Quantitative Analysis and Antimicrobial Susceptibility of Enteric Bacteria Isolated from Surfaces of Canned Soft Drinks and Handlers from Selected Institutions in Zaria, Kaduna State

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ABSTRACT

The potentially major importance but currently unstudied trend of enteric bacterial contamination on surfaces of canned drinks forms the basis of the research. A study was carried out to determine the antimicrobial susceptibility of selected enteric bacteria on the surface of canned soft drinks and its handlers (palms of consumers) from selected tertiary institutions in Zaria. Two hundred and sixteen swabs were collected from the external surfaces of canned soft drinks and its handlers, thirty-six swabs each from cans and hand swabs were also collected from designated whole sellers known as Distributors. The samples were screened and analyzed using Spread Plate Method, Microgen GNA System for identification for enteric bacteria in three institutions in Zaria, Kaduna State. Kirby-Bauer disk diffusion method was employed for antimicrobial susceptibility test. The results showed that the total bacteria count of canned soft drinks and its handlers in the three institutions range from 1.0×10^4 to 3.0×10^7 cfu/ml. Fourteen species of the Family Enterobacteriaceae were identified. The predominant species observed on canned soft drinks were *Citrobacter freundii* and *Klebsiella ozaenae* while that of its handlers were *Citrobacter freundii* and *Escherichia coli*. Perfloracin (66.7%) in ABU, perfloracin (66.7%) in FCE and ciprofloxacin (88.9%) in POLY were highly sensitive against the isolates, while imipenem and ampicillin had (57.6%) in ABU, ofloxacin (73.3%) in FCE and Amoxicillin-Clavulanic (66.7%) in POLY were found to be non-susceptible. The study showed that surfaces of canned soft drinks and its handlers in Zaria are significantly contaminated with enteric bacteria. Hence, incorporation of routine check of canned drinks in the health system is required. There is low antimicrobial activity by the antibiotic against the isolates, this poses serious health implications and requires further investigation.

Keywords: Enteric bacteria, contamination, antimicrobial, susceptibility.

Introduction

Soft drinks are sweetened, water-based beverages, usually with balancing acidity. Soft drinks are consumed worldwide and it represents a significant market within the food industry (Mooji, 2018) and is largely consumed by

children and the working class daily, especially in the rush hours (Pettigrew *et al.*, 2015). Its origin is dated back to two thousand years ago when Greeks and Romans recognized the medicinal value of mineral water and bathed it for relaxation

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(Sordu *et al.*, 2016). Soft drink constitutes a diverse group of products that can be classified based on their sugar and fruit juice content, flavouring, carbonation level, ingredients, and functionality (Pranita *et al.*, 2015; Kregiel, 2015).

There are many ways of producing soft drinks as producers find inventive ways of breathing new life into old-time brands by updating the products that are appealing to consumer (Lantos, 2015). According to Salako *et al.* (2016), non-alcoholic drinks are admittedly vital vehicles of a balanced diet and have also turned out to be a drink preferred in most social, health or religious gathering (Romo *et al.*, 2015). These drinks are easily absorbed providing hydration, refreshment, and energy as well as replenishing the human body with all the key vitamins and minerals (Salako *et al.*, 2016). Soft drinks are packed in bottles, laminated paper packs, pouches, cups and cans (Kregiel, 2015). These packaging materials is a means of storage and prevention from damage (Brockgreitens and Abbas, 2016).

Cans are one of the most accepted forms of packaging today due to its numerous advantages, such as it is one of the least expensive and most recyclable metal substances in the market. Therefore, many health concerns for this packaging method have been overlooked due to the financial and sustainability benefits (Stenberg *et al.*, 2017). Consumers frequently drink straight from the package, and, in this case, the package enters into direct contact with the consumer's mouth. According to Pranita *et al.* (2015), cans and bottles can serve as sources of contamination, leading to large-scale disease outbreaks.

Infectious diseases caused by pathogenic enteric bacteria are the most common and widespread health risk diseases (Singer *et al.*, 2017). These organisms can live on inanimate surfaces for an extended period (Russotto *et al.*, 2015). It has also been observed that bacteria can grow in a low nutrient medium and exhibit resistance even to harsh conditions and antibiotics (Moretro and Langsrud, 2017). Food, hands, utensils and

clothing are associated with domestic sanitation and poor hygiene. Also, the type of microflora on the hands, arms, nasal cavities, mouth or garments of vendors, reflects their sanitary state (Alum *et al.*, 2016). Enteric diseases are common cause of illness and death in the developing world. They can cause disease when they gain access to the host, adhere to host tissue, penetrate host defenses and damage host tissue (Scallan *et al.*, 2015). *Bacillus* species are one of the most virulent and destructive pathogens. Food-poisoning are attributed to *Bacillus cereus* since 1906 (Kigigha and Jonathan, 2012). The Gram-negative bacteria including *Alcagenes*, *Pseudomonas*, *Escherichia coli*, *Serratia* and *Proteus*, indicating probable faecal contamination causes severe diarrhoea (Kigigha and Jonathan, 2012).

There is paucity of reports on the health risk assessment that could arise from the consumptions of soft drinks directly from the orifice of canned soft drinks. This study aims to determine the bacterial load, isolate, identify, and evaluate the antimicrobial susceptibility of selected enteric bacteria on the surfaces of canned soft drinks and its handlers in the study area.

