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# Microbial Assessment of Surfaces of Canned Drinks Sold Within Federal University of Technology, Owerri, Imo State, Nigeria and Its Associated Health Implications

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## Abstract

**Background and Objective:** Over the years, numerous studies have been conducted on the possible links between canned drink intake and medical problems. The qualitative assessment of microorganisms on the surfaces of canned drinks sold within FUTO environment and its health implications were evaluated in this study.

**Materials and Methods:** A total of twenty canned drink (alcoholic and non-alcoholic brands) were collected from the refrigerator and the packs of retailer's shop. Standard microbiological methods were adopted using Gram staining, biochemical tests and lacto phenol cotton blue staining.

**Results:** The canned drinks from refrigerator showed total viable bacterial count which ranged from 2.0 × 10 1 cfu to 1.4× 102 cfu while total fungal count ranged from 2.0× 10 1 cfu to 4.0 × 10 1 cfu were recorded. There was no coliform and total Staphylococci count recorded. The canned drinks from the packs showed total viable bacterial count ranged from 2.0× 10 1 cfu to 4.0 × 10 2 cfu, total coliform count recorded ranged from 1.0× 10 1 cfu to 4.0 × 10 1 cfu, while total Staphylococci count ranged from 1.0× 10 1 cfu to 4.0 × 10 1 cfu. Total fungal count ranged from 2.0× 10 1 cfu to 8.0 × 10 1 cfu. Bacillus species had the highest bacterial occurrence while Aspergillus species had the highest fungi occurrence (40.7%). Corynebacterium, Pseudomonas, Bacillus, Klebsiella were resistant to Norfloxacin while Micrococcus species were resistant to chloramphenicol, Norfloxacin and streptomycin. Penicillium and Aspergillus species were resistant to both ketonazole and fluconazole antifungal drugs. The implications of antibiotic resistance on healthcare systems are enormous as resistance leads to limitation of treatment options. Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality.

**Conclusion:** This research demonstrated that no relationship exists between the physical appearance of canned drink sold at FUTO and its microbial load. Some of the isolated microbes were of public health importance. There is need to ensure proper washing of the surfaces of canned drinks before refrigeration and consumption.

**Keywords:** Canned drinks; microorganism; health implications

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## Introduction

A drink can (or beverage can) is a metal container designed to hold a fixed portion of liquid such as carbonated soft drinks, alcoholic drinks, fruit juices, teas, herbal teas, energy drinks, etc.1. Drink cans are made of aluminum (75% of worldwide production) or tin-plated steel **[1].** The spread of infectious diseases was influenced by various steps in human civilization such as canning of drinks. Cans are exposed to various environments during production, storage and shipping during which the lid may be contaminated with microorganisms. Beverages in aluminum cans are widely available, and for convenience, drinks are often consumed directly from the can. When drinking from a can, one's mouth comes in direct contact with the can lid allowing possible transfer of microorganisms. Infectious diseases are the major cause of human suffering in terms of both morbidity and mortality throughout human history. Of the approximately 53 million deaths worldwide in 2009, at least a third was due to infectious

 

diseases2. Infection can be defined as the multiplication of microbes (from viruses to multicellular parasites) in the tissues of the host2. The host may or may not be symptomatic, for example Clostridium botulinum may grow in certain improperly processed foods and produce a toxin that can be lethal on ingestion2. Microbial diseases are sicknesses or ailments caused in animals and humans by the introduction of one of four different types of microbes; Bacteria, Viruses, Fungi and Protozoa (also known as protoctista). Microbes are far better at adapting to new environments than human3. Poor food preparation or preservative methods can permit microbes to grow in food and go on to infect a person. Escherichia coli bacteria at times persist in food products such as unpasteurized fruit juice or undercooked meat. The bacteria infection may have fatal consequences in people who are vulnerable, particularly children and the elderly. Each year, millions of people around the world become ill from eating foods or taking drinks that are contaminated3. Microbes can cause these illnesses, some of which may be fatal if they are not treated appropriately3. A number of microbes are developing new properties to resist drug treatments that were once effective at destroying them. Drug resistance has become a serious issue around the world today. Micro-organisms may be classified in the following large biological groups: Algae, Protozoa, and Slime moulds, Fungi, Bacteria, Archaea and Viruses4. They can survive under all types of environment, ranging from ice cold climate to hot springs and deserts to marshy lands **[2-4].**

To cause an infection, microbes must enter the body. The site at which they enter is known as the portal of entry. Microbes can enter the body through any of these four sites;

- Respiratory tract (mouth and nose) e.g. influenza virus which causes the flu.
- Gastrointestinal tract (mouth oral cavity) e.g. Vibrio cholerae which causes cholera.
- Urogenital tract e.g. Escherichia coli which causes cystitis.
- Breaks in the skin surface e.g. Clostridium tetani which causes tetanus.

