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Microbiological Analysis, Antibiogram and Different Washing Treatments of Reusable Plastic Bottles Used in Packaging Food Products Within Ikot Ekpene Metropolis

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Abstract *:Traditional foods such as fermented cereal drinks, locally brewed beverages, cooking oils, and thick sauces are commonly stored in reusable plastic bottles that support rapid bacterial growth. This study examined 80 reusable plastic bottles used for packaging these products using standard microbiological methods. Inner surfaces were swabbed with sterile nutrient broth, suspended in 9 ml saline, serially diluted, and plated on Nutrient Agar, MacConkey Agar, and Sabouraud Dextrose Agar before incubation at 37 °C for 24 – 48 hours. All samples were contaminated. Unwashed bottles had the highest total bacterial counts of 1.1 × 10^4 – 9.5 × 10^4 CFU/ml, while washing with sterile water, soapy hot water (>60 ^{0}C, and 3 % hydrogen peroxide reduced counts to 1.0 – 7.0× 10^4, 1.0 – 5.0 × 10^4, and 1.0 – 1.2 × 10^4 CFU/ml, respectively, with 3 % hydrogen peroxide being most effective. Bacterial solates included Streptococcus spp., Klebsiella spp., Escherichia coli, Staphylococcus aureus, Bacillus spp., and Pseudomonas aeruginosa. Fungal counts in unwashed and sterile water-washed bottles ranged from 0.0 – 3.0 × 10^4 CFU/ml, dominated by Aspergillus and Penicillium spp., but no fungi were recovered after hot soapy water or hydrogen peroxide treatment. Antibiotic susceptibility testing showed that S. aureus, Streptococcus spp., Bacillus spp., and E. coli were sensitive to agents including Ciprofloxacin, Streptomycin, Gentamicin, Levofloxacin, and Ampiclox, while several isolates were resistant to others. The findings highlight significant microbial risks from reused bottles and the need for effective decontamination before reuse.*

Keywords: *Reusable plastic bottles; Traditional foods; Microbial contamination; Foodborne pathogens; Hydrogen peroxide; Antibiotic susceptibility.*

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1.0 Introduction

Plastic materials have become indispensable in modern food packaging because of their low cost, light weight, durability, ease of processing, and resistance to breakage. Among these materials, polyethylene terephthalate (PET) is one of the most widely used polymers for packaging beverages and food products due to its excellent mechanical properties, transparency, and recyclability. Despite growing concerns regarding the environmental and health impacts associated with plastic waste and repeated reuse, the global demand for PET containers continues to increase because of their affordability and widespread availability (Revathi et al., 2017; Rafey & Siddiqui, 2021). In many developing countries, including Nigeria, used plastic bottles are routinely collected, washed, and reused for packaging traditional food

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products such as fermented cereal drinks, locally brewed beverages, edible oils, liquid condiments, and thick sauces.

The reuse of plastic bottles offers important economic and environmental advantages by reducing packaging costs and minimizing plastic waste. However, repeated use without adequate cleaning and sanitation presents a significant food safety challenge. The narrow neck, threaded cap, scratches on the internal surface, and complex geometry of reusable bottles provide suitable sites for the attachment and persistence of microorganisms. Residual food materials and moisture remaining after use further promote microbial growth, particularly when bottles are stored under ambient tropical conditions. Consequently, inadequately cleaned reusable bottles may serve as reservoirs for pathogenic microorganisms capable of contaminating subsequently packaged food products.

Food packaging is intended to preserve product quality and protect foods from physical, chemical, and microbiological contamination throughout storage and distribution. However, packaging materials themselves may become sources of contamination when they are improperly handled, inadequately cleaned, or repeatedly reused without effective decontamination. Microbial contamination may occur through direct contact with contaminated food-contact surfaces, processing equipment, handlers, or inadequately sanitized packaging containers (Lau & Wong, 2000; Mohanty & Swain, 2017; Akanele et al., 2016). Consequently, the microbiological quality of reusable food packaging materials is an important determinant of food safety and consumer health.

One of the major concerns associated with reusable plastic bottles is the formation of microbial biofilms on their internal surfaces. Biofilms are structured communities of microorganisms enclosed within self-produced extracellular polymeric substances that firmly adhere to surfaces. Once established, biofilms protect microorganisms from environmental stress and substantially reduce the effectiveness of routine washing procedures. As a result, pathogenic bacteria and fungi may survive within reusable containers despite repeated rinsing, thereby increasing the likelihood of cross-contamination of newly packaged food products. The persistence of biofilm-associated microorganisms has been recognized as an important contributor to foodborne disease outbreaks and represents a major challenge in food hygiene (Bartram et al., 2004).

Several pathogenic microorganisms have previously been isolated from inadequately cleaned food-contact surfaces and reusable containers. These include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Klebsiella* spp., *Bacillus* spp., and various fungal genera such as *Aspergillus* and *Penicillium*. These microorganisms are capable of causing food spoilage and a wide range of foodborne illnesses, including gastroenteritis, diarrhea, vomiting, abdominal pain, septicemia, and opportunistic infections, particularly among young children, older adults, pregnant women, and immunocompromised individuals. The World Health Organization estimates that approximately 600 million people suffer from foodborne diseases annually, resulting in about 420,000 deaths worldwide, with developing countries bearing a disproportionate share of the disease burden (WHO, 2022). In Nigeria, foodborne diseases remain an important public health problem, accounting for substantial morbidity and mortality each year (Jimoh et al., 2021).

In addition to microbial contamination, the emergence of antimicrobial-resistant foodborne pathogens has become a major global public health concern. Bacteria contaminating food-contact surfaces and packaging materials may harbor resistance to commonly used antibiotics, thereby increasing the difficulty of treating foodborne infections. Determination of the antibiotic

susceptibility profiles of bacterial isolates recovered from reusable plastic bottles is therefore important for assessing their potential public health significance and providing baseline epidemiological information for antimicrobial resistance surveillance.

Proper cleaning and sanitation are essential for minimizing microbial contamination in reusable packaging materials. Various cleaning approaches, including rinsing with water, washing with hot soapy water, and the use of chemical disinfectants such as hydrogen peroxide, have been employed to reduce microbial loads on food-contact surfaces. However, the effectiveness of these methods varies depending on the type of contaminating microorganisms, the extent of biofilm formation, and the cleaning procedure employed. Comparative evaluation of commonly used washing methods is therefore necessary to identify effective decontamination practices that can enhance the microbiological safety of reusable plastic bottles used for food packaging.

Although several studies have investigated microbial contamination of drinking water containers, food-contact surfaces, and reusable beverage bottles, relatively limited information is available regarding the microbiological quality of reusable plastic bottles employed for packaging traditional food products within Ikot Ekpene Metropolis, Akwa Ibom State, Nigeria. Furthermore, comparative information on the effectiveness of commonly used washing treatments and the antibiotic susceptibility patterns of bacterial isolates recovered from these bottles remains scarce. Generating such information is essential for improving food hygiene practices, protecting consumer health, and supporting evidence-based recommendations for the safe reuse of plastic food packaging materials.

Therefore, this study aimed to evaluate the microbiological quality of reusable plastic bottles used for packaging traditional food products within Ikot Ekpene Metropolis, determine the antibiotic susceptibility patterns of the recovered bacterial isolates, and compare the effectiveness of selected washing treatments, namely sterile water, hot soapy water, and 3% hydrogen peroxide, in reducing microbial contamination.

2.0 Materials and Methods

2.1. Study Area

Ikot Ekpene local government area, Akwa Ibom State is located in the South–South geopolitical zone of Nigeria. It covers about 7, 081 km2 lands mass and has an estimated population of 5.5 million (NBS, 2021). The study area is made up of covered ten (10) local government areas situated between latitude 5° 151′ and 5° 173′N and longitude 7° 41′ and 7° 42′E. The area is characterized by long wet season (March – October) and short dry season (November – February), with a mean annual rainfall of 2,200 mm. The area experiences significant seasonal variations, with temperature ranging from 22 °C during dry months to 35 °C during wet months.

