-forming units per millilitre (CFU/mL) and calculated using standard microbiological procedures.

### *2.3.2 Purification of Isolates*

Distinct colonies exhibiting different morphological characteristics were selected from primary culture plates and purified by repeated streaking on freshly prepared Nutrient Agar plates. The inoculated plates were incubated at 37°C for 24 hours. Pure cultures obtained were maintained on Nutrient Agar slants in McCartney bottles and stored at 4°C for subsequent characterization and identification.

### *2.4 Characterization and Identification of Bacterial Isolates*

Bacterial isolates were identified based on their cultural, morphological, microscopic, and biochemical characteristics according to standard microbiological procedures described by Cheesbrough (2006). Colony morphology, including colour, shape, margin, elevation, and texture, was recorded. Gram staining was performed to determine cellular morphology and Gram reaction. Biochemical tests conducted included catalase, coagulase, oxidase, citrate utilization, urease activity, indole production, motility, methyl red, Voges–Proskauer, and carbohydrate fermentation tests. Identification of isolates was achieved by comparing the observed characteristics with established taxonomic keys.

### *2.5 Fungal Analysis*

### *2.5.1 Macroscopic Characterization of Fungal Isolates*

Fungal cultures were examined after incubation for 5–7 days. Macroscopic characteristics including colony colour, texture, shape, margin, surface appearance, growth pattern, and reverse pigmentation were observed and recorded.

### *2.5.2 Microscopic Characterization of Fungal Isolates*

Microscopic examination of fungal isolates was carried out using the lactophenol cotton blue staining technique. A small portion of each fungal colony was mounted on a clean grease-free glass slide containing a drop of lactophenol cotton blue stain and covered with a cover slip. The preparation was examined under a light microscope using ×10 and ×40 objectives for the observation of hyphal structures, spores, conidia, and other diagnostic features necessary for identification.

### *2.6 Antibiotic Susceptibility Testing*

The antibiotic susceptibility patterns of the bacterial isolates were determined using the

Kirby–Bauer disc diffusion method on Mueller–Hinton Agar. Fresh bacterial cultures were standardized to 0.5 McFarland turbidity standard and uniformly inoculated onto the surface of sterile Mueller–Hinton Agar plates using sterile swabs.

Commercially prepared antibiotic discs were aseptically placed on the inoculated agar surface and gently pressed to ensure proper contact. The plates were incubated at 37°C for 18–24 hours. Following incubation, the diameters of inhibition zones surrounding each antibiotic disc were measured in millimetres using a transparent ruler. The results were interpreted as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (formerly NCCLS).

### *2.7 Statistical Analysis*

Microbial counts obtained from the reusable plastic bottles were converted to logarithmic values ($log_{10}$ CFU/mL) before analysis. Descriptive statistics including means and standard deviations were calculated using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 25. Statistical significance was determined at $p < 0.05$.

## 3.0 Results and Discussion

The microbiological quality and antibiotic susceptibility profiles of reusable plastic bottles used for packaging food products within Ikot Ekpene metropolis were investigated. The findings revealed substantial microbial contamination of the sampled bottles, indicating potential public health risks associated with their reuse for food and beverage packaging.

The total bacterial counts obtained from the reusable plastic bottles are presented in Table 1. Out of the thirty samples analyzed, twenty-five (83.3%) exhibited bacterial growth, while five samples showed no detectable bacterial contamination. The bacterial load ranged from $3.0 \times 10^5$ to $5.9 \times 10^5$ CFU/mL, with the highest count recorded in Sample 4 ($5.9 \times 10^5$ CFU/mL), followed closely by Sample 30 ($5.5 \times 10^5$ CFU/mL). These findings demonstrate widespread bacterial contamination of the reusable plastic bottles examined.

**Table 1: Total bacterial count of the samples**

| Samples | Total bacterial count of samples (CFU/ml) |
|---|---|
| 1 | 3.7 x 105 |
| 2 | 3.4 x 105 |
| 3 | 3.3 x 105 |
| 4 | 5.9 x 105 |
| 5 | 3.3 x 105 |
| 6 | - |
| 7 | 4.1 x 105 |
| 8 | 3.8 x 105 |
| 9 | - |
| 10 | 3.0 x 105 |
| 11 | 3.1 x 105 |
| 12 | 3.1 x 105 |
| 13 | - |
| 14 | 3.3 x 105 |
| 15 | 3.6 x 105 |
| 16 | 3.5 x 105 |
| 17 | 30 x 105 |
| 18 | 4.8 x 105 |
| 19 | 3.9 x 105 |
| 20 | 4.0 x 105 |
| 21 | 3.5 x 105 |
| 22 | 3.0 x 105 |
| 23 | 4.4 x 105 |
| 24 | - |
| 25 | 3.0 x 105 |
| 26 | - |
| 27 | 3.0 x 105 |

