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Research Article

Bacteriological Assessment of Canned Drinks Surface

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Abstract

Numerous types of food and beverages such as beer or soft drinks are commonly packaged in so-called "tin-cans". The adhesion and persistence of microorganisms to the surfaces can spread pathogens and spoilage microorganisms to foods, influencing their shelf-life and safety. Due to the fact that the bacteriology of the external orifice of canned drinks have not been extensively studied in various geographical locations and in this region, this study is limited to the investigation of the bacteria present on the external orifice of canned drinks in Ekpoma and to ascertain what group of bacteria the people of the locality consuming these drinks are possibly going to be exposed to. Ware houses and shops were randomly selected in Ekpoma to be used in this study. Forty (40) canned drinks towels were randomly used for this study from which twenty (20) were gotten from wholesalers (warehouses) and twenty (20) were gotten from retailers. Those from the retailers were divided into two (2) groups i.e. ten (10) from the refrigerator and ten (10) from unrefrigerated. Samples were taken by cotton swab which was scrubbed on the top surface of the canned drinks. Swabs were cultured on different Nutrient agar and incubated at 37°C and sub-cultured into relevant agars. Moreover, different biochemical tests were applied; catalase test, coagulase test and oxidase test. Microorganisms were recognized on the basis of macroscopic, microscopic and differential biochemical tests. The swabs were inoculated into phosphate buffer saline and incubated for a week before the swabs were streaked on various agar. Swabs were cultured on Nutrient agar, Blood agar, Saboraud dextrose agar (SDA) and Maconkey agar and incubated at 37°C. The different biochemical tests were applied; catalase test, Indole test, Oxidase test, Citrate test and Coagulase test. Microorganisms were identified on the basis of macroscopic, microscopic and differential tests. With regards to pathogenic bacteria, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia* and *Bacillus cereus* was analyzed in about thirty-five (35) of the cans. With respect to the presence of microorganisms indicating general contamination, 35 out of 40 (87.5%) of the cans analyzed presented positive to aerobic microorganisms. With respect to contamination by fungi, 15 cans (37.5%) presented positive to *Aspergilus* spp and *Candida albicans*.

Keywords: Canned Drinks; Orifice *Staphylococcus aureus; Escherichia coli; Klebsiella pneumonia* and *Bacillus cereus*

Introduction

Numerous types of food and beverages such as beer or soft drinks are commonly packaged in so-called "tin-cans." The misnomer "tin-can" is a hold-over from the early days of the canning industry when tin-plated steel was used to fabricate the cans [1]. In modern canning, tin-plated steel is still sometimes used, although aluminum or an aluminum alloy is more commonly used. In some instances, steel coated with a plastic or synthetic elastomer is also used [2].

After filling and seaming the cans, they are normally processed by pasteurization or sterilization in the beer and food industries, or warming for package protection from humidity damage in the soft-drink industry [3]. But these processes may cause contamination of the outside surface of the can, such contamination being either bacteriological or, simply, dirt. There, therefore, has been a need for a way to clean the ends of the cans for both sanitary reasons and aesthetic reasons [4].

Quite often, the cans are processed by washing them to remove surface dirt and contamination, but the wash water remains on the cans, especially in crevices. Thus, any dirt coming in contact with the wet surface remains, and residual water, on steel-based cans especially, can cause corrosion. Corrosion is also a problem with the so-called "ecological tab end" cans, or cans with opening tabs which, after being opened, fold out of the way instead of breaking off. The pre-cut area of the ecological tab is thinner than the formerly used pull tab pre-cut area, and consequently is more prone to corrosion damage. There still exists, therefore, a need for a method and means to effectively clean the can ends without leaving a residue [5].