2.2 Materials

The materials used for this study included eighty (80) reusable plastic bottles previously used for packaging food products, sterile cotton swabs, sterile saline solution (0.85% NaCl), nutrient broth, Nutrient Agar (NA), MacConkey Agar (MCA), Sabouraud Dextrose Agar (SDA), Mueller–Hinton Agar (MHA), Gram staining reagents, Lactophenol Cotton Blue stain, biochemical test reagents (catalase, oxidase, coagulase, indole and motility test reagents), commercially prepared antibiotic discs, sterile distilled water, mild liquid detergent, 3% hydrogen peroxide, sterile Petri dishes, sterile pipettes, micropipettes with sterile tips, conical flasks, test tubes, measuring cylinders, incubator, autoclave, laminar airflow cabinet, analytical balance, vortex mixer, colony counter, and other routine microbiological laboratory equipment.

2.3 Experimental Design

A completely randomized experimental design was adopted for the study. A total of eighty (80) reusable plastic bottles were randomly assigned into four treatment groups consisting of twenty (20) bottles per group as follows:

(i) Group I: Unwashed reusable plastic bottles (control).
(ii) Group II: Reusable plastic bottles washed with sterile distilled water.
(iii) Group III: Reusable plastic bottles washed with hot soapy water (>60 °C).
(iv) Group IV: Reusable plastic bottles washed with 3% hydrogen peroxide.

The effectiveness of each washing treatment was evaluated by comparing the microbial loads and the occurrence of bacterial and fungal isolates recovered from the bottles.

2.4 Study Area and Sample Collection

The study was conducted in Ikot Ekpene Metropolis, Akwa Ibom State, Nigeria. Eighty reusable plastic bottles previously used for packaging traditional food products, including fermented cereal drinks, locally brewed beverages, edible oils, and sauces, were obtained from food vendors and retailers within the metropolis. Bottles showing evidence of previous use were aseptically collected while visibly damaged bottles were excluded from the study.

Each bottle was handled using sterile disposable gloves and individually placed in sterile polyethylene bags appropriately labelled according to the sampling code. Samples were transported to the Microbiology Laboratory in insulated containers and processed within two hours of collection to minimize changes in the microbial population.

2.5 Washing Procedures

The reusable plastic bottles were subjected to the following washing treatments prior to microbiological analysis.

2.6 Sterile Water Treatment

Each bottle was rinsed thoroughly with 100 mL of sterile distilled water for approximately 30 seconds. Excess water was drained, and the bottles were allowed to air-dry inside a laminar airflow cabinet for 15–20 minutes before microbiological sampling.

2.7 Hot Soapy Water Treatment

Each bottle was washed with approximately 100 mL of hot water maintained at temperatures above 60 °C containing approximately 2 mL of mild liquid detergent. The bottles were capped, shaken vigorously for 30 seconds, thoroughly rinsed with sterile distilled water to remove detergent residues, and air-dried in a laminar airflow cabinet for 15–20 minutes.

2.8 Hydrogen Peroxide Treatment

Each bottle was rinsed with 100 mL of 3% hydrogen peroxide solution and allowed to remain in contact with the inner surfaces for approximately 5 minutes. Excess solution was discarded, and the bottles were air-dried inside a laminar airflow cabinet for 15–20 minutes before analysis.

2.9 Unwashed Control

The bottles assigned to the control group received no washing treatment before microbiological examination.

2.10 Enumeration of Microbial Population

The microbial population was determined using standard microbiological procedures. Sterile cotton swabs previously moistened with sterile nutrient broth were used to thoroughly swab the entire internal surface of each reusable plastic bottle. The swab was immediately transferred into a test tube containing 9 mL of sterile physiological saline and vortexed for approximately one minute to dislodge the attached microorganisms.

Serial ten-fold dilutions (10^{-1}–10^{-5}) were prepared using sterile physiological saline. Aliquots (0.1 mL) from appropriate dilutions were inoculated onto Nutrient Agar for total viable bacterial count, MacConkey Agar for coliform bacteria, and Sabouraud Dextrose Agar for fungal enumeration using the spread plate technique. All inoculations were performed in duplicate.

Nutrient Agar plates were incubated at 37 °C for 24 hours, MacConkey Agar plates at 37 °C for 24–48 hours, while Sabouraud Dextrose Agar plates were incubated at 28 ± 2 °C for 5–7 days.

2.11 Determination of Microbial Load

Following incubation, plates containing between 30 and 300 colonies were selected for enumeration. Colonies were counted using a digital colony counter, and the microbial load was expressed as colony-forming units per millilitre (CFU/mL) according to equation (3.1):

$$CFU(mL) - \frac{N_{counted} \times Dilution\ factor}{Volume\ plated\ (mL)} \quad \{1\}$$

where CFU/mL = Colony-forming units per millilitre and $N_{counted}$ is the number of colony counted.

2.12 Identification of Bacterial Isolates

Distinct bacterial colonies were purified by repeated sub-culturing on Nutrient Agar. Preliminary identification was based on colonial characteristics including colony size, pigmentation, elevation, margin, surface texture and haemolytic characteristics where applicable. Gram staining was subsequently performed to determine Gram reaction and cellular morphology.

Further identification of bacterial isolates was carried out using standard biochemical tests including catalase, oxidase, coagulase, indole, citrate utilization and motility tests following the procedures described by Cheesbrough (2006). Identification was confirmed by comparing the observed characteristics with standard microbiological identification keys.

2.13 Identification of Fungal Isolates

Fungal isolates were identified based on both macroscopic and microscopic characteristics. Macroscopic examination included observations of colony colour, texture, pigmentation, margin and growth pattern on Sabouraud Dextrose Agar. Microscopic examination was carried out by preparing fungal mounts stained with Lactophenol Cotton Blue and observing the hyphal structures, conidiophores, spores and other reproductive structures under a light microscope. Identification was accomplished by comparison with standard fungal identification manuals as described by Winn et al. (2006).

2.14 Antibiotic Susceptibility Testing

The antibiotic susceptibility patterns of the bacterial isolates were determined using the Kirby–Bauer disc diffusion method. Fresh bacterial cultures were adjusted to the turbidity of a 0.5 McFarland standard before inoculation. Sterile cotton swabs were used to uniformly spread the standardized inoculum onto Mueller–Hinton Agar plates.

Commercially prepared antibiotic discs were aseptically placed on the inoculated agar surfaces using sterile forceps and gently pressed to ensure complete contact with the agar. The plates were incubated aerobically at 37 °C for 24 hours. Following incubation, the diameters of the zones of inhibition surrounding each antibiotic disc were measured in millimetres using a transparent ruler.

The bacterial isolates were classified as susceptible, intermediate or resistant according to the interpretative criteria of the Clinical and Laboratory Standards Institute (CLSI, current edition).

2.15 Quality Assurance

All culture media were prepared according to the manufacturers' instructions and sterilized by autoclaving at 121 °C for 15 minutes. Sterility of prepared media was confirmed by incubating uninoculated control plates before use. All microbiological analyses were performed under aseptic conditions within a laminar airflow cabinet, and all determinations were carried out in duplicate to ensure accuracy and reproducibility.

2.`6 Statistical Analysis

Data obtained from the study were expressed as mean ± standard deviation. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25.0. Differences among the washing treatments were analysed using one-way

analysis of variance (ANOVA), and significant differences between treatment means were separated using Tukey's post hoc test. Statistical significance was established at $p < 0.05$.

3.0. Results and Discussion

This chapter presents the results obtained from the microbiological examination of reusable plastic bottles collected from food vendors within Ikot Ekpene Metropolis and discusses their public health implications. The study evaluated the microbial quality of eighty (80) reusable plastic bottles subjected to four different treatments: unwashed bottles (control), bottles washed with sterile water, bottles washed with hot soapy water (>60 °C), and bottles washed with 3% hydrogen peroxide. The effectiveness of each washing treatment was assessed by comparing the total bacterial and fungal loads, identifying the bacterial and fungal species isolated, and determining the antibiotic susceptibility profiles of the bacterial isolates.