Jace tested soda cans for bacteria, and what he found was pretty gross **[5].** The cans tested came from a variety of places, including grocery stores, convenience stores, vending machines and cans stored in a home. It was discovered that all but one of the cans tested had mold on it. The highest mold count was 600 colonies of mold. That can were from a grocery store. Although microbiologist, Helene Ver Eecke with the Metropolitan State University of Denver, argued that the 600 colonies of mold isn't really a cause for concern, yet this type of exposure could make people with compromised immune systems or lung disease sick.

In another study conducted to detect and Isolate Bacteria from the surfaces of canned drinks Sold in Ugbor, Benin City, it was shown that surfaces of canned drinks tested were contaminated irrespective of whether they were kept in the refrigerator or not **[6].** The isolated organisms were identified to be S. aureus, P. aeruginosa, Enterococcus sp., Escherichia, and members of the spore forming Bacillus genus. It has been reported that some of these identified pathogens can survive on hands, sponges and surfaces of stainless steel materials for several days and weeks after contact. The research further revealed that the isolated organisms were of public health importance and that they have been shown to be multi-resistant pathogens. None of the isolated pathogens was within the safe range of 0.2. FDA7 also reported the isolation of certain microorganisms from refrigerators.

Study conducted on both food and beverage cans revealed that there was no correlation between the visual appearance of cleanliness on the tops of aluminum cans and the level of microbial contamination8. The study also reported that an effective way to clean the surface on the can lids was to rinse the can top and then wipe it with either a paper towel or a napkin. A study conducted by Dawson et al. **[7]** used ATP- bioluminescence (ATP-B) swabs to test the levels of contamination on the surface of aluminum can lids, as the presence of ATP can indicate the presence of bacteria. Out of the 194 randomly selected cans, 90 (46.39%) had RLU readings of greater than 30 which is in the dangerous unsanitary category, 60 (30.93%) had RLU between 10 and 30 which were considered cautionary and 44 (22.68%) had a RLU of <10 and were categorized as clean. Another study was carried out by Ezemba et al.10 to isolate and identify microorganisms from the surfaces of canned and bottled drinks. Out of 64 sample cans not washed, 28 samples were found to contain Leptospira spp, by the formation of circular haze or disc formation known as Dinger's ring. Other pathogenic bacteria were also isolated and identified; Coliform bacteria such as E. coli, Klebsiella, and Enterobacter were seen to have this percentage of occurrence 71.8%, 43.6% and 30.9% respectively, while pathogens like Salmonella and Shigella species have their percentage of occurrence to be 39.4% and 22.3% respectively **[10].**

This study was carried out in a school environment. The findings will help to curb some of the diseases the students suffer from that might have been contracted from microbes in canned drink surfaces.

# Methodology

## Study design

Descriptive design study was used for this study where a total of twenty (20) canned drink comprising (both alcoholic and non-alcoholic brands) were sampled. Study area: The study was carried out in Federal University of technology Owerri (FUTO), Imo State Nigeria.

## Data Collection

Samples of canned drinks were randomly collected at shops within FUTO environment. For every store or shops, two samples were obtained; each from the refrigerator and the packs. Sample collections were from ten (10) shops randomly selected within the school premises. Sterile Amie's swab sticks, aseptically soaked with tryptone soya broth (Oxoid) were used to swab the upper surfaces of canned drinks which come in direct contact with the mouth (with an approximate 10cm2 area). The swab sticks were properly labeled, aseptically packaged and taken to the laboratory for analysis **[11].**