The adhesion and persistence of microorganisms to the surfaces can spread pathogens and spoilage microorganisms to foods, influencing their shelf-life and safety [3]. Several studies have showed the ability of microorganisms to attach to all the surfaces commonly found in the food processing environment, such as stainless steel, polystyrene, rubber, glass, wood and so on [6-9]. Additionally, if microorganisms remain on a given surface for a relatively long time, they can multiply and, eventually, form biofilms. Although no literature reports are available on the survival of microorganisms on packaging materials, several studies showed that various foodborne pathogens, including *Escherichia coli* and *Listeria monocytogenes*, can survive on utensils and equipment surfaces for hours or days [4,10-12]. The wide variability is mainly due to the differences in physico-chemical features of packaging materials but also in logistic such as transportation. The few literatures data show that spore-forming bacteria (belonging to the genera *Bacillus, Geobacillus, Alicyclobacillus,* and *Clostridium*) and molds (belonging mainly to the species *Aspergillus niger, A. cinnamomeus,* and *Cladosporium herbarum*) prevail on packaging microbiota. They are wide spread microorganisms, resistant to adverse environmental conditions and endowed with high spoilage potential [13,14]. However, also yeast and other spoilage bacteria can be present on packaging materials. To avoid and/or minimize this issue, the use of appropriate packaging is essential, since it acts as a barrier that can protect fresh food from contamination [15].

Microbial cross-contamination refers to the transfer, direct or indirect, of microorganisms (bacteria, virus, parasites, or fungi) from a contaminated item to a non-contaminated one [16]. In food, cross contamination of foodborne pathogens is a major concern since it increases the health risk for humans due to the intake of contaminated food. Otherwise, cross-contamination of foodborne pathogens from inert surfaces to foods is well documented [4,10] (Erickson., *et al.* 2015). The adhesion and persistence of microorganisms to the surfaces can spread pathogens and spoilage microorganisms to foods, influencing their shelf-life and safety [1,3]. Consequently, controlling the permanence of microorganisms on surfaces, including packaging materials, is fundamental in reaching food safety standards and improving the overall quality (i.e., texture, flavor, aroma) and shelf-life of fresh produce. In addition, the microbial survival, growth or death on the packaging materials, and consequently their role in cross contamination of packed fruits, are affected by environmental conditions, including storage temperature, relative humidity and nutrient availability [5,9,17]. The aim of this study is to determine the bacteriology of canned drinks external orifice.

Materials and Methods

Area of study

This study was carried out in Ekpoma, The Headquarter of Esan West Local Government area of Edo State. It is located at latitude 6^{o} 45^{I}N and longitude 6^{o} 08^{I}E. It is moderately populated with the peoples' occupation being farming and trading. The main sources

of water in the locality are rainfall and well. The well is augmented by irrigation scheme provided by the Government for public use. University is situated in this region. It is usually cold at night and very hot during the day. It also has undulating topography [24].

Research design

This study was a descriptive/analytical study. It was designed to evaluate the bacteria present on the surface of canned drinks. Specimens such as cnned drinks swab were collected and analyzed in the laboratory using standard methods. Results were presented in tables. This study was carried out within three (3) months.

Sampling criteria

Canned drinks from the wholesalers and retailers without rust while canned drinks with rust and canned drinks from individuals.

Sample collection

Forty (40) canned drinks towels were randomly used for this study from which twenty (20) were gotten from wholesalers (warehouses) and twenty (20) were gotten from retailers. Those from the retailers were divided into two (2) groups i.e. ten (10) from the refrigerator and ten (10) from unrefrigerated. Samples were taken by cotton swab scrubbed on the top external orifice of the canned drinks. The swabs were inoculated into phosphate buffer saline and incubated for a week before the swabs were streaked on various agar. Swabs were cultured on Nutrient agar, Blood agar, Saboraud dextrose agar (SDA) and MacConkey agar and incubated at 37°C. The different biochemical tests were applied; catalase test, Indole test, Oxidase test, Citrate test and Coagulase test. Microorganisms were identified on the basis of macroscopic, microscopic and differential tests.