The reuse of plastic bottles for packaging locally processed food products such as fermented cereal drinks, locally brewed beverages, edible oils, and sauces is a common practice in many developing countries because it reduces packaging costs and minimizes environmental waste. However, inadequate cleaning before reuse may promote the accumulation of microbial biofilms and food residues on the internal surfaces of bottles, thereby creating suitable conditions for the survival and multiplication of spoilage organisms and pathogenic microorganisms. Consequently, the microbiological quality of reusable plastic bottles is an important determinant of food safety and consumer health.

3.1 Total Bacterial Count of Reusable Plastic Bottles

The total bacterial counts obtained from the reusable plastic bottles following the different washing treatments are presented in Tables 4.1–4.4. The results show marked differences in bacterial loads among the treatment groups, indicating that the efficiency of microbial removal depended largely on the washing method employed. In general, unwashed bottles exhibited the highest bacterial loads, while bottles treated with 3% hydrogen peroxide recorded the lowest bacterial counts. These findings demonstrate the importance of effective cleaning and disinfection in reducing microbial contamination of reusable food packaging containers.

3.1.1 Total Bacterial Count of Unwashed Reusable Plastic Bottles

The bacterial counts obtained from the twenty unwashed reusable plastic bottles are presented in Table 1.

The results presented in Table 1 indicate that all twenty unwashed reusable plastic bottles were contaminated with viable bacteria, giving a contamination rate of 100%. The total bacterial counts ranged from 1.1×10^4 CFU/mL to 9.5×10^4 CFU/mL, with Sample 7 recording the highest bacterial load (9.5×10^4 CFU/mL) and Sample 16 the lowest (1.1×10^4 CFU/mL). The wide variation in bacterial counts among the samples suggests differences in the extent of previous use, storage conditions, type of food previously packaged, duration before reuse, and cleaning history of the bottles.

The consistently high bacterial counts observed in the unwashed bottles indicate substantial microbial colonization of the bottle surfaces. Food residues adhering to the internal surfaces of the bottles likely provided nutrients that supported bacterial multiplication. Furthermore, repeated reuse without adequate cleaning may have encouraged the development of microbial biofilms, which protect microorganisms from environmental stress and facilitate their persistence on plastic surfaces.

The findings demonstrate that reusable plastic bottles that are not properly cleaned may constitute significant reservoirs of foodborne microorganisms capable of contaminating freshly packaged food products. Similar observations have been

reported by Bayoi et al. (2014), who found high microbial loads in beverages packaged in recovered plastic bottles in Cameroon. Likewise, Gonzalez-Rivas et al. (2018) reported that reusable food containers frequently harbour large populations of bacteria because of inadequate sanitation during repeated use. The present findings further support reports by Mohanty and Swain (2017) that improperly sanitized food packaging materials can become important sources of microbial contamination.

3.1.2 Total Bacterial Count of Reusable Plastic Bottles Washed with Sterile Water

The total bacterial counts obtained after washing reusable plastic bottles with sterile distilled water are presented in Table 2.

As shown in Table 2, washing the reusable plastic bottles with sterile water reduced the bacterial population compared with the unwashed bottles. The bacterial counts ranged from 1.0×10^4 CFU/mL to 7.0×10^4 CFU/mL, indicating that although sterile water removed part of the microbial population, none of the bottles became free of bacterial contamination. The persistence of viable bacteria after washing demonstrates that simple rinsing with water is insufficient to eliminate microorganisms firmly attached to bottle surfaces.

Table 1:Total bacterial count of samples (Unwashed)

Sample ID	Colonies Counted	Total Bacterial Count (CFU/mL)
1.	15	1.5×10^4
2.	82	8.2×10^4
3.	56	5.6×10^4
4.	31	3.1×10^4
5.	25	2.5×10^4
6.	12	1.2×10^4
7.	95	9.5×10^4
8.	42	4.2×10^4
9.	68	6.8×10^4
10.	18	1.8×10^4
11.	21	2.1×10^4
12.	51	5.1×10^4
13.	85	8.5×10^4
14.	35	3.5×10^4
15.	92	9.2×10^4
16.	11	1.1×10^4
17.	65	6.5×10^4
18.	48	4.8×10^4
19.	72	7.2×10^4
20.	28	2.8×10^4

The reduction in bacterial count observed after sterile water washing is attributable primarily to the mechanical removal of loosely attached microorganisms and food particles. However, microorganisms embedded within biofilms remain attached to the bottle surface because sterile water possesses no antimicrobial activity capable of destroying microbial cells or disrupting the extracellular polymeric matrix of biofilms.

These findings agree with those of Flores et al. (2015), who reported that water alone mainly removes loosely attached

microorganisms through physical action without effectively destroying microbial biofilms. Similarly, Bayoi et al. (2014) observed that rinsing reused plastic bottles with water produced only limited reductions in microbial contamination, emphasizing the need for more effective sanitizing procedures before bottles are reused for food packaging.

3.1.3 Total Bacterial Count of Reusable Plastic Bottles Washed with Hot Soapy Water

The bacterial counts obtained from reusable plastic bottles washed with hot soapy water maintained above 60 °C are presented in Table 3. The results presented in Table 3 show that washing with hot soapy water further reduced bacterial contamination compared with sterile water washing. The bacterial counts ranged from 1.0×10^4 CFU/mL to 5.0×10^4 CFU/mL, representing a noticeable decrease in microbial load. Nevertheless, viable bacteria were still recovered from all the treated bottles, indicating that washing with hot soapy water alone was unable to achieve complete microbial elimination.

Table 2: Total bacterial count of reusable plastic bottles washed with sterile water

S/N	Number of Colonies	Total Bacterial Count (CFU/mL)
1.	13	1.3×10^4
2.	50	5.0×10^4
3.	40	4.0×10^4
4.	28	2.8×10^4
5.	20	2.0×10^4
6.	10	1.0×10^4
7.	68	6.8×10^4
8.	35	3.5×10^4
9.	50	5.0×10^4
10.	15	1.5×10^4
11.	20	2.0×10^4
12.	40	4.0×10^4
13.	50	5.0×10^4
14.	30	3.0×10^4
15.	70	7.0×10^{4s}
16.	11	1.1×10^4
17.	50	5.0×10^4
18.	45	4.5×10^4
19.	50	5.0×10^4
20.	25	2.5×10^4

The improved performance of hot soapy water may be attributed to the combined effects of elevated temperature and detergent action. Hot water enhances the removal of grease, food residues, and organic matter, while detergents lower the surface tension, allowing microorganisms to be detached more easily from bottle surfaces. However, microorganisms enclosed within mature biofilms may remain protected from complete removal, particularly when the contact time is relatively short.

The present findings corroborate those of Flores et al. (2015), who demonstrated that soaps and detergents primarily facilitate the physical removal of microorganisms rather

than directly killing them. Similar observations have been reported in food hygiene studies, where detergent washing significantly reduced bacterial loads but did not completely eliminate biofilm-associated microorganisms from reusable food-contact surfaces. Consequently, detergent washing should be complemented with appropriate disinfectants when reusable plastic bottles are intended for packaging ready-to-eat foods.

3.1.4 Total Bacterial Count of Reusable Plastic Bottles Washed with 3% Hydrogen Peroxide

The bacterial counts obtained after treatment of reusable plastic bottles with 3% hydrogen peroxide are presented in Table 4.

The results presented in Table 4 demonstrate that washing reusable plastic bottles with 3% hydrogen peroxide was the most effective treatment among all the washing methods investigated. Eight of the twenty bottles (40%) showed no detectable bacterial growth after treatment, while six bottles produced fewer than ten colonies, indicating very low levels of contamination. The remaining bottles recorded bacterial counts ranging from 1.0×10^4 CFU/mL to 1.2×10^4 CFU/mL, which were considerably lower than those observed in the other treatment groups.

The remarkable reduction in bacterial load confirms the strong antimicrobial activity of hydrogen peroxide. Hydrogen peroxide acts as a powerful oxidizing agent that generates reactive oxygen species capable of damaging microbial cell membranes, proteins, enzymes, and nucleic acids, ultimately leading to cell death. Unlike sterile water and detergent washing, hydrogen peroxide not only removes microorganisms from surfaces but also inactivates many bacterial cells that remain attached after washing.