| | |
|---|---|
| 28 | 4.0 x 105 |
| 29 | 3.5 x 105 |
| 30 | 5.5 x 105 |

The relatively high bacterial counts observed in the present study suggest inadequate cleaning, poor handling practices, and possible biofilm formation on the inner surfaces of the bottles. Reused plastic containers often develop microscopic surface irregularities that facilitate microbial attachment and biofilm development, making complete decontamination difficult. The bacterial counts obtained are comparable to those reported by Ezeigbo (2015) and Risiquat (2013), who reported microbial loads ranging from $1.2 \times 10^2$ to $1.2 \times 10^6$ CFU/mL in reused plastic beverage containers. Similarly, Enetimi et al. (2020) reported significant microbial contamination of reused plastic bottles used for packaging locally processed drinks. The presence of such microbial loads indicates poor hygienic conditions and may compromise the microbiological safety of food products packaged in these containers (Table 1).

The cultural, morphological, and biochemical characteristics of the bacterial isolates are presented in Tables 2 and 3. Based on colony morphology, Gram staining reactions, and biochemical test results, six bacterial genera were identified, namely *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Streptococcus* spp. The diversity of bacterial contaminants observed in this study indicates multiple sources of contamination, including environmental exposure, contaminated water, inadequate washing procedures, and poor personal hygiene during handling.

The isolation of *Escherichia coli* and *Klebsiella* spp. is particularly significant because these organisms are recognized indicators of faecal contamination. Their presence suggests possible contamination arising from the use of poor-quality water during bottle washing, improper sanitation practices, or contamination from handlers. The occurrence of these coliform bacteria is consistent with reports by Enetimi et al. (2020), who isolated similar organisms from reused plastic beverage containers.

**Table 2: Cultural and morphological identification of bacteria**

| Sample number | Macroscopic examination on Nutrient Agar | Grams reaction/ shape | Probable organisms |
|---|---|---|---|
| 9 | Milk coloured | Gram + rod | Bacillus spP |
| 5 | Yellow grapelike | Gram + cocci | Staphylococcus aureus |
| 1 | Circular whitish | Gram - rod | E.coli |
| 4 | Milk yellow, irregular shape | Gram - cocci | Streptococcus spp |
| 21 | Circular whistish | Gram - rod | E. coli |
| 27 | Circular dome shape, Greyish white | | Klesiella spP |
| 30 | Opaque, greenish yellow | Gram - rod | Pseudomonas aeruginosa |

**Table 3 : Results of biochemical identification of bacterial isolates**

| Catalase test | Coagulase test | Urease test | Indole test | Citrate test | Motility test | Probable organisms |
|---|---|---|---|---|---|---|
| + | - | - | + | - | + | E. coli |
| + | + | - | - | + | + | Bacillus sp |
| + | + | + | - | + | - | Staphylococcus aureus |
| + | - | + | - | + | - | Klebsiella sp |
| - | - | - | + | - | - | Streptococcus sp |
| + | - | - | - | + | - | Pseudomonas aeruginosa |

Key: + = positive, - = negative

TThe detection of coliform organisms in food-contact materials is undesirable because it indicates inadequate hygienic conditions and the potential presence of enteric pathogens capable of causing gastroenteritis and other foodborne illnesses.he recovery of *Pseudomonas aeruginosa* from some of the bottles further highlights the poor microbiological quality of the containers. *Pseudomonas* species are widely distributed in water and moist environments and are capable of surviving under adverse conditions. Their presence may be attributed to contaminated washing water, ineffective cleaning procedures, or prolonged storage of bottles under humid conditions. Although generally regarded as opportunistic pathogens, *P. aeruginosa* can cause respiratory tract infections, urinary tract infections, wound infections, and septicemia, particularly among immunocompromised individuals.

Similarly, the isolation of *Staphylococcus aureus* indicates contamination arising from human handling. Human skin, nasal passages, and hands constitute major reservoirs of this organism, and poor personal hygiene among handlers may facilitate its transfer to reusable bottles. The presence of *S. aureus* is of public health importance because certain strains produce heat-stable enterotoxins capable of causing food poisoning. The findings support previous observations that repeated handling of reusable containers without proper sanitation significantly increases the likelihood of contamination.

The occurrence of *Bacillus* spp. and *Streptococcus* spp. may be associated with environmental contamination from soil, dust, air, water, and contaminated surfaces. *Bacillus* species are spore-forming organisms capable of surviving harsh environmental conditions and may persist even after routine washing. Their presence in reusable bottles suggests inadequate sanitation and storage practices. Some species of *Bacillus* are known to cause food spoilage and foodborne intoxications, thereby posing potential health risks to consumers.

