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Qualitative Detection and Isolation of Bacteria from Surfaces of Canned Drinks Sold in Ugbor, Benin City

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Abstract

The qualitative assessment of putative bacterial pathogens on the surfaces of canned drinks sold in Benin metropolis was evaluated in this study. Standard bacteriological culture-based techniques employing the use of selective and differential media (Oxoid) such as *Bacillus cereus* agar, mannitol Salt agar, *Pseudomonas* cetrimide agar, bile esculin agar and MacConkey agar were used for isolation and identification of bacteria from swabbed surfaces of canned drinks. Kirby-Bauer disc diffusion technique was used for antibacterial susceptibility testing. The multiple antibiotic resistance (MAR) index was deduced from the antibiogram characterization to evaluate the public health importance of the bacterial isolates. Refrigerated samples had 25% contamination while 75% were not contaminated and about 15.39% contamination was observed for non-refrigerated samples (stored in crates or cartons) compared to the counterpart 84.61%. The bacterial species include *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Bacillus* sp. and *Enterococcus* sp. The bacteria were found to be sensitive to ciprofloxacin (92.5%) and gentamicin (90.1%) and least susceptible to cefixime (23.1%) and vancomycin (26.4%). They were found to be multi-resistant because they have an MAR index above the tolerable permissible limit (0.2) for common antibiotics usually used for their eradication. It is important to ensure that the surfaces of canned drinks must be rinsed with water before consumption.

Keywords: canned drinks; contamination; public health; qualitative detection; surfaces of canned drinks.

1.0 Introduction

Soft drinks, according to Kigighai and Jonathan, (2012) are defined as packaged beverages which might be non-alcoholic, non-carbonated or carbonated and flavored. They are best enjoyed when served cold. Different variations or variants of soft drinks exist depending on the flavored ingredients which include: lemon, orange, lime, assorted fruit-juices, colas, ginger ales, sodas and root beers according to CSDA, (1996). It is often a cliché in microbiology that "microorganisms are ubiquitous" in that they are able to survive and live everywhere they deem fit. Thus there might not be any place on the earth surface that is devoid of microbial forms. Bacteria in particular like other microbial forms are known for their ability to survive in different environments such as on soil, air, water and foods. More so, they have been found to grow on certain materials which are deplete in nutrients and moisture such as clothing, glassware and other inanimate objects (Pelczar *et al.*, 1993).

Several studies have shown that microbes have been able to colonize inanimate objects such as beverage packages and other items used to consume beverages (Dantas *et al*., 2006); mobile phones of health care staff/workers (Kilic *et al*., 2009) and household surfaces as well as food surfaces (Othman, 2015). To say the least, microbes are not just ubiquitous in nature but a plethora of their activities in the environment can lead to positive or negative consequences on humans, animals and plants.

There are few published work on the associated health risk to consumers of canned or bottle soft drinks due to microbial contamination from the surfaces and orifces especially in the Third World and developing countries. Kigighai and Jonathan (2012), carried out the microbiological survey of non-alcoholic carbonated beverages and reported the presence of bacterial pathogens such as *Staphylococcus, Bacillus, Enterococcus, Micrococcus Proteus* and *Pseudomonas* species which are of public health significance. Griffiths *et al*. (1997) (cited in Kigigha and Jonathan, 2012) carried out an analysis of the quality of the ingredients used in the Soft-drink Industries. In the City of Ibadan, Amusa *et al.* (2005) carried out microbiological and nutritional studies on the quality of hawked sorrel drinks popularly known as zobo and the report revealed a plethora of microbial contaminants of public health importance which include *Bacillus cereus, B. subtilis, Staphylococcus, Streptococcus* and *Escherichia*. Fungal contaminants were also reported which are basically species in the genus *Aspergillus, Penicillium, Fusarium* and *Rhizopus.* Oranusi *et al.* (1994) in a technical report, studied the microbial contaminants of commercially bottled non-alcoholic beverage available in Nigeria at the time and posited that 50% of a total of analyzed 90 samples were contaminated mainly with saprophytic and non-pathogenic bacterial species such as *Bacillus*, *Lactobacillus, Pediococcus*, *Staphylococcus epidermidis* and *Micrococcus*.