Sample analysis/methods

The sample analysis was carried out for bacteriological examination in laboratory of the Department of Microbiology, Faculty of Life Science, Ambrose Alli University, Ekpoma, Edo State.

- **Macroscopic Examination:** Exterior can condition: leaker, dented, rusted, buckled, paneled, bulge etc.

Microscopic Examination/Bacteriological Examination

Culture of canned drink swab

The swab stick was inoculated on each plate of Nutrient and Blood agar by making a primary inoculum on a small area of the agar plate and then streaked out. The growth from the nutrient agar was then sub-cultured into SDA and maconkey agar. The inoculated media was incubated aerobically at 37°C for 24 hours. Those inoculated on chocolate agar were incubated anaerobically. Identification of bacteria was done by carrying out biochemical tests [18].

Identification of Fungi

- **Identification of *Candida Albicans*:** For the identification of Candida germ tube test was carried out.
- **Procedure:** A very small inoculum of yeast cell from an isolate was suspended in 0.5ml of human plasma in the test tube and incubated at 35°C for not longer than 3 hours. The suspension was removed after incubation period and a drop of the suspension was placed on glass microscope glass slide. It was examined under lower power magnification for the presence of pseudohyphae showing production of germ tube [19].
- **Identification of *Aspergillus* spp:** Identification of *Aspergillus* was also carried out using Lactose phenol cotton blue reagent.
- **Procedure:** A drop of lactose phenol cotton blue was placed on a clean grease free slide. With a sterile straight sharp needle, a small portion of the colony was picked and placed on the glass slide in which a drop of lactose phenol cotton blue has been added. It was properly teased and cover with clean glass cover-slip. The preparation was examined microscopically using low magnification.

Statistical analysis

The results was analysed statistically. The mean values of each microorganism isolated were determined. Analysis of variance was used to determine any significant difference between the products. The least significance (LSD) was used to compare the means of any significance difference [20].

Results

The present study investigates the bacteria on the external orifice of canned drinks sold in Ekpoma.

Table 1 shows the Samples collected, number of positive samples and percentage prevalence in the study. Twenty (20) samples were collected from Retailers and Wholesalers each making a total of Forty (40) samples out of which thirty-five (35)

were positive. Out of the thirty-five (35) positive samples, twenty (20) were from retailers and fifteen (15) were from wholesalers. The total percentage prevalence from the study was 87.5%.

From both the retailers and wholesalers**,** four (4) types of bacteria were isolated which include**;** *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia* and *Bacillus cereus. Candida albicans* and *Aspergillus spp* were the fungi isolated (Table 2). The prevalence of *Staphylococcus aureus* from the study is 28.6%, *Escherichia coli* 31.4%, *Klebsiella pneumonia* 22.9% and *Bacillus cereus* is 17.1%. *Escherichia coli* have the highest prevalence while *Bacillus cereus* has the lowest prevalence in the study (Table 3). *Escherichia coli* (35%) had the highest prevalence followed by *Klebsiella pneumonia* (30%) while *Staphylococcus aureus* (15%) had the lowest prevalence in the study (Table 4). *Staphylococcus aureus* (46.7%) had the highest prevalence followed by *Escherichia coli* (26.7%) while *Klebsiella pneumonia* and *Bacillus cereus* (13.3%) were the organisms with equal prevalence and had the lowest prevalence in the study (Table 5).

Table 6 identifies the total number of each fungi isolated from each location in the study and to address which accommodates more fungi. From the retailers, samples were collected from the refrigerator (8) and the non-refrigerated (3) canned drinks and a total of eleven (11) canned drinks were positive to fungi while four (4) canned drinks were positive to fungi from the wholesalers.

Table 7 describes the morphological and cultural characteristics of fungal isolates in the study.

Table 8 describes the cultural characteristics and biochemical analysis of bacterial isolates in the study.