The fungal counts recorded in the reusable plastic bottles are presented in Table 4. Fungal growth was observed in twenty-two (73.3%) of the thirty samples examined. The fungal counts ranged from $0.1 \times 10^5$ to $2.0 \times 10^5$ CFU/mL, with Samples 18 and 30 recording the highest counts of $2.0 \times 10^5$ CFU/mL. The widespread occurrence of fungi in the samples indicates that the bottles provide favorable conditions for fungal colonization and persistence.

The fungal counts observed may be attributed to inadequate drying after washing, storage under humid conditions, and the presence of residual nutrients from previously packaged beverages.

Fungal contamination is particularly important because several fungal species are capable of producing allergens, mycotoxins, and opportunistic infections, thereby reducing the microbiological quality and safety of packaged foods (Table 4).

**Table 4: Total fungal count of the samples**

| Samples | Total fungal count (CFU/ml |
|---|---|
| 1 | 1.0 x 105 |
| 2 | 0.2 x 105 |
| 3 | - |
| 4 | - |
| 5 | 1.1 x 105 |
| 6 | 1.1 x 105 |
| 7 | 1.2 x 105 |
| 8 | - |
| 9 | 1.3 x 105 |
| 10 | - |
| 11 | 0.2 x 105 |
| 12 | 1.5 x 105 |
| 13 | 1.3 x 105 |
| 14 | 1.5 x 105 |
| 15 | - |
| 16 | 1.0 x 105 |
| 17 | 0.4 x 105 |
| 18 | 2.0 x 105 |
| 19 | - |
| 20 | 1.6 x 105 |
| 21 | 0.3 x 105 |
| 22 | 1.0 x 105 |
| 23 | 1.2 x 105 |
| 24 | 0.1 x 105 |
| 25 | - |
| 26 | 1.0 x 105 |
| 27 | 0.3 x 105 |
| 28 | - |
| 29 | 1.4 x 105 |
| 30 | 2.0 x 105 |

The morphological and microscopic examination of fungal isolates revealed the presence of *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Candida* spp. (Table 5). These fungi are common environmental contaminants and have been frequently reported in food processing environments and reusable containers. Their presence may result from airborne spores, contaminated water sources, improper storage, and poor sanitation practices.

The isolation of *Aspergillus* spp. and *Penicillium* spp. is of particular concern because some species within these genera are capable of producing mycotoxins that may adversely affect human health. Likewise, *Cladosporium* spp. are known allergens and may contribute to respiratory complications in susceptible individuals. The detection of *Candida* spp. suggests contamination from human handlers or environmental sources. Although *Candida* species normally exist as commensals, they can cause opportunistic infections in immunocompromised individuals. Similar fungal contaminants have been reported in reused beverage containers and food-contact surfaces by previous investigators, supporting the assertion that inadequate sanitation facilitates fungal colonization of reusable packaging materials.

The antibiotic susceptibility profiles of the bacterial isolates are presented in Table 6. The results revealed varying susceptibility patterns among the isolates. *Escherichia coli* and *Streptococcus* spp. exhibited susceptibility to all the antibiotics tested, indicating the absence of detectable multidrug resistance among these isolates. Similarly, *Staphylococcus aureus* showed susceptibility to most antibiotics but exhibited resistance to amoxicillin. *Pseudomonas aeruginosa* was susceptible to ciprofloxacin, amoxicillin, erythromycin, augmentin, streptomycin, and chloramphenicol but resistant to gentamicin and ampicillin. *Klebsiella* spp. demonstrated resistance to gentamicin, erythromycin, chloramphenicol, and ampicillin, while remaining susceptible to ciprofloxacin, amoxicillin, augmentin, and streptomycin.

The varying antibiotic susceptibility patterns observed among the isolates may reflect differences in exposure to antimicrobial agents within the environment. The resistance exhibited by *Klebsiella* spp. and *Pseudomonas aeruginosa* is noteworthy because these organisms are frequently associated with antimicrobial resistance in both environmental and clinical settings. Their occurrence in reusable plastic bottles highlights the potential role of contaminated food-contact materials as reservoirs for antibiotic-resistant microorganisms.