With the recent news of leptospirosis on the internet (Gompf, 2017; CDC, 2017), there are concerns over the safety of consumers who consumes soft drinks most especially canned drinks directly from the orifice. Just as the definition of canned drinks goes, it is best consumed when cold or chilled thus most consumers do not take cleaning the canned drink surfaces seriously before consumption while others are under the assumption and illusion of the fact that when it is brought out of the refrigerator cold or chilled, it is apparently safe for consumption. Contamination of canned drinks surface can occur anywhere including environment where it was manufactured to the point of storage in the refrigerator as well as point of consumption. Studies have shown that certain bacterial and fungal species of public health importance are able to survive refrigeration temperature. Kregiel, (2015) reported that Soft drinks consumption is still a controversial issue for public health and public policy. Through the years, several studies have been carried out to evaluate the possible links between the consumption of soft drink and associated health problems. The findings, however, remain highly contested. Nonetheless, there is an increasing emphasis being placed on the health properties of soft drinks, by both the industry and the consumers. Moreover, there are extant rules already in place to ensure that manufacturers of soft drinks conform to established national and international standards. Most consumers believe that the soft drinks are safe for consumption in whatever form and that their quality is guaranteed; but little do they understand that there is also another reason to worry over our public health as it concerns canned drinks or food. Hygiene is one aspect of science that most persons do not give much credence to. Notwithstanding, in the science of hygiene, everything is a potential infection vehicle including the orifice or surfaces of canned drinks.

The aim of the study is to isolate and characterize putative bacterial isolates from surfaces of canned drinks sold at different points in Benin metropolis, Benin City. The objectives of the study include to isolate and biochemically characterize bacterial species from surfaces of canned drinks sold in shopping complexes in university of Benin, determine the antibiogram of the putative identified bacterial isolates, and determine the public health risk of the isolated pathogen using the multiple antibiotic resistance index tool.

2.0 Materials and Methods

2.1 Materials

All materials such as glass wares were manufactured by Pyrex® in England. The media and reagents used for the research were obtained Vicdomstell limited, Lagos State, Nigeria, Pyrex- IG Scientific Company Benin and Equator Medics International limited, Lagos. The media used in this study were manufactured by Oxoid Limited, Basingstoke, Hampshire, England. They include tryptone soya broth CM0129, Mueller Hinton agar, manitol salt agar, oxacillin resistant screening agar base, *Salmonella Shigella* agar, bile esculin agar, *Bacillus cereus* agar and MacConkey agar.

2.2 Study Design

The study was designed in such a way that a total of ninety-six (96 samples) were collected from retail kiosks, shops and other outlets in Benson Idahosa University (BIU) and environs, Ugbor, Benin City. For every store or shops, three sets of samples were obtained, one each from the crates, refrigerator and the last rinsed with distilled water before swabbing was done (this was to evaluate the importance of rinsing the surfaces on the burden of microorganisms present). Samples collected from the distributors were also of three sets. The first was swab obtained from the crates inside the store, the next was the swab obtained from the crates usually outside the store and more so, a third sample rinsed with water. Rinsed with water samples were those obtained from refrigerators (from shops and kiosks) and from crates (strictly from distributors). Negative control experiment was set up in the research as sterile swab sticks soaked with tryptic soya broth were streaked onto petri dishes containing Mueller Hinton agar. The prepared broths were made to stand for seven days to ensure that they pass the test of sterility before they were used for sample collection as well as for control experiment.

2.3 Preparation and Sterilization of Culture Media

All culture media were prepared according to the manufacturer's instructions. Sterilization was at 121ºC at 15ppi for 15 min unless otherwise opined by manufacturer.

2.4 Sample Collection

Samples were collected from shops around Benson Idahosa University, Ugbor road in Benin City. Sterile Amies swab sticks, aseptically soaked with tryptone soya broth (Oxoid) were used to swab the upper surfaces of canned drinks which comes in direct contact with the mouth (with an approximate 10cm^2 area). A total of ninety-six (96) samples from twenty eight (28) shops, and four (04) distributors (basically to trace the source of bacterial contaminants on the surfaces of canned drinks) were evaluated in this research. A total of twenty-eight (28) samples were collected from the refrigerator, thirty six (36) from crates from and thirty-two samples whose surfaces were rinsed with distilled water before swabbing and dully labelled rinsed with water (RWW). Ten swab sticks were kept aside after soaking with the broth and were used as control during culturing. The tryptone soya broth (Oxoid) were prepared and allowed to stand for seven (7) days without showing any sign of turbidity to ensure that all broths passed test of sterility.

2.5 Sample Processing and Qualitative Detection of Bacteria

Samples were processed in the Department of Microbiology, University of Benin immediately after collection. They were cultured directly by streaking onto Mueller Hinton agar (Oxoid), staphylococcal species were cultivated using mannitol salt agar, *Pseudomonas aeruginosa* were screened for using *Pseudomonas* cetrimide agar, coliform bacteria were recovered using MacConkey agar, and enterococcal species using bile esculin as described by Oxoid manual (2006). The swab sticks after sample collection were streaked onto respective selective and differential agar plates for isolation and cultivation of bacterial species of public health importance. Relevant staining and biochemical tests were carried out on the isolated organisms recovered from the samples.