Location	Number examined	Number of positive samples	Percentage prevalence of infection (%)
Retailers	20	20	100
Wholesalers	20	15	75
TOTAL	40	35	87.5

Table 1: Samples examined, number of positive samples and percentage prevalence in the study.

Location	Bacteria	Fungi
Retailers	*Staphylococcus aureus, Escherichia coli*	*Candida albicans*
	Klebsiella pneumonia, Bacillus cereus	*Aspergillus* spp
Wholesalers	*Staphylococcus aureus, Klebsiella pneumonia*	*Candida albicans*
	Bacillus cereus, Escherichia coli	*Aspergillus* spp

Table 2: Organisms isolated from the study according to the location of sample collection.

Organisms	Retailers (n = 20)		Wholesalers (n = 20)	Percentage prevalence (%)
	Refrigerator	Non-refrigerated		
Staphylococcus aureus	1	2	7	28.6
Escherichia coli	3	4	4	31.4
Klebsiella pneumonia	4	2	2	22.9
Bacillus cereus	2	2	2	17.1
TOTAL	10	10	15	100

Table 3: Prevalence of Bacterial Isolates from both retailers and wholesalers in the study.

Bacterial Isolates	Frequency		Total Percentage of prevalence (%)
	Refrigerator (%)	Non-refrigerated (%)	
Staphylococcus aureus	1 (10)	2 (20)	15
Escherichia coli	3 (30)	4 (40)	35
Klebsiella pneumonia	4 (40)	2 (20)	30
Bacillus cereus	2 (20)	2 (20)	20
TOTAL	10	10	100

Table 4: The Prevalence of Bacterial Isolates in Samples from Retailers in the study.

Bacterial Isolates	Frequency		Total Percentage of prevalence (%)
	Refrigerator (%)	Non-refrigerated (%)	
Staphylococcus aureus	1 (10)	2 (20)	15
Escherichia coli	3 (30)	4 (40)	35
Klebsiella pneumonia	4 (40)	2 (20)	30
Bacillus cereus	2 (20)	2 (20)	20
TOTAL	10	10	100

Table 4: The Prevalence of Bacterial Isolates in Samples from Retailers in the study.

Organisms	Frequency	Percentage of prevalence (%)
Staphylococcus aureus	7	46.7
Escherichia coli	4	26.7
Klebsiella pneumonia	2	13.3
Bacillus cereus	2	13.3
TOTAL	15	100

Table 5: The Prevalence of Bacterial Isolates in Samples from Wholesalers in the study.

Organisms	Retailers		Wholesalers
	Refrigerator	Non-refrigerated	
Candida albicans	5	2	2
Aspergillus spp	3	1	2
TOTAL	8	3	4

Table 6: Number of each Fungus isolated from the location.

Fungal Isolates	Macroscopy	Microscopy
Aspergillus spp	Greenish, filamentous with profuse proliferation of black velvety spores.	Septate hyphae, branched condiophore with secondary branches. The condiophore isenlarged at the tip forming rounding vesicle-like chains.
Candida albicans	Grows quickly and cover agar surface with white fluffy that later turns grey, reverse side is white.	Hyphae practically non-septate, sporangiophores are long, often branched

Table 7: Morphological and Cultural Characteristics of Fungal Isolates.

Organism	Cultural characteristics			Biochemical analysis							
	Shape	Consistency	Colour	Gram	Catalase	Coagulase	Indole	Motility	Oxidase	Citrate	Urease
Staph. aureus	Cocci	Moist	Pink in MacConkey	+	+	+	-	-	-	+	+
E. coli	Bacillus	Mucoid	Rose Pink in MacConkey	-	+	-	+	+	-	-	-
Klebsiella pneumonia	Rod	Mucoid	Light Pink in MacConkey	-	-	-	-	-	-	+	+
Bacillus cereus	Rod	Moist	Grey	+	+	-	-	+	-	+	-

Table 8: Cultural Characteristics and Biochemical Analysis Bacterial Isolates.