Table 3: Total bacterial load (Washed with soapy hot Water)

S/N	Number of Colonies	Total Bacterial Count (CFU/mL)
1.	30	3.0×10^4
2.	45	4.5×10^4
3.	40	4.0×10^4
4.	40	4.0×10^4
5.	10	1.0×10^4
6.	50	5.0×10^4
7.	30	3.0×10^4
8.	45	4.5×10^4
9.	35	3.5×10^4
10.	29	2.9×10^4
11.	15	1.5×10^4
12.	40	4.0×10^4
13.	30	3.0×10^4
14.	50	5.0×10^4
15.	19	1.9×10^4
16.	20	2.0×10^4
17.	20	2.0×10^4
18.	30	3.0×10^4
19.	48	4.8×10^4
20.	30	3.0×10^4

The superior performance of hydrogen peroxide observed in this study agrees with the findings of Juven and Pierson (1996), who attributed the antimicrobial activity of

hydrogen peroxide to oxidative damage of essential cellular components. Similarly, Hooker et al. (2020) demonstrated that hydrogen peroxide significantly reduced populations of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* on contaminated hospital mattresses following routine washing. Comparable reductions have also been reported for food-contact surfaces, where hydrogen peroxide effectively disrupted microbial biofilms and reduced bacterial contamination to safe levels.

Overall, the results of the bacterial enumeration clearly demonstrate that the effectiveness of the washing treatments followed the order: 3% hydrogen peroxide > hot soapy water > sterile water > unwashed control.

This trend highlights the importance of incorporating effective chemical disinfectants into cleaning protocols for reusable plastic bottles intended for food packaging. The use of hydrogen peroxide, either alone or following detergent washing, may substantially reduce microbial contamination and thereby minimize the risk of foodborne disease transmission associated with reused plastic containers.

Following the enumeration of bacterial populations, representative colonies were purified and identified using cultural characteristics,

Table 4: Total bacterial count of reusable plastic bottles washed with 3% hydrogen peroxide

S/N	Number of Colonies	Total Bacterial Count (CFU/mL)
1.	<10	$<10.0 \times 10^4$
2.	<10	$<10.0 \times 10^4$
3.	0	0
4.	10	1.0×10^4
5.	<10	$<1.0 \times 10^4$
6.	<10	$<1.0 \times 10^4$
7.	0	0
8.	10	1.0×10^4
9.	0	0
10.	<10	$<1.0 \times 10^4$
11.	11	1.1×10^4
12.	0	0
13.	0	0
14.	11	1.1×10^4
15.	<10	$<1.0 \times 10^4$
16.	12	1.2×10^4
17.	0	0
18.	0	0
19.	10	1.0×10^4
20.	0	0

4.2 Identification, Distribution and Frequency of Occurrence of Bacterial Isolates

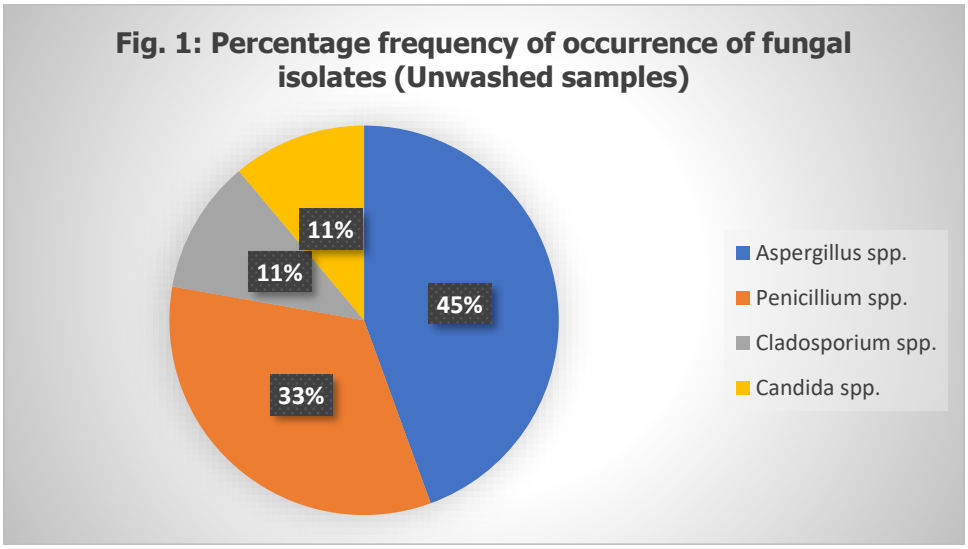
Gram staining, cellular morphology, and standard biochemical tests. The results of the cultural, microscopic, and biochemical characterization of the bacterial isolates are presented in Table 5, while the percentage frequency of occurrence of the identified bacterial species under the different washing treatments is illustrated in Figs. 1 to 4. These results provide information on the diversity of bacterial contaminants associated with reusable plastic bottles and the effect of the

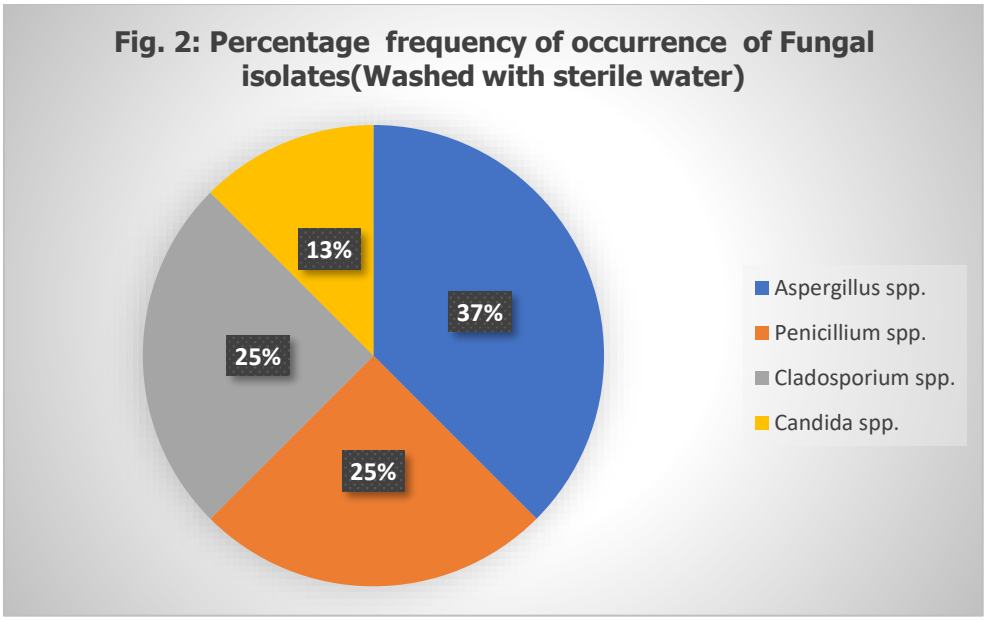
different washing treatments on the distribution of bacterial species.