2.6 Antibiotics Susceptibility Test

The antimicrobial agents were chosen on the basis of their importance in treating human or animal infections caused by Gram positive and Gram negative bacteria as well as a broad spectrum antibiotic of choice for multi-resistant pathogen. The already identified bacterial isolates were made to undergo antibiotics susceptibility testing using the standard Kirby-Bauer disc diffusion technique (Bauer, 1966). A loopful of each test bacterial corresponding to 10^8 cells/ml were evenly streaked on Mueller-Hinton agar and the streaked plate was impregnated with different antibiotic discs manufactured by Oxoid Limited which include: Cephazolin (30µg), Ampicillin (10µg), Cefixime (5µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Erythromycin (15µg), Vancomycin (30µg), Gentamicin (10µg), Cefuroxime (30µg) and Amoxycillin (25µg). The plates were incubated at 37ºC for 24 hours after which the zones of inhibition were measured and interpreted as Resistant (R), Intermediate resistant (I) or Sensitive (S) in conformity with the recommended standards established by the Clinical Laboratory Standards Institute (2016; 2017).

2.7 Multiple Antibiotic Resistances (MAR) Index

The MAR index is a good tool for health risk assessment which identifies if the isolates are from a region of high or low antibiotic usage. A MAR index of 0.2 and above indicates a 'high-risk' source of contamination (Davis and Brown 2016). The multiple antibiotic resistance MAR index was determined for each isolate using the methods delineated by Chitanand *et al.* (2010) by dividing the percentage of antibiotic resistance of the total percentage of antibiotics used in the study. However the total percentage resistance of certain multi-resistant organisms' will be calculated and used as the numerator.

2.8 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.

3.0 Results

The Frequency of bacterial contaminants present in the surfaces of refrigerated and non-refrigerated canned drink sold in shops and kiosks around Benson Idahosa University (BIU) as well as some distributors are shown in table 1. The frequency of contamination to non-contamination of the surfaces of analyzed canned drinks samples were 25% to 75% for refrigerated samples while 15.39% contamination was observed for non-refrigerated samples (stored in crates or cartons) compared to the counterpart 84.61% non-contamination that was observed for samples in crates or cartons. However, 100% contamination was obtained for analysed samples from kiosks while all samples obtained from distributors were found to be devoid of contamination. An important observation in this study was that all samples rinsed with water before analysis were found to be non-contaminated. This is a testament of the fact that rinsing has a role to play in reducing microbial contaminants from the surfaces of canned drinks. Meanwhile, samples obtained from distributors were found to be non-contaminated as all samples showed no growth of cultivable bacterial isolates.

Table 1 Frequency of bacterial contaminants of refrigerated canned drinks sold in shopping complexes in Benson Idahosa University and environs.

Sample location (n)	Refrigerated (%)		Non-refrigerated (%)	
	Non-contaminated	Contaminated	Non-contaminated	Contaminated
BIU shopping complex (40)	85.00	15.00	100.00	0.00
Open kiosks (16)	0.00	100.00	0.00	100.00
Distributors (8)	NA	NA	100.00	0.00
Rinse with water (32)	100.00	0.00	100.00	0.00
Total (96)	75.00	25.00	84.61	15.39

Key: NA = not applicable where n/2 is obtained for refrigerated and non-refrigerated samples except for samples tagged NA.

This further reveal that the contamination obtained from the kiosks and from refrigerated samples were never from the factory or distributors. The contamination however could be as a result of the unhygienic state of the vendors. The isolated bacterial species include *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Bacillus* sp., and *Enterococcus* sp. Figure 1 shows the percentage of occurrence of bacterial isolates obtained from the surfaces of canned drinks evaluated in the area of study. *Staphylococcus aureus* were found to have 63.6% of occurrence while *Bacillus* species were found to have 72.72% of occurrence.