## Sterilization of Glassware's and Media

The media used were; nutrient agar, eosin methylene blue agar, sabouraud dextrose agar, peptone water, triple sugar iron agar, Simmon's citrate agar, Mannitol salt agar and Mueller-Hinton agar. Nutrient served as general purpose media for the isolation of different types of aerobic bacteria. Eosin methylene blue agar served for the isolation of coliform bacteria while sabouraud dextrose agar was used in the isolation of fungi (yeasts and molds). Triple sugar iron agar was used for the identification of sugar fermentation test, acid production, gas production and hydrogen sulphide production. Peptone water was used together with Kovac's reagent for identification of indole producing bacteria while Simmon's citrate agar was in the identification of citrate utilizing bacteria. Mannitol salt agar was used for the isolation of Staphylococcus species. All the glass wares used were sterilized using laboratory hot air oven at temperature of 1600C for 1 hour and the media used was sterilized using the autoclave at a temperature of 121oC at 15psi for 15 minutes. After the sterilization, the media were brought out together with the glassware and kept on a clean laboratory bench. The media were poured into the Petri-dishes when cooled to 45oC and were allowed to solidify. This method was adopted from the work of Cheese brough **[12].**

## Culture technique

The swab sticks were introduced into a test-tube containing sterile nutrient broth and properly labeled. Each of the test-tubes were shaken and loosely capped. All the samples were prepared using the same procedure. Thereafter, each sample was streaked onto Nutrient agar, MacConkey agar, Eosin methylene blue agar and Mannitol salt agar plates successively **[13].**

## Incubation of Cultured Plates and Microbial Plate Count

Bacteria plates were incubated for 24 hours at 37ºC for bacterial growth while fungi plates were incubated for 3-7 days at 28ºC. After incubation, the colonies that developed on the plates were counted using hand tally and magnifying lens. The numbers of colonies counted were expressed as colony forming unit (cfu).

## Colonial Morphology Identification

The method described by Cheesbrough 11 was adopted in the colonial morphology identification. Presumptive identification of the colonies were done by observing their individual shape, colour, elevation, edge, surface, consistency and appearance on the media used for isolation.

## Purification and Preservation of Isolates

After the Colony counts, bacterial isolates were picked with a wire loop based on their cultural and morphological characteristics. The picked colonies were sub-cultured onto freshly prepared nutrient agar plates to obtain pure cultures. They were further incubated for 24hrs at 37°C. After incubation, pure cultures were stored in McCartney Bottle slants and refrigerator **[14].**

## Gram Staining of Isolates

A smear of the colony from pure culture was made on a clean grease-free glass slides to be stained. The smears were allowed to air dry and later heat fixed. Crystal violet was added to the slide and allowed for 1 minute. The slide was rinsed with a gentle stream of water for a maximum of 5 seconds. Lugol's iodine was added for 1 minute before the slide was rinsed again with water. The slide was rinsed with acid alcohol for 3 seconds and with water. The secondary stain, safranin, was added to the slide and allowed for 1 minute. The slide was rinsed with gentle stream of water for a maximum of 5 seconds. The stained slides were allowed to air dry and were viewed under a microscope using x40 and x100 objective lenses. Gram positive bacteria retained the primary stain (Crystal violet) and appear purple under the microscope. Gram negative, lost the primary stain and take the secondary stain, causing it to appear pink when viewed under a microscope.

## Biochemical Test

Biochemical test was carried out to test the motility test, catalase test, coagulase test, indole test, oxidase test, sugar fermentation test and citrate utilization test.

Statistical Analysis: The obtained data in this research were exposed to version 21.0 of SPSS statistical package. Descriptive statistics were employed to both determine the level of contamination as well as the susceptibility profile of obtained isolates.

# Results

## Microbial plate count of the surfaces of the canned drinks

Table 1 showed the results of the microbial plate count from the surfaces of the canned drinks sold within Federal University of Technology, Owerri. All the samples showed total viable bacterial count. Out of twenty samples used four **[4]** had coliform and Staphylococci counts while fourteen **[14]** had fungal count. For the canned drinks from refrigerator, total viable bacterial count ranged from 2.0 x 101 cfu to 1.4 x 102 cfu. There was no coliform count and total Staphylococci count recorded. Total fungal count ranged from 2.0 x 101 cfu to 4.0 x101 cfu. For the canned drinks from the packs, total viable bacterial count ranged from 2.0 x 101 cfu to 4.2 x 102 cfu. Total coliform count recorded ranged from 1.0 x 101 cfu to 4.0 x 101 cfu while total Staphylococci count ranged from 1.0 x 101 cfu to 4.0 x 101 cfu. Total fungal count ranged from 2.0 x 101 cfu to 8.0 x101 cfu **(Table 1).**