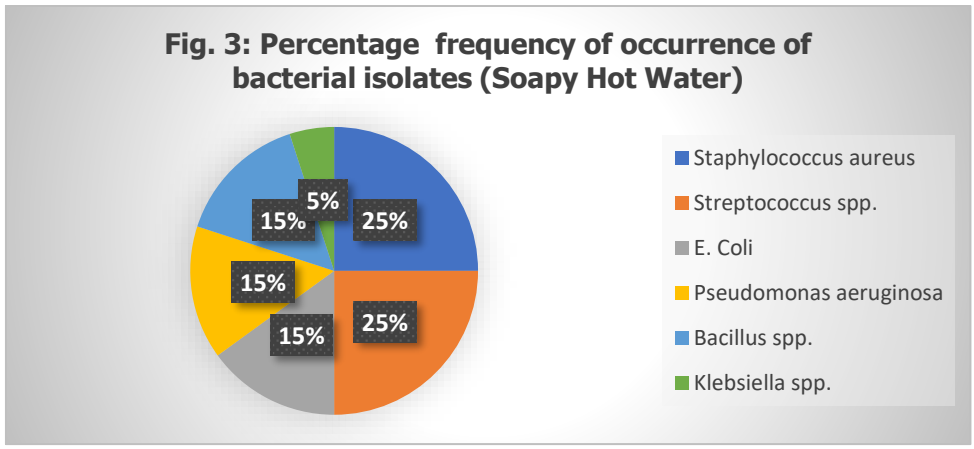
Table 5: Cultural, morphological, and biochemical characteristics of bacterial isolates

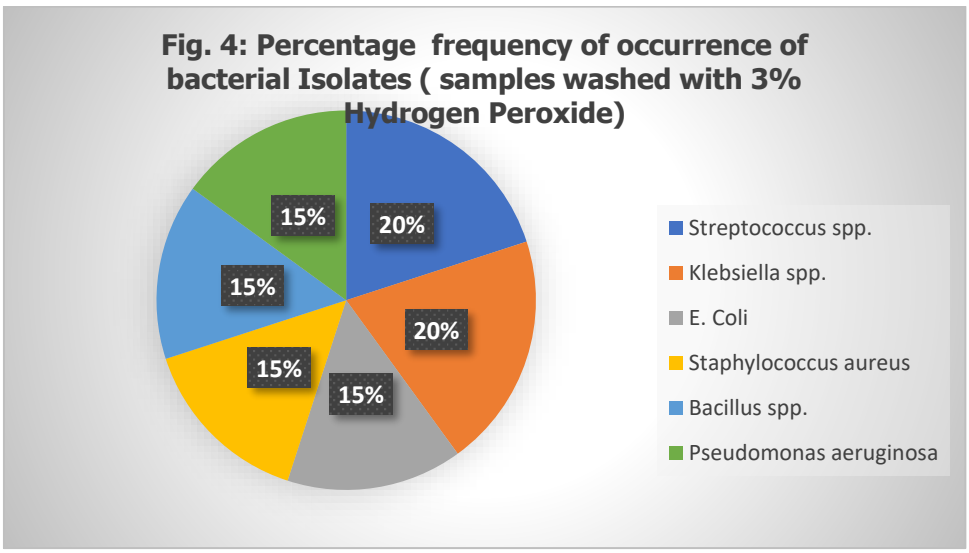
Characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Cultural characteristics	Milky, circular, large, raised, entire, opaque	Milky, filamentous, crater form, curled, transparent	Milky, filamentous, crater form, curled, transparent	Pink, circular, convex, entire, opaque	Green, spindle, umbonate, undulate, opaque
Gram reaction	+	+	+	–	–
Cell shape	Cocci in clusters	Cocci in chains	Rods	Rods	Rods
Coagulase	+	–	–	–	–
Spore	–	–	+	–	–
Catalase	+	–	+	+	+
Oxidase	–	–	–	–	+
Citrate	+	+	+	+	+
Urease	+	–	–	+	–
Motility	–	–	+	–	+
Glucose	AG	AG	AG	AG	AG
Maltose	AG	AG	AG	OO	AG
Lactose	AG	AG	AO	AG	AG
Sucrose	AG	AG	AG	AG	AG
Probable organism	*Staphylococcus* sp.	*Streptococcus* sp.	*Bacillus* sp.	*Klebsiella* sp.	*Pseudomonas* sp.

Keys: AG= Acid and Gas AO= Acid, No gas OO= No Acid, No gas- = Negative + = Positive









3.2.1 Cultural, Morphological and Biochemical Characteristics of the Bacterial Isolates

The cultural, microscopic and biochemical characteristics used for the identification of the bacterial isolates recovered from the

reusable plastic bottles are presented in Table 5.

The bacterial isolates recovered from the reusable plastic bottles were identified as *Staphylococcus aureus*, *Streptococcus* spp., *Bacillus* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa* based on their colonial morphology, Gram reaction, cellular morphology and biochemical characteristics. The isolates exhibited distinct cultural features on Nutrient Agar and MacConkey Agar, including differences in colony colour, size, elevation, margin, opacity and pigmentation.

Gram staining revealed that *Staphylococcus aureus*, *Streptococcus* spp., and *Bacillus* spp. were Gram-positive organisms, whereas *Klebsiella* spp. and *Pseudomonas aeruginosa* were Gram-negative bacteria. The isolates also demonstrated characteristic biochemical reactions that facilitated their identification. For instance, *Staphylococcus aureus* was catalase- and coagulase-positive, *Bacillus* spp. produced endospores and exhibited motility, whereas *Pseudomonas aeruginosa* was oxidase-positive and motile. These characteristics are consistent with the standard descriptions reported by Cheesbrough (2006).

The recovery of these bacterial species demonstrates that reusable plastic bottles can harbour both Gram-positive and Gram-negative bacteria, including organisms recognized as important foodborne pathogens and opportunistic pathogens. Their presence indicates inadequate sanitation of reusable bottles before refilling and emphasizes the potential risk of cross-contamination of food products.

3.2.2 Frequency of Occurrence of Bacterial Isolates in Unwashed Reusable Plastic Bottles

The percentage frequency of occurrence of bacterial isolates recovered from the unwashed reusable plastic bottles is presented in Fig.1. Fig. 1: Percentage frequency of occurrence of bacterial isolates recovered from unwashed reusable plastic bottles

As illustrated in Fig. 1, *Klebsiella* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* each accounted for 20% of the bacterial isolates recovered from the unwashed bottles, making them the predominant bacterial contaminants. In contrast, *Streptococcus* spp. and *Bacillus* spp. each constituted 10% of the total bacterial isolates.

The relatively high occurrence of *Escherichia coli* and *Klebsiella* spp. is of considerable public health significance because these organisms are indicators of poor hygiene and possible faecal contamination. Their presence suggests that the bottles may have been exposed to contaminated environments or handled under unhygienic conditions during collection, storage or reuse. Similarly, the recovery of *Staphylococcus aureus* indicates contamination from human handlers, as this organism commonly colonizes the skin, nose and hands of healthy individuals. The occurrence of *Pseudomonas aeruginosa*, an environmental bacterium capable of forming persistent biofilms, further demonstrates the ability of reusable plastic bottles to support microbial attachment and long-term survival. These findings agree with those reported by Bayoi et al. (2014), who isolated *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* species from recovered plastic bottles used for packaging beverages in Cameroon. Similar bacterial contaminants have also been reported from reusable food-contact surfaces in other developing countries, where inadequate sanitation practices contribute significantly to food contamination.

3.2.3 Frequency of Occurrence of Bacterial Isolates in Bottles Washed with Sterile Water

The percentage frequency of occurrence of bacterial isolates recovered from reusable plastic bottles washed with sterile water is presented in Fig.2.

Fig. 2: Percentage frequency of occurrence of bacterial isolates recovered from reusable plastic bottles washed with sterile water

Fig. shows that *Bacillus* spp. recorded the highest frequency of occurrence (25%) following washing with sterile water, while *Pseudomonas aeruginosa* accounted for 20% of the isolates. *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus* spp. each represented 15%, whereas *Klebsiella* spp. showed the lowest occurrence (10%).

The predominance of *Bacillus* spp. after washing may be attributed to the ability of these bacteria to produce highly resistant endospores that survive adverse environmental conditions and routine cleaning procedures. Likewise, the persistence of *Pseudomonas aeruginosa* can be explained by its remarkable capacity to form biofilms and resist removal from moist surfaces. These characteristics enable both organisms to survive simple rinsing with water.

The persistence of bacterial contaminants after sterile water washing confirms that rinsing alone is inadequate for sanitizing reusable food containers. Water effectively removes loose debris but lacks antimicrobial properties necessary to destroy microorganisms embedded within biofilms or attached firmly to plastic surfaces.

3.2.4 Frequency of Occurrence of Bacterial Isolates in Bottles Washed with Hot Soapy Water

The percentage frequency of occurrence of bacterial isolates recovered from reusable plastic bottles washed with hot soapy water is shown in Fig. 3.