KEY

+ = Positive

- = Negative

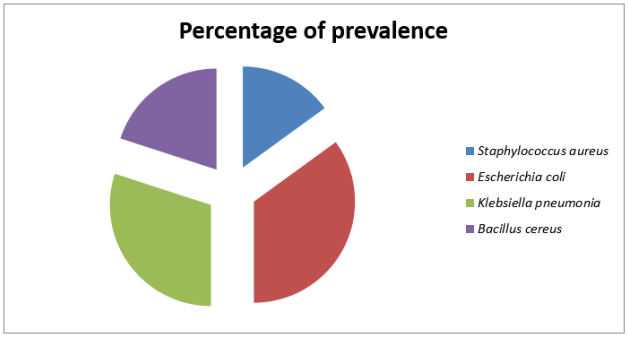

Figure 1: Percentage of prevalence of bacteria isolates from canned drinks sampled from retailers.

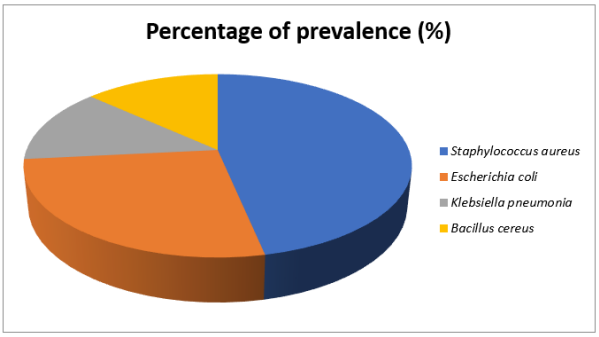

Figure 2: Percentage of prevalence of bacteria isolates from canned drinks sampled from wholesalers.

Discussion and Conclusion

With regards to pathogenic bacteria, No can was contaminated by *Salmonellai* and *Leptospira*. *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia* and *Bacillus cereus* was analyzed in about thirty-five (35) of the cans. With respect to the presence of microorganisms indicating general contamination, 35 out of 40 (87.5%) of the cans analyzed presented positive to aerobic microorganisms. With respect to contamination by fungi, 15 cans (37.5%) presented positive to *Aspergilus* spp and *Candida albicans*.

Sokari and Kigigha [21] had indicated the probable incidence of microbiological health risk arising from environmental contamination in medicine dispensing bottles in Port Harcourt metropolis. The study showed that medicine dispensing bottles were either not washed at all or only sparingly so with water of doubtful quality; in which frequency of characterized bacterial species was *Bacillus* and other gram-positive spp (4.9%); *S. aureus* (18.2%); *Staphylococcus spp* (13.3%) etc.

There is a correlation between coagulase production and ability to cause infection especially in food poisoning outbreaks in *S. aureus* [22]. There was about 28.6% frequency of occurrence of coagulase positive *S. aureus* in this study. Moreover the occurrence of 31.4% frequency of occurrence of *E. coli* indicated a serious health implication. The *E. coli* is known to survive under very harsh environmental conditions [23].

The frequency of more contaminated cans at these collection points reflects inadequate handling. One of these practices is the conditioning of cans in polystyrene boxes containing ice, in which the microbiological quality of the ice was much lower than recommended.

In conclusion, this study permitted verification of the following: The contamination level of the cans can be considered to be negligible despite the fact that (87.5%) presented a high level of contamination. At the collection points for retail sales, the microbiological condition of aluminum cans was found to be high (100%) and the microbiological condition of the cans from the wholesalers was quite better than that from the retailers. The points at which contamination was higher were the retailers in both the refrigerated and non-refrigerated cans as well.

The points at which the contamination was higher were the retailers in both the refrigerated and non-refrigerated cans. All the Leptospira analyses carried out on the aluminum cans were negative. Therefore, there is evidence that the commercialization and consumption of beverages direct from cans represents a potential focus for contamination of the population.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

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