Key: NG = No growth

TVBC = Total viable bacterial count

TCC = Total coliform count

TFC = Total fungal count

TSC = Total Staphylococci count

Cfu = Colony forming unit

A – E = canned drink samples from different vendors

**Table 1:** Microbial plate count of the surfaces of the canned drinks.

| Samples/ Vendors | TBVC (cfu) | TSC (cfu) | TCC (cfu) | TBVC (cfu) |
|---|---|---|---|---|
| **Non-alcoholic** (From Refrigerator) | | | | |
| A | $2.0 \times 10^2$ | NG | NG | NG |
| B | $6.0 \times 10^1$ | NG | NG | NG |
| C | $1.2 \times 10^2$ | NG | NG | NG |
| D | $8.0 \times 10^1$ | NG | NG | $3.0 \times 10^1$ |
| E | $1.0 \times 10^2$ | NG | NG | NG |
| **Non-alcoholic** (From Packs) | | | | |
| A | $2.6 \times 10^2$ | $1.0 \times 10^1$ | NG | |
| B | $4.2 \times 10^1$ | NG | $3.0 \times 10^1$ | |
| C | $1.8 \times 10^2$ | NG | NG | $6.0 \times 10^1$ |
| D | $1.6 \times 10^2$ | $3.0 \times 10^1$ | NG | |
| E | $2.0 \times 10^2$ | NG | $4.0 \times 10^1$ | |
| **Alcoholic** (From Refrigerator) | | | | |
| A | $1.4 \times 10^2$ | NG | NG | $4.0 \times 10^1$ |
| B | $4.0 \times 10^1$ | NG | NG | $3.0 \times 10^1$ |
| C | $1.4 \times 10^2$ | NG | NG | NG |
| D | $6.0 \times 10^1$ | NG | NG | $2.0 \times 10^1$ |
| E | $4.0 \times 10^1$ | NG | NG | NG |
| **Alcoholic** (From Packs) | | | | |
| A | $6.0 \times 10^2$ | NG | NG | $2.0 \times 10^1$ |
| B | $1.0 \times 10^2$ | $2.0 \times 10^1$ | NG | |
| C | $1.4 \times 10^2$ | NG | $1.0 \times 10^1$ | |
| D | $2.4 \times 10^2$ | NG | $2.0 \times 10^1$ | |
| E | $8.0 \times 10^1$ | $4.0 \times 10^1$ | | |

## Cultural morphology, Gram staining and biochemical characterization of the bacterial isolates from the surfaces of the canned drinks

The cultural morphology, Gram staining and biochemical characteristics of the bacterial isolates from the canned drinks were shown. The colonies were characterized culturally on mannitol salt agar, eosin methylene blue agar and nutrient agar. Gram staining was carried out using Gram staining stains; crystal violet, Lugol's iodine, acetone and safranin. The biochemical tests carried out were; catalase, coagulase, indole, motility, citrate utilization, sugar fermentation and oxidase tests. A total of seven **[7]** bacterial isolates, comprising four **[4]** Gram positive and three **[3]** Gram negative bacteria were isolated. The Gram positive bacteria were; *Bacillus, Staphylococcus,* Corynebacterium and Micrococcus species while Gram negative bacteria were; *Pseudomonas,* Klebsiella and Proteus species.

## Frequency and percentage occurrence of the bacterial isolates from the surfaces of the canned drinks

From table 2 below, Bacillus species had the highest bacterial occurrence (27.0%) followed by *Pseudomonas* species (18.9%) while *Proteus* species had the least occurrence (8.1%). Surfaces of non-alcoholic canned drinks collected from the packs had the highest bacterial occurrence (70%). Bacillus species were isolated from all the surfaces of both alcoholic and non-alcoholic packs of the canned drinks. *Proteus* species were isolated from three 3 out of twenty 20 samples used **(Table 2). [15].**

**Key:** % = Percentage occurrence

+ = Presence of bacteria

- = Absence of bacteria

AF = Surfaces of alcoholic canned drinks from the refrigerator

AP = Surfaces of alcoholic canned drinks from the packs

NAF = Surfaces of non-alcoholic canned drinks from the refrigerator

NAP = Surfaces of non-alcoholic canned drinks from the packs

## Cultural morphological and microscopic characteristics of the fungal isolates from the surfaces of the canned drinks

A total of four **[4]** fungal isolates were isolated. They were; Mucor, Aspergillus, Penicillium and Rhizopus species **(Table 3).**

## Frequency and percentage occurrence of the fungal isolates from the surfaces of the canned drinks

From the study, Aspergillus species had the highest fungi occurrence (40.7%) followed by Mucor species (25.9%) while Penicillium species was the least occurring fungi 3(11.1%).