As presented in Fig. 3, *Staphylococcus aureus* and *Streptococcus* spp. were the predominant bacterial isolates, each accounting for 25% of the total isolates. *Escherichia coli*, *Bacillus* spp., and *Pseudomonas aeruginosa* each constituted 15%, while *Klebsiella* spp. recorded the lowest frequency (5%).

The reduced occurrence of *Klebsiella* spp. following washing with hot soapy water suggests that this treatment was relatively more effective against this organism than sterile water alone. Nevertheless, the continued isolation of several bacterial species indicates that detergent washing, although capable of removing food residues and reducing microbial loads, does not completely eliminate bacteria attached to bottle surfaces.

The persistence of *Staphylococcus aureus* following detergent washing is likely associated with contamination from human handling after washing or incomplete removal of biofilm-associated cells. Similar observations have been reported by Flores et al. (2015), who demonstrated that soaps primarily facilitate the physical removal of microorganisms rather than their complete destruction.

3.2.5 Frequency of Occurrence of Bacterial Isolates in Bottles Washed with 3% Hydrogen Peroxide

The percentage frequency of occurrence of bacterial isolates recovered from reusable plastic bottles treated with 3% hydrogen peroxide is presented in Fig. 4.

Fig. 4: Percentage frequency of occurrence of bacterial isolates recovered from reusable plastic bottles washed with 3% hydrogen peroxide

The results presented in Fig. 4 indicate that *Streptococcus* spp. and *Klebsiella* spp. each accounted for 20% of the bacterial isolates recovered after hydrogen peroxide treatment, whereas *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp., and *Pseudomonas aeruginosa* each represented 15% of the isolates.

Although these percentages describe the relative distribution of the bacteria that survived treatment, they should be interpreted together with the bacterial counts presented in Table 4. Hydrogen peroxide treatment substantially reduced the number of contaminated bottles and the overall bacterial load, with 40% of the bottles showing no detectable bacterial growth. Consequently, the percentage distribution shown in Fig. 4 reflects only the few isolates that survived

treatment and does not imply that hydrogen peroxide was ineffective.

The broad reduction in bacterial contamination observed following hydrogen peroxide treatment is attributable to its strong oxidizing activity, which damages bacterial cell membranes, proteins and nucleic acids through the production of reactive oxygen species. The effectiveness of hydrogen peroxide against both Gram-positive and Gram-negative bacteria has been widely documented in food processing and healthcare environments. Juven & Pierson (1996) demonstrated that hydrogen peroxide exerts rapid bactericidal activity through oxidative stress, while Hooker et al. (2020) reported significant reductions in *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* on contaminated hospital surfaces following hydrogen peroxide disinfection.

Overall, the bacterial identification results demonstrate that reusable plastic bottles used for packaging food products harbour a diverse population of potentially pathogenic microorganisms. The occurrence of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Streptococcus* spp., and *Bacillus* spp. highlights the potential public health risks associated with the reuse of inadequately sanitized plastic bottles. These findings reinforce the bacterial count results, confirming that 3% hydrogen peroxide was the most effective washing treatment, followed by hot soapy water, while sterile water alone provided only limited decontamination.

3.3 Fungal Load, Identification and Frequency of Occurrence of Fungal Isolates

In addition to bacterial contamination, reusable plastic bottles were examined for the presence of fungal contaminants because fungi are important spoilage organisms capable of producing mycotoxins and causing opportunistic infections. The total fungal counts obtained from the different washing treatments are presented in Tables 6 and 7, while the morphological and microscopic characteristics of the fungal isolates are presented in Table 7. The percentage frequency of occurrence of the fungal isolates recovered from the bottles is illustrated in Figs. 5 and 6. The results provide information on the effectiveness of the different washing treatments in controlling fungal contamination associated with reusable plastic bottles.

3.3.1 Total Fungal Count of Unwashed Reusable Plastic Bottles

The total fungal counts recovered from the unwashed reusable plastic bottles are presented in Table 6.

The results presented in Table 6 reveal that fungal contamination was detected in the majority of the unwashed reusable plastic bottles. The fungal counts ranged from 0.0×10^4 CFU/mL to 3.0×10^4 CFU/mL, indicating varying degrees of fungal colonization among the sampled bottles. While some bottles showed no detectable fungal growth, others recorded relatively high fungal populations, suggesting that the level of contamination depended on the previous contents of the bottles, duration of storage, environmental exposure, and sanitation practices before reuse.

The occurrence of fungi in unwashed bottles demonstrates that reusable plastic containers provide favourable conditions for fungal growth, particularly when food residues and moisture remain on the internal surfaces. These conditions encourage the attachment, germination and proliferation of fungal spores, many of which are ubiquitous in the environment. In tropical regions such as southern Nigeria, high temperature and relative humidity further enhance fungal survival and multiplication on inadequately cleaned food-contact surfaces.

Table 6:Total fungal count of samples (Washed with sterile water)

Sample ID	Colonies Counted	Fungal Count (CFU/ml)
1	2	2.0×104
2	2	3.0×104
3	3	2.0×104
4	2	2.0×104
5	3	1.0×104
6	1	1.0×104
7	3	3.0×104
8	2	2.0×104
9	2	2.0×104
10	1	1.0×104
11	0	0.0×104
12	0	0.0×104
13	2	2.0×104
14	3	3.0×104
15	0	2.0×104
16	1	1.0×104
17	0	0.0×104
18	2	2.0×104
19	0	0.0×104
20	1	1.0×104

The fungal loads observed in this study are consistent with previous reports that recovered plastic bottles and reusable food containers frequently harbour fungal contaminants capable of causing food spoilage and reducing the microbiological quality of packaged foods. The findings therefore emphasize the importance of effective cleaning and disinfection before the reuse of plastic bottles intended for food packaging.

3.3.2 Total Fungal Count of Reusable Plastic Bottles Washed with Sterile Water

The total fungal counts obtained from reusable plastic bottles washed with sterile water are presented in Table 6.

As shown in Table 6, fungal counts in bottles washed with sterile water ranged from 0.0×10^4 CFU/mL to 3.0×10^4 CFU/mL. Although sterile water washing reduced fungal contamination in some bottles, fungal growth was still detected in several samples, indicating that rinsing alone was insufficient to completely eliminate fungal propagules attached to the bottle surfaces.

The persistence of fungal contamination after sterile water washing may be attributed to the ability of fungal spores to adhere strongly to plastic surfaces and survive under adverse environmental conditions. Unlike vegetative bacterial cells, fungal spores possess protective structural components that confer resistance to desiccation and mechanical removal. Consequently, simple rinsing with water may remove loose contaminants but is generally ineffective against firmly attached fungal spores and developing biofilms.

Table 7: Morphology and microscopic characteristics of fungal isolates

Characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Surface pigmentation	Gray	Green with white border	Brown	Yellow
Colony appearance	Moist and shiny	Velvety	Powdery	Cottony
Reverse side pigmentation	Black	White	Black	Creamy
Microscopy	Septate hyphae with heavy clusters of oval spores along the hyphae; spores mainly budding cells	Septate hyphae with long, erect, multi-branched conidiophores; flask-shaped phialides bearing round conidia in chains, forming a brush-like appearance	Aseptate stalk-like conidiophores expanding into a dome-shaped swollen vesicle bearing round and globose conidia	Pseudohyphae surrounded by oval yeast cells
Probable organism	*Cladosporium* sp.	*Penicillium* sp.	*Aspergillus* sp.	*Candida* s

The present findings indicate that washing with sterile water alone provides only limited protection against fungal contamination and therefore cannot be recommended as an effective decontamination procedure for reusable plastic bottles used in food packaging.

3.3.3 Effect of Hot Soapy Water and 3% Hydrogen Peroxide on Fungal Contamination

The effects of hot soapy water and 3% hydrogen peroxide on fungal contamination were also evaluated during this study. Following microbiological analysis, no fungal growth was detected in bottles washed with either hot soapy water or 3% hydrogen peroxide.