**Table 5: Morphology and microscopic characteristics of fungal isolates**

| Isolates | Surface pigmentation | Colony appearance | Microscopy | Probable organisms |
|---|---|---|---|---|
| 1. | Gray | Moist and shiny | Septate hyphae with heavy clusters of spores along the hyphae and the spores are oval and mainly of budding cells. | Cladosporium spp. |
| 2. | Green with white boarder | Velvety | Septate hyphae with long, erect and multi-branched conidiophores developing at the tip, flask-shaped phialides with round conidia in chains in a brush-like appearance | Penicillium spp. |
| 3. | Brown | Powdery | Aseptate stalk-like conidiophores expanding at the apex to a dome-shaped swollen vesicle harbouring round and globose conidia | Aspergillus spp. |
| 4. | Yellow | Cottony | Pseudohyphae surrounded by oval yeast cells. | Candida spp. |

Overall, ciprofloxacin, augmentin, and streptomycin demonstrated broad-spectrum effectiveness against most bacterial isolates recovered in this study. The complete susceptibility of *E. coli* and *Streptococcus* spp. to the tested antibiotics suggests that these organisms remain largely responsive to conventional antimicrobial therapy within the study area. However, the resistance patterns observed among *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* underscore the need for continuous monitoring of antimicrobial resistance among microorganisms associated with food packaging materials.

**Table 6 : Antibiotics sensitivity and resistance against bacterial isolate (mm)**

| Zones of inhibition (mm) Antibiotics | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Bacterial isolates | CPX | CN | AMX | E | AU | S | CH | AMP |
| Bacillus spp | S | R | R | S | S | S | S | S |
| Klebsiella spp | S | R | S | R | S | S | R | R |
| Pseudomonas aeruginosa | S | R | S | S | S | S | S | R |
| E. coli. | S | S | S | S | S | S | S | S |
| Staphylococcus aureus | S | S | R | S | S | S | S | S |
| Streptococcus spp | S | S | S | S | S | S | S | S |

**Key: Sensitive= ≤ 21 ; Resistant = ≥ 15, CPX = Ciprofloxacin; CN=Gentamycin; AMX= Amoxilin;E= Erythromycin; AU= Augmentin; S = Streptomycin; CH= Chloramphenicol; AMR= Ampicillin**.

The findings of this study demonstrate that reusable plastic bottles used for packaging food products within Ikot Ekpene metropolis harbour diverse bacterial and fungal contaminants, including potentially pathogenic and opportunistic microorganisms. The presence of faecal indicator organisms, opportunistic pathogens, and antibiotic-resistant bacteria highlights significant food safety concerns and emphasizes the need for improved cleaning, disinfection, and regulatory oversight of reusable plastic bottles used in food packaging.

## 4.0 Conclusion

This study evaluated the microbial contamination and antibiotic susceptibility patterns of microorganisms associated with reusable plastic bottles used for packaging food products in Ikot Ekpene metropolis. The findings revealed that a high proportion of the bottles examined were contaminated with bacteria and fungi, with bacterial counts ranging from $3.0 \times 10^5$ to $5.9 \times 10^5$ CFU/mL and fungal counts ranging from $0.1 \times 10^5$ to $2.0 \times 10^5$ CFU/mL. These microbial loads indicate poor microbiological quality and suggest inadequate cleaning, handling, and storage practices among users of reusable plastic bottles.

Microbiological characterization identified the presence of potentially pathogenic and opportunistic bacterial species including *Escherichia coli, Staphylococcus aureus, Bacillus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Streptococcus* spp., as well as fungal species such as *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Candida* spp. The occurrence of coliform bacteria and other pathogens suggests possible faecal contamination, poor personal hygiene, and the use of contaminated water during bottle washing and reuse. The isolation of these microorganisms highlights the potential public health risks associated with the continued use of inadequately sanitized plastic bottles for food and beverage packaging.

The antibiotic susceptibility test revealed varying resistance patterns among the bacterial isolates. While *Escherichia coli* and *Streptococcus* spp. were susceptible to all the antibiotics tested, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* exhibited resistance to one or more antibiotics, indicating the presence of antimicrobial resistance among some contaminants. This finding further emphasizes the health risks associated with contaminated reusable plastic bottles, particularly as potential reservoirs for antibiotic-resistant microorganisms.

Overall, the study demonstrates that reusable plastic bottles used for packaging food products within Ikot Ekpene metropolis constitute potential vehicles for the transmission of microbial pathogens and may compromise consumer health. Therefore, proper cleaning and disinfection of reusable bottles, the use of potable water during washing, adherence to good hygienic practices by food vendors, and regular monitoring by relevant public health and food safety agencies are recommended to minimize microbial

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**Declarations**

Ethical Approval and Consent to Participate

This study did not involve human participants, human tissues, or live animals. Therefore, ethical approval and consent to participate were not required.

**Consent for Publication**

Not applicable.

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**Availability of Data and Materials**

All data generated or analyzed during this study are included in this published article. Additional information may be made available by the corresponding author upon reasonable request.

**Competing Interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Authors' Contributions**

Eteyen A. Uko and Imaobong Adenugba jointly conceived and designed the study, conducted sample collection, laboratory analyses, microbial identification, antibiotic susceptibility testing, data analysis, interpretation of results, manuscript preparation, critical revision, and approved the final version for publication. Both authors contributed equally to the research and writing process.