**Table 2.** Frequency occurrence of the bacterial isolates from the surfaces of the canned drinks.

| Bacteria Species | AF | AP | NAF | NAP | Total | Percentage |
|---|---|---|---|---|---|---|
| Micrococcus species | 0 | 2 | 0 | 3 | 5 | 13.5 |
| Corynebacterium species | 1 | 1 | 0 | 2 | 4 | 10.8 |
| Pseudomonas species | 2 | 2 | 2 | 1 | 7 | 18.9 |
| Proteus species | 0 | 1 | 0 | 2 | 3 | 8.1 |
| Staphylococcus species | 0 | 2 | 0 | 2 | 4 | 10.8 |
| Bacillus species | 0 | 5 | 0 | 5 | 10 | 27 |
| Klebsiella species | 0 | 2 | 0 | 2 | 4 | 10.8 |
| **Total** | 3 | **15** | **2** | **17** | **37** | **100** |

**Table 3.** Identification and characterization of the fungal isolates from the surfaces of the canned drinks.

| Cultural Morphology | Microscopy | Possible fungi |
|---|---|---|
| Green brown and white surface light yellow base and conidia and short aerial mycelium | Filamentous, septate conidia | Penicillium species |
| Whitish, fluffy raised colonies that covered the plate | Non-septate hyphae with spores at one end | Rhizopus species |
| Whitish, fluffy, enlarged colonies with grey powdery surface | Non-branched hyphae | Mucor species |
| Dark-brown, enlarged, circular smooth-edged powdery colonies with light yellow reverse | Septate globose hyphae | Aspergillus species |

Surfaces of non-alcoholic canned drinks collected from the packs had the highest fungi occurrence **[11]** followed by that of alcoholic drinks collected from the packs **[9].** Surfaces of alcoholic canned drinks collected from the refrigerator had the least fungi occurrence **[2] (Table 4).**

**Key:** % = Percentage occurrence

+ = Presence of fungi

- = Absence of fungi

AF = Surfaces of alcoholic canned drinks from the refrigerator

AP = Surfaces of alcoholic canned drinks from the packs

NAF = Surfaces of non-alcoholic canned drinks from the refrigerator

NAP = Surfaces of non-alcoholic canned drinks from the packs

## Health Implications of the Bacteria Isolated from the Surfaces of the Canned Drinks

The health implications of the bacterial isolates were determined through the susceptibility of the bacterial isolates to commercial antibiotics. The zones of inhibition recorded with the antibiotics against the Gram positive bacterial isolates ranged from 14mm to 30mm. Levofloxacin antibiotics was the most effective antibiotics against the bacterial isolates. Staphylococcus species was the most susceptible bacterial isolate compared to the other isolates. Norfloxacin showed no zones of inhibition against *Corynebacterium, Pseudomonas,* Bacillus, and Klebsiella while Micrococcus species was resistant to chloramphenicol, Norfloxacin and streptomycin **(Table 5).**

Key: mm = millimetre

- = No inhibition

**Table 4.** Frequency occurrence of the fungal isolates from the surfaces of the canned drinks.

| Fungal Species | AF | AP | NAF | NAP | Total | Percentage |
|---|---|---|---|---|---|---|
| Aspergillus species | 3 | 3 | 1 | 4 | 11 | 40.7 |
| Penicillium species | 0 | 1 | 0 | 2 | 3 | 11.1 |
| Mucor species | 1 | 3 | 0 | 3 | 7 | 25.9 |
| Rhizopus species | 1 | 2 | 1 | 2 | 6 | 22.2 |
| **Total** | **5** | **9** | **2** | **11** | **27** | **100.0** |