The complete absence of fungal growth following these treatments demonstrates that both washing methods were highly effective in controlling fungal contamination. The elevated temperature of the hot soapy water, together with the detergent action, likely facilitated the removal of fungal spores and residual organic matter from the bottle surfaces. In contrast, hydrogen peroxide not only removed fungal cells but also exerted a fungicidal effect through oxidative damage to essential cellular components.

Hydrogen peroxide generates reactive oxygen species capable of oxidizing proteins, membrane lipids and nucleic acids, thereby causing irreversible cellular damage and death. This mechanism explains the complete elimination of fungal growth observed after treatment with 3% hydrogen peroxide. Similar observations have been reported by Juven and Pierson (1996), who demonstrated the broad-spectrum antimicrobial activity of hydrogen peroxide against bacteria, yeasts and filamentous fungi. Likewise, Hooker et al. (2020) reported that hydrogen peroxide-based disinfectants effectively reduced microbial contamination on reusable healthcare surfaces

by destroying both vegetative cells and resistant microbial forms. The present findings therefore demonstrate that hot soapy water and particularly 3% hydrogen peroxide are considerably more effective than sterile water in preventing fungal contamination of reusable plastic bottles.

3.3.4 Morphological and Microscopic Identification of Fungal Isolates

The cultural, morphological and microscopic characteristics of the fungal isolates recovered from the reusable plastic bottles are presented in Table 7.

Based on their colonial morphology and microscopic characteristics following Lactophenol Cotton Blue staining, the fungal isolates were identified as *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Candida* spp. These fungal genera are commonly associated with environmental contamination and food spoilage and have frequently been isolated from food-contact surfaces and improperly stored food products.

Aspergillus spp. were characterized by septate hyphae bearing conidiophores terminating in swollen vesicles with globose conidia. *Penicillium* spp. exhibited characteristic brush-like conidiophores with flask-shaped phialides, while *Cladosporium* spp. produced pigmented septate hyphae with chains of conidia. *Candida* spp. were identified by the presence of oval budding yeast cells and pseudohyphae.

The recovery of these fungal genera indicates environmental exposure of the bottles during collection, storage and reuse. The presence of *Aspergillus* and *Penicillium* species is of particular concern because certain species within these genera are capable of producing mycotoxins that may contaminate food products and pose significant health risks to consumers. Similarly, *Candida* species are opportunistic pathogens that may cause infections in immunocompromised individuals, while *Cladosporium* species are known allergens and spoilage organisms.

3.3.5 Frequency of Occurrence of Fungal Isolates

The percentage frequency of occurrence of fungal isolates recovered from the unwashed and sterile water-washed reusable plastic bottles is presented in Figs. 5 and 6, respectively.

Fig. 5 shows that *Aspergillus* spp. were the predominant fungal isolates recovered from the unwashed bottles, accounting for 40% of the total fungal isolates. *Penicillium* spp. constituted 30%, while *Cladosporium* spp. and *Candida* spp. each accounted for 10% of the isolates. The predominance of *Aspergillus* spp. is not unexpected because members of this genus produce abundant airborne spores that readily contaminate food products and food-contact surfaces. Their ability to grow over a wide range of environmental conditions further contributes to their widespread distribution.

As presented in Fig. 6, *Aspergillus* spp. remained the predominant fungal isolate following washing with sterile water, accounting for 37.5% of the total fungal isolates. *Penicillium* spp. and *Cladosporium* spp. each represented 25%, whereas *Candida* spp. accounted for 12.5% of the isolates.

Although the relative distribution of fungal species changed slightly after sterile water washing, the persistence of these fungal genera indicates that rinsing with water alone was unable to eliminate fungal contamination. In contrast, no fungal isolates were recovered from bottles washed with hot soapy water or 3% hydrogen peroxide, confirming the superior effectiveness of these treatments in preventing fungal survival.

Overall, the fungal results complement the bacterial findings by demonstrating that reusable plastic bottles can serve as reservoirs for a wide range of fungal contaminants capable of compromising food quality and

consumer safety. The predominance of *Aspergillus* and *Penicillium* species further emphasizes the importance of effective bottle sanitation because these fungi are among the most significant spoilage organisms associated with stored foods. The complete elimination of fungal growth following treatment with hot soapy water and 3% hydrogen peroxide demonstrates that these washing methods are highly effective for reducing fungal contamination and improving the microbiological safety of reusable plastic bottles intended for food packaging.

3.4 Antibiotic Susceptibility Profile of Bacterial Isolates

The increasing occurrence of antibiotic-resistant bacteria in food and food-contact materials has become a major public health concern because contaminated foods may serve as vehicles for the transmission of resistant microorganisms to humans. To evaluate the potential health risk associated with the bacterial contaminants isolated from reusable plastic bottles, the antibiotic susceptibility patterns of the identified bacterial isolates were determined using the Kirby–Bauer disc diffusion method. The interpretation of inhibition zone diameters was based on the susceptibility criteria presented in Table 9, while the susceptibility profiles of the bacterial isolates are presented in Table 10.

3.4.1 Interpretation of Antibiotic Susceptibility Testing

The inhibition zones produced by each antibiotic against the bacterial isolates were interpreted according to the standard susceptibility criteria shown in Table 9.

The interpretation criteria classify bacterial isolates as Sensitive (S), Intermediate (I) or Resistant (R) based on the diameter of the inhibition zone surrounding each antibiotic disc. Isolates classified as sensitive are expected to respond to treatment with the corresponding antibiotic at recommended therapeutic doses, whereas resistant isolates are unlikely to be effectively treated. Intermediate isolates exhibit reduced susceptibility and may require higher drug concentrations or may respond only under specific clinical conditions. These criteria provide a standardized approach for evaluating the effectiveness of antimicrobial agents against the bacterial isolates recovered from reusable plastic bottles.

3.4.2 Antibiotic Susceptibility Pattern of the Bacterial Isolates

The antibiotic susceptibility profiles of the bacterial isolates recovered from reusable plastic bottles are presented in Table 10.

The results presented in Table 10 show considerable variation in the susceptibility of the bacterial isolates to the antibiotics tested. Overall, the isolates demonstrated varying degrees of sensitivity, intermediate susceptibility and resistance, reflecting differences in their intrinsic resistance mechanisms and previous exposure to antimicrobial agents.

Among the Gram-positive isolates, *Staphylococcus aureus* exhibited high susceptibility to ciprofloxacin (CPX), streptomycin (S), gentamicin (CN), levofloxacin (LEV), ampiclox (APX), rifampicin (RD), erythromycin (E) and chloramphenicol (CH). However, resistance was observed against amoxicillin (AMX), indicating that this antibiotic may be less effective in treating infections caused by the isolate recovered during this study. Resistance of *Staphylococcus aureus* to β-lactam antibiotics has been widely reported and is frequently associated with the production of β-lactamase enzymes or alterations in penicillin-binding proteins that reduce antibiotic efficacy.

Similarly, *Streptococcus* spp. demonstrated broad susceptibility to nearly all the antibiotics evaluated, including ciprofloxacin, gentamicin, streptomycin, erythromycin, chloramphenicol, ampiclox and levofloxacin. The high

susceptibility observed suggests that these isolates have not yet acquired extensive antimicrobial resistance and therefore remain susceptible to commonly prescribed antibiotics.

The *Bacillus* spp. isolate exhibited susceptibility primarily to ciprofloxacin, streptomycin and selected fluoroquinolones, whereas reduced susceptibility or absence of activity was observed against gentamicin, ampiclox, rifampicin, chloramphenicol and amoxicillin. The relatively narrow susceptibility profile may reflect the inherent physiological characteristics of the isolate rather than acquired resistance, since many environmental *Bacillus* species naturally exhibit variable responses to antimicrobial agents.