Table 2. Antibiotic sensitivity and resistance pattern of putatively isolated bacteria from the surfaces of canned drinks

Isolates (n)	Antibiotic susceptibility of isolates (%)									
	CIP	AML	AMP	CFM	CXM	E	CRO	CN	VA	KZ
B. cereus (5)	100	80.0	60.0	0.0	60.0	60.0	100	100	0.0	60.0
Staphylococcus (7)	100	71.40	57.1	28.5	14.3	28.6	71.4	85.7	42.8	28.6
Enterococcus (3)	100	66.7	66.7	0.0	66.7	33.3	100	100	66.7	100
Pseudomonas (4)	75.0	25.0	50.0	0.0	0.0	25.0	50.0	75.0	50.0	50.0
E. coli (5)	100.0	40.0	40.0	60.0	40.0	60.0	60.0	80.0	20.0	20.0
Bacillus sp. (8)	100	87.5	75.0	50.0	50.0	87.5	50.0	100.0	12.5	25.0
Total (%)	92.5	61.8	58.1	23.1	32.9	49.1	71.9	90.1	26.4	47.3

The antibiotic sensitivity and resistance pattern of isolated bacteria from the surfaces of canned drinks is shown in table 2. Most bacterial isolates were found to be sensitive to ciprofloxacin and gentamicin with 92.5 and 90.1% respectively. The isolates were least susceptible to cefixime (23.1%) and vancomycin (26.4%).

All isolates were found to be multi-resistant as most of the putative species were resistant to more than three antibiotics commonly employed in their eradication. *Pseudomonas aeruginosa* and *Escherichia coli* putatively identified in this study were found to be resistant to most of the antibiotics used in the study. The multiple antibiotic resistance patterns exhibited by bacterial isolates from the surfaces of canned drinks is shown in table 4.

Table 3. Multiple antibiotic resistance pattern exhibited by bacterial isolates isolated from surfaces of canned drinks

Isolates	Antibiotics resistance patterns	Percentage (%)
B. cereus	AML, AMP, CFM, CXM, E, VA, KZ	54.29
Staphylococcus	CIP, AML, AMP, CFM, CXM, CRO, E, CN, VA, KZ	52.40
Enterococcus	AML, AMP, CFM, CXM, E, VA,	49.60
Pseudomonas	CIP, AML, AMP, CFM, CXM, CRO, E, CN, VA, KZ	65.00
E. coli	AML, AMP, CFM, CXM, CRO, E, CN, VA, KZ	53.33
Bacillus sp.	AML, AMP, CFM, CXM, CRO, E, VA, KZ	45.31

The MAR index of the putative bacterial isolates are shown in figure 2. The results revealed that the bacterial species were obtained from high risk sources where antibiotics have been used and they are of public health importance. All bacterial isolates were found to have an index of above the tolerable permissible limit for common antibiotics usually used for their eradication. *Enterococcus, Staphylococcus aureus* has an index on 0.40 and 0.653 respectively while *Pseudomonas aeruginosa* and *Escherichia coli* had an index of 1.00.

4.0 Discussion

This study has shown that surfaces of canned drinks can be readily contaminated irrespective of whether they are kept in the refrigerator or

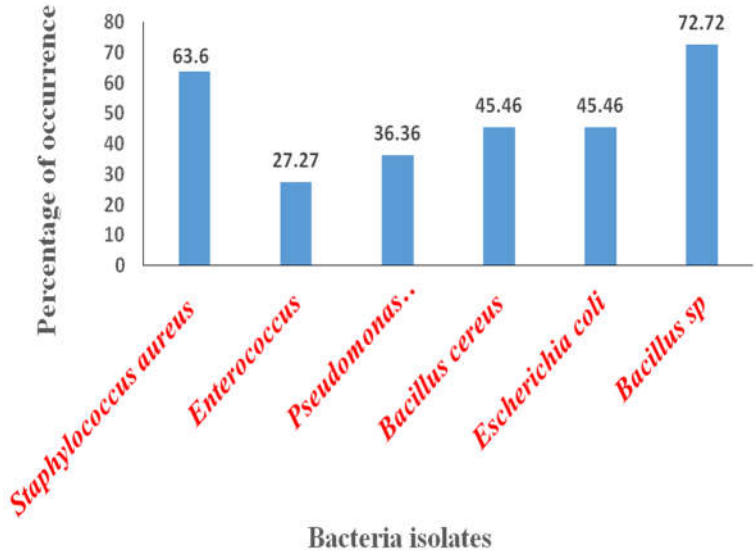

Figure 1. *Frequency of occurrence of bacteria isolated from the surfaces of canned drinks*