**Table 5.** Health Implications of the Bacteria Isolated from the Surfaces of the Canned Drinks.

| Organisms | Zones of inhibition (mm)/Antibiotics used | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NB | CH | CPX | E | LEV | CN | RD | AMX | S | APL |
| **Staphylococcus species** | 26 | 28 | 28 | 28 | 30 | 28 | 28 | 28 | 24 | 26 |
| **Klebsiella species** | 14 | 18 | 15 | 26 | 25 | 18 | 26 | 18 | 24 | 20 |
| **Bacillus species** | 14 | 18 | 15 | 24 | 25 | 18 | 26 | 18 | 24 | 20 |
| **Corynebacterium species** | - | 30 | 25 | 30 | 30 | 28 | 25 | 18 | 18 | 20 |
| **Pseudomonas species** | - | 30 | 25 | 30 | 30 | 28 | 25 | 18 | 18 | 20 |
| **Proteus species** | - | 30 | 25 | 30 | 30 | 28 | 25 | 18 | 18 | 20 |
| **Micrococcus species** | - | - | 15 | 28 | 22 | 22 | 20 | 20 | - | 10 |

mcg = microgram

CLSI standard = Clinical laboratory standard institute

R= Resistant (0-12mm)

I= Intermediate (12-16mm)

S= Susceptible (16mm and above)

NB = Norfloxacin (10mcg) CH = Chloramphenicol (30mcg)

CPX = Ciproflax (10mcg) E = Erythromycin (30mcg)

LEV = Levofloxacin (20mcg) CN = Gentamycin (10mcg)

RD = Rifampicin (20mcg) AMX = Ampiclox (20mcg)

S = Streptomycin (30mcg) APL = Amoxil (20mcg)

### Health Implications of the Fungi Isolated from the Surfaces of the Canned Drinks

The health implications of the fungi isolates were determined through testing their susceptibility to antifungal drugs. The zones of inhibition recorded ranged from 10mm to 24mm with ketonazole and 12mm to 28mm with fluconazole. Penicillium and Aspergillus species were resistant to both ketonazole and fluconazole antifungal drugs **(Table 6).**

Key: mm = millimetre CLSI guidelines = Clinical laboratory standard institute

R = Resistant (0-12mm) S = Susceptible (16mm and above)

## Discussion

The findings of this study showed that surfaces of canned drinks can be readily contaminated irrespective of whether they are kept in the refrigerator or in packs. Table 1 showed that the cans from refrigerator had viable bacteria on it (2.0 x 101 cfu to 1.4 x 102 cfu) while the cans from pack had a total viable bacterial count ranged from 2.0 x 101 cfu to 4.2 x 102 cfu. This was consistent with the findings of Michaels et al.8 which noted that there was no correlation between the visual appearance of cleanliness on the tops of aluminum cans and the level of microbial contamination. The findings of Ogofure et al.,6 further supported this research finding. The researchers discovered that the surfaces of canned drinks tested were contaminated irrespective of whether they were kept in the refrigerator or not **[16].**

The frequency and percentage occurrence of the bacterial isolates from the surfaces of the canned drinks were analyzed and reported (Table 3). Bacillus species had the highest bacterial occurrence (27.0%) followed by Pseudomonas species (18.9%). Bacillus species were isolated from all the surfaces of both alcoholic and non-alcoholic packs of the canned drinks. Since the early 1970s, a number of reports have implicated Bacillus species other than B. cereus as the cause of foodborne disease, mainly involving species were B. subtilis group (B. subtilis, B. pumilus, and B. licheniformis) 12. Common symptoms for these foodborne illness incidents are vomiting and diarrhea and in some cases abdominal pain/cramps. Additional symptoms are headache, flushing, sweating, and dizziness. The duration of the disease is reported from 1.5 hours to 24 hours depending on the species involved12. Stomach cramps and diarrhea, which lasted for several days might developed later. Bacillus species produce endospores that have the ability to resist heat, cold, radiation, and chemical treatments13. The Gram positive bacteria isolated were; Bacillus, Staphylococcus, Corynebacterium and Micrococcus species. Gram negative bacteria were; Pseudomonas, Klebsiella and Proteus species. Similar bacterial isolates were reported by Ogofure et al. 6 from surfaces of canned drinks sold in Ugbor, Benin City. Their results reported the isolation of Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Bacillus species and Enterococcus species.