Table 9: Interpretation Criteria for Antibiotic Susceptibility Testing of Bacterial Isolates

Antibiotic	Abbreviation	Disc Potency (µg)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Ciprofloxacin	CPX	10	>21	16–20	<15
Norfloxacin	NB	10	>18	14–17	<13
Gentamicin	CN	10	>17	13–17	<12
Amoxil (Amoxicillin)	AMX	30	>18	14–16	<13
Streptomycin	S	30	>21	15–17	<14
Rifampicin	RD	20	>18	14–22	<13
Erythromycin	E	10	>23	14–17	<13
Chloramphenicol	CH	10	>18	13–17	<12
Ampiclox	APX	30	>18	14–17	<13
Levofloxacin	LEV	30	>18	14–18	<13
Ceporex	CEP	10	>19	14–15	<13
Tarivid (Ofloxacin)	OFX	20	>16	13–15	<12
Nalidixic acid	NA	20	>19	14–18	<13
Reflacin (Pefloxacin)	PEF	20	>18	14–17	<13
Augmentin	AU	10	>19	14–18	<13
Septrin	SXT	30	>17	14–16	<13
Ampicillin	PN	10	>19	14–18	<13

Key: Susceptibility was interpreted based on the diameter of the inhibition zone around each antibiotic disc. Values are expressed in millimetres (mm). S = Sensitive; I = Intermediate; R = Resistant.

spp. demonstrated intermediate susceptibility to ciprofloxacin, ofloxacin and pefloxacin, while little or no activity was observed for several other antibiotics. This finding is consistent with the increasing prevalence of multidrug-resistant *Klebsiella* species reported in both clinical and environmental settings. The reduced susceptibility of *Klebsiella* spp. may result from the production of extended-spectrum β-lactamases (ESBLs), alterations in membrane permeability and the presence of multidrug efflux pumps that reduce intracellular antibiotic concentrations.

The *Pseudomonas aeruginosa* isolate showed susceptibility to ciprofloxacin, gentamicin, norfloxacin, ofloxacin, cefepime (or cephalexin) and augmentin, but resistance to streptomycin and several other antibiotics. This

pattern agrees with the well-documented intrinsic resistance of *Pseudomonas aeruginosa*, which possesses multiple resistance mechanisms including low outer membrane permeability, efflux pumps and antibiotic-degrading enzymes. Consequently, infections caused by *Pseudomonas aeruginosa* often require treatment with carefully selected antibiotics based on susceptibility testing

Table 10: Antibiotic Susceptibility Pattern of Bacterial Isolates

Antibiotic	Staphylococcus sp.	Streptococcus sp.	Bacillus sp.	Klebsiella sp.	Pseudomonas sp.
CPX (Ciprofloxacin)	S	S	S	I	–
NB (Norfloxacin)	S	S	–	I	S
CN (Gentamicin)	S	S	–	I	S
AMX (Amoxicillin)	R	S	–	–	–
S (Streptomycin)	S	S	S	–	R
RD	S	S	–	–	–
E (Erythromycin)	S	S	–	–	–
CH (Chloramphenicol)	S	S	–	–	–
APX	S	S	–	S	–
LEV (Levofloxacin)	S	S	–	S	–
OFX (Ofloxacin)	—	—	—	I	S
PEF (Pefloxacin)	—	—	—	I	S
PN	—	—	—	–	R
AU	—	—	—	–	–
CEP (Cefepime/Cephalexin*)	—	—	—	–	S
SXT (Trimethoprim–Sulfamethoxazole)	—	—	—	–	–

Key: S = Sensitive; R = Resistant; I = Intermediate; – = No activity/Not tested; — = Data not provided in the original table.

Among the Gram-negative bacteria, *Klebsiella*

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3.4.3 Discussion of Antibiotic Susceptibility Profiles

The antibiotic susceptibility results demonstrate that although several bacterial isolates remained susceptible to commonly used antibiotics, evidence of resistance was also observed among some of the organisms. The coexistence of susceptible and resistant bacteria on reusable plastic bottles has important public health implications because contaminated food containers may serve as reservoirs for antimicrobial-resistant bacteria that can be transferred to food products and ultimately to consumers.

The excellent activity of ciprofloxacin against almost all bacterial isolates confirms the broad-spectrum efficacy of fluoroquinolone antibiotics against both Gram-positive and Gram-negative organisms. Ciprofloxacin inhibits bacterial DNA gyrase and topoisomerase IV, thereby preventing DNA replication and cell division. Similar high susceptibility of foodborne bacterial isolates to ciprofloxacin has been reported by numerous investigators, making it one of the most effective antibiotics for treating bacterial gastroenteritis and other foodborne infections.

Likewise, gentamicin, levofloxacin, streptomycin and ampiclox demonstrated good antibacterial activity against most of the Gram-positive isolates recovered in this study. These antibiotics interfere with bacterial protein synthesis or cell wall formation and have remained effective against many environmental bacterial isolates despite the increasing prevalence of antimicrobial resistance.

Conversely, the reduced susceptibility of *Klebsiella* spp. and *Pseudomonas aeruginosa* to several antibiotics reflects the growing global challenge of antimicrobial resistance among opportunistic pathogens. Members of these genera possess sophisticated resistance mechanisms that enable them to survive exposure to multiple classes of antibiotics. Their presence on reusable food packaging materials therefore represents an additional public health concern, particularly for immunocompromised individuals, children and the elderly.

The isolation of potentially antibiotic-resistant bacteria from reusable plastic bottles emphasizes the need for improved hygiene practices during bottle collection, washing and reuse. Effective decontamination procedures not only reduce microbial loads but may also limit the dissemination of antibiotic-resistant bacteria through the food chain. Similar observations have been reported by the World Health Organization (WHO, 2023), which identified foodborne transmission as one of the important pathways contributing to the spread of antimicrobial-resistant microorganisms worldwide.

Overall, the antibiotic susceptibility profiles indicate that ciprofloxacin, gentamicin, streptomycin, levofloxacin, ampiclox, rifampicin and augmentin were among the most effective antimicrobial agents against the bacterial isolates recovered from reusable plastic bottles in this study. However, the observed resistance among some isolates underscores the importance of performing antibiotic susceptibility testing before initiating antimicrobial therapy. From a food safety perspective, prevention of contamination through proper sanitation of reusable plastic bottles remains a more effective strategy than relying on antibiotic treatment after infection has occurred.

Summary of Findings

The microbiological assessment conducted in this study demonstrated that reusable plastic bottles used for packaging food products within Ikot Ekpene Metropolis were extensively contaminated with bacteria and fungi. Washing with sterile water reduced microbial loads only marginally, whereas hot soapy water produced greater reductions. Treatment with 3% hydrogen peroxide proved to be the most effective decontamination method, substantially reducing bacterial populations and completely eliminating fungal growth. The identification of foodborne and opportunistic pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Streptococcus* spp., *Bacillus* spp., *Aspergillus* spp. and *Penicillium* spp., highlights the significant health risks associated with the reuse of inadequately sanitized plastic bottles. Although many of the bacterial isolates remained susceptible to commonly used antibiotics, the detection of resistant organisms further underscores the necessity for improved hygiene practices and effective disinfection protocols to safeguard public health.

4.0 Conclusion

The reuse of recovered plastic bottles for direct food packaging in Nigeria presents clear health risks due to widespread microbial contamination. This study isolated several potentially pathogenic bacteria and fungi from the bottles, with contamination being most prevalent in unwashed samples.

The distribution of microbial species shifted with different washing treatments, indicating

that residual contaminants and biofilms can persist on bottle surfaces collected from waste environments. Among the washing methods tested, 3% hydrogen peroxide was the most effective at reducing microbial growth, including complete elimination of fungi, followed by hot soapy water and sterile water. Antibiotic susceptibility testing further revealed varied resistance patterns among the bacterial isolates, highlighting concerns for treatment of possible infections linked to these contaminants. Therefore, the findings support the need to discourage the use of scavenged plastic bottles for food packaging. Effective decontamination is essential where reuse occurs, and stronger public health regulations and consumer awareness are required to prevent foodborne disease transmission associated with this common practice

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Compliance with Ethical Standards

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Availability of Data and Materials

The datasets generated and analysed during this study are available from the corresponding author upon reasonable request.

Author Contributions

Eteyen Anthony Uko conceived and designed the study, supervised laboratory investigations, interpreted the results, and drafted the manuscript. Imaobong Timothy Adenugba carried out sample collection, microbiological and antibiotic susceptibility analyses, validated the data, contributed to statistical evaluation, critically revised the manuscript, and approved the final version for publication.