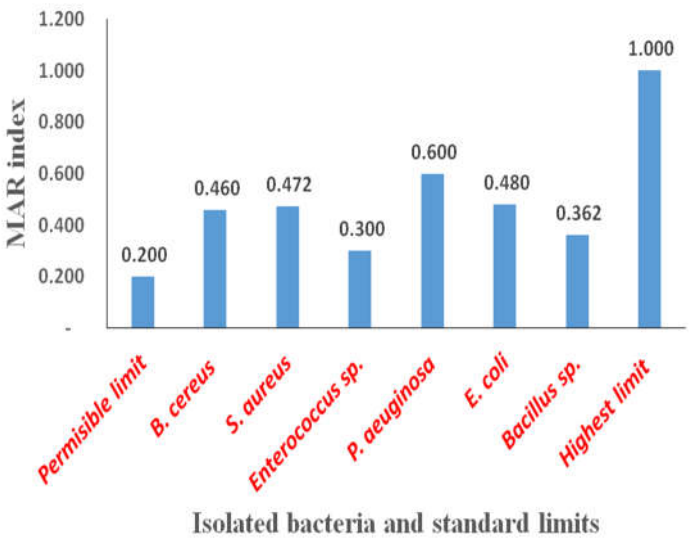

Figure 2. *Multiple antibiotic resistance index of presumptively identified bacteria from the surfaces of canned drinks*

not (Tables 1 and 2) and this finding agrees with the work of FDA (105) and Out-Bassey *et al.* (2017) who reported the isolation of certain microorganisms from refrigerators. The isolated organisms in this study were identified to be *S. aureus, P. aeruginosa, Enterococcus* sp., *Escherichia,* and members of the spore forming *Bacillus* genus. It has been reportd that some of these identified pathogens can survive on hands, sponges and surfaces of stainless steel materials for several days and weeks after contact.

Kusumaningrum *et al*. (2002) indicated that some bacteria, such as *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* may either directly contaminate surfaces or indirectly do same through aerosolized droplets and could survive on hands, sponges, and other objects such as mobile phones (Kilic *et al*., 2009) and external beverage packaging materials (Dantas *et al*., 2006) for up to several days after contact. This is not oblivious of the fact that spore forming *Bacillus* can survive for several years. More so, the fact that most Gram-positive bacteria, such as *S. aureus* and *Enterococcus* contaminate inanimate environment has been well established. They can survive for months on an inanimate surfaces as reported by Ekrami *et al*. (2010); and this is in agreement with the results obtained from this study as four of the isolated bacteria were Gram positives.

Other frequently occurring bacteria like *Escherichia coli* have been associated with diarrhea; and could get to the surfaces of canned drinks either 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 supply not forgetting the fact that often times, most of these refrigerators are not adequately sanitized. Sample with considerable levels of contamination were found to be non-contaminated after rinsing with sterilized distilled water. This underscores the fact that rinsing surfaces of canned drinks with portable water can result to a gross reduction in the number of contamination. More importantly, samples obtained from distributors were found to be non-contaminated with any culturable bacterial pathogen. Hence, this points to the fact that the contamination seen in the analysed samples were not from the manufacturers of canned drinks products but rather from the vendors. The 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. *E. coli* is widely or generally regarded as indicator of fecal contamination suggesting that the refrigerator internal surfaces are frequently contaminated by import of contaminated raw foods or by poor personal hygiene. The harmless or less virulent species or strains are definitely no threats to public health but if the MAR index is greater than 0.2 as stipulated by Krupernman (1983), Chitanand *et al*. (2010) and Davis and Brown (2016) there is every reason to worry about the public health of the community. The MAR index according to Chitanand *et al*. (2010) reflects the pathogen's importance as a public health threat and more so, its origin (whether or not antibiotics have been used). Figure 2 from this research revealed that the isolated organisms were of public health importance and that they have been shown to be multiresistant pathogens. None of the isolated pathogens was within the safe range of 0.2. As a result, this leaves us with plenty reasons to worry about the community health status of the isolated pathogens from the surfaces of canned drinks.

5.0 Conclusion

It is important to ensure that the surfaces of canned drinks are cleansed before being consumed as their surfaces have been known to harbour a plethora of bacterial pathogens. Surfaces of canned drinks have been found to harbour multiple drug resistant bacteria therefore, cleaning the surfaces of canned drinks before consumption is essential to reducing or removing microbial contaminants. In the exact word of Othman (2015) "cleaning a surface helps you remove all signs of germs". It is better to be on the safe side hence proper cleansing of canned drink surfaces is key to safeguarding the health of consumers.

6.0 Conflict Of Interest

Authors declare no conflict of interest.

Authors Contribution

O.A.G, B.I.O and EAO prepared the manuscript together

B.I.O. analyzed and interpret the data

A.B.U., O.A.G, and B.I.O carried out the laboratory experiment

I.V.E corrected and proofread the final copy of the manuscript

E. A.O. supervised the entire project and manuscript preparation

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