**Table 6.** Zones of Inhibition of Antifungal Drugs against the Fungal Isolates from the Surfaces of the Canned Drinks.

| Organism | Antifungal drugs/Zones of inhibition (mm) | |
|---|---|---|
| | Ketonazole | Fluconazole |
| Mucor species | 24 | 28 |
| Aspergillus species | 12 | 14 |
| Penicillium species | 10 | 12 |
| Rhizopus species | 18 | 24 |

These bacteria species on the surface of canned drinks sampled could get there through food products stored in the refrigerator or as a result of vendor's poor personal hygiene practices. The presence of P. aeruginosa could be attributed to improperly packed food sources (raw milk). This organism has been shown to grow inside the refrigerator due to incessant power outage and inconsistent power supply6. Moreover, most of these refrigerators were not adequately sanitized. Surface contamination observed is a clear reflection of the poor hygienic practices of the vendors as well as the surrounding environmental conditions which favour the survival and proliferation of the bacterial pathogens. Klebsiella species is widely or generally regarded as indicator of fecal contamination. This implied unhygienic practices among the retailers. This bacterium can be sourced from the nose and excreta. It might have been introduced into the surfaces of the canned drinks through nose drilling with hands of the retailers and subsequent touching of the surfaces of the cans.

Frequency and occurrence of the fungal isolates from the surfaces of the canned drinks showed that Aspergillus species had the highest fungi occurrence (40.7%) followed by Mucor species (25.9%) while Penicillium species was the least occurring fungi 3(11.1%). Surfaces of non-alcoholic canned drinks from the packs had the highest fungi occurrence (11) followed by that of alcoholic drinks collected from the packs (9). Diseases associated with Aspergillus species included invasive pulmonary aspergillosis, aspergilloma, and different forms of hypersensitivity diseases such as allergic asthma, hypersensitivity, pneumonitis, and allergic broncho pulmonary aspergillosis (ABPA) 14. There is considerable concern regarding the potential health outcomes of exposure to biological materials existing in the air. Molds constitute an important threat to human health; their effects range from moderate allergies and severe asthma to disseminated infections 15. Fungal counts recorded in this study could be as a result of the air quality of the environment where these canned drinks were displayed for sale in the shops. Fungi spores present in the air can be deposited on the surfaces of materials such as can drinks.

Some of these microbes can survive under all types of environment, ranging from ice cold climate to hot springs and deserts to marshy lands. It has been reported that some of these identified pathogens can survive on hands, sponges and surfaces of stainless steel materials for several days and weeks after contact 6.

The health implications of the bacterial isolates were determined by determining the susceptibility of the bacterial isolates to commercial antibiotics. Norfloxacin showed no zones of inhibition

against Corynebacterium, Pseudomonas, Bacillus, Klebsiella; while Micrococcus species were resistant to chloramphenicol, Norfloxacin and streptomycin. Resistant of bacteria to antibiotics is of public health significance. According to Chitanand et al.16, antibiotic resistance reflects the pathogen's importance as a public health threat. The zones of inhibition recorded ranged from 10mm to 24mm with ketonazole and 12mm to 28mm with fluconazole. Penicillium and Aspergillus species were resistant to both ketonazole and fluconazole antifungal drugs. The implications of antibiotic resistance on healthcare systems as a whole are enormous as resistance leads to limitation of treatment options. Within the healthcare system, there are cases in which antibiotic resistance may limit available and often lifesaving treatment options. Drug resistance is a growing hazard as many microorganisms have become multi-resistant to several drugs. This drug resistance could be observed both in bacteria, fungi and viruses. This failure of drugs to treat some microbial infections has been associated with abuse and misuse. Therefore, it is necessary to ensure that the surfaces of canned drinks are properly washed to avoid fomite-mediated transmission of infectious agents to humans during consumption of canned drinks.

## Conclusion

From this study, the surfaces of canned drinks sold within Federal University of Technology, Owerri, were contaminated with microorganisms of public health importance. The results showed that the canned drinks collected from the packs of canned drinks had higher microbial plate count as well as higher bacterial and fungal isolates. Some of the bacteria and fungi isolated from the surfaces of the canned drinks were found to be resistant to two or more antibiotics and antifungal drugs. However, some were susceptible to the drugs. The study showed that the cleanliness of the surface of canned drink cannot guarantee absence of microbes. Adequate washing of any canned drink irrespective of whether it is from refrigerator or not will reduce or eliminate these microbes from the canned surfaces.

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