Materials and Methods

Study area

This study was conducted in three tertiary institutions in Zaria which included; Ahmadu Bello University Main Campus (11°02.268'N, 007°40.397'E), Federal College of Education Kongo (11°05.020'N, 007°43.791'E) and Nuhu Bamali Polytechnic (11°02.140'N, 007°40.336'E). The sample size was determined using the formula described by Kogi (2019).

$$\text{Thus, } N = \frac{Z^2(1-P)}{2d^2}$$

Where

N = Sample size

Z = 1.96 at 95%

P = Prevalence = 78.9% (Kigigha and Jonathan, 2012)

d = Level of precision (0.05)

Therefore, $N = \frac{Z^2(1-P)}{2d^2} = \frac{1.96^2(1-0.789)}{2 \times 0.05^2} = 162$

Bacteriological analysis was conducted at the Microbiology Laboratory, Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

Collection of Samples

A total of 216 samples was collected which includes 108 swabs from cans of soft drinks obtained from kiosks stored in the refrigerators or in cooler box with ice blocks placed on them and 108 swabs of consumer's palms that bought the drink at the time of the study. From each of the tertiary institutions, 36 swabs of cans and 36 hand swabs were taken from designated whole sellers known as Distributors in the institutions were randomly selected. Each swab stick was moistened using sterile normal saline then the moistened swab stick was used to swab the surface of the canned soft drinks and the handler's hand that gave their approval by filling the consent form and immediately placed in sterile plastic bags and taken to the laboratory for analysis.

Preparation of Culture Media and Total Bacteria Counting

The media was prepared according to the manufacturer's specification. The swab sticks used to swab the cans and its handlers hand were cut aseptically into 9 ml of sterile peptone water and was shaken vigorously for 15 – 20 seconds. Afterwards, several dilutions were made up to dilution 10^3 and 0.1 ml was plated out of the dilution 10^3 . The diluent was inoculated in nutrient agar by spread method and incubated for 24 hours at 37°C (Barrow and Feltham, 2004). Discrete single colonies were counted as cfu/ml (Cheesbrough, 2006).

Sample Processing and Qualitative Detection of Bacteria

The diluent, 0.1 ml was transferred to selective and differential agar media (Eosin methylene blue agar and MacConkey agar respectively) and a sterile wire loop was used to streak the diluent by streaking in two directions at right angles over the surfaces of the agar media and incubated at 37°C for 24 hours (Murugalatha *et al.*, 2012). Discrete single colonies were picked and inoculated onto nutrient agar slant bottle, isolation and cultivation of bacterial species of public health importance was carried out. Relevant staining and biochemical tests were carried out on the isolated organisms recovered from the samples (Cheesbrough, 2006).

Antibiotics Susceptibility Test

The modified Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility of the isolates to the various antibiotics, according to Turnidge *et al* (2006). Discrete colonies of the isolates were emulsified in 5ml of sterile normal saline and the turbidity adjusted to 0.5 McFarland standard (approximately a cell density of 1.5×10^8 cfu/ml). The standardized suspension was inoculated on Mueller Hinton agar and spread using a sterile swab to ensure even distribution and confluent growth. The sensitivity disc of the various selected antibiotics was aseptically placed using a sterile forceps on the dried inoculated agar surface. The plates were then incubated at 37°C for 18 hours. The plates were examined for the zones of inhibition, and were measured in micrometre and the result interpretation according to EUCAST (2013). The antibiotics used include: Ciprofloxacin (5µg), Trimethoprim-sulfamethoxazole (30µg), Amikacin (30µg), Gentamycin (10µg), Amoxycillin/clavulanic acid (30µg), Imipenem (10µg), chloramphenicol (30µg), Perfloracin (5µg), Ofloxacin (5µg) and Ampicillin (10µg). EUCAST (2013).

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

The canned drink surfaces and the handlers showed the highest mean microbial load among the kiosk located in FCE Kongo (15.65×10^5 cfu/ml) and (6.49×10^5 cfu/ml). While the least were among kiosks located in ABU Main Campus (9.84×10^5 cfu/ml) and (4.73×10^5 cfu/ml). (Table 1).

Table 1: Microbial load of Cans and their Handlers in the three Institutions

Schools	CAN Colony count (cfu/ml $\times 10^5$)	HANDLER Colony count (cfu/ml) $\times 10^5$
	Mean $\pm$ SEM	Mean $\pm$ SEM
ABU	5.11 $\pm$ 0.59 ^a	4.73 $\pm$ 0.90
FCE	9.16 $\pm$ 1.64 ^b	6.49 $\pm$ 1.45
POLY	4.38 $\pm$ 0.98 ^a	5.93 $\pm$ 1.48
F-value	4.978	0.475
P-Value	0.009	0.623

n=216. Values on the same column with different superscripts, ^{a,b}, are significantly different ($p < 0.05$).

Bacterial load on Cans and distributors hands in the selected Institutions

Among the schools sampled, it was observed that swabs from the external surfaces of canned soft drinks had the highest isolation of enteric organisms. In contrast, samples obtained from distributors (samples in crates before removal of cover) were found to be devoid of contamination although hand swabs taken from a representative of the distributors' stores had bacterial growth (Figure 1).

Figure 1: Cans and Handlers bacterial load of distributors of soft drinks and the selected Institutions.

Isolation and Identification of Isolates

Fourteen bacterial were identified belonging to the family Enterobacteriaceae and several genera subsumed under, *Klebsiella* 3, *Serratia* 2, and *Proteus* 2 while other genera occurred only once. The isolation was highest in *Citrobacter freundii* (26.30%) and the least was (0.9%) among *Serratia marcescens*, *Proteus mirabilis*, *Klebsiella oxytoca* and *Proteus vulgaris* (Figure 2).

Figure 2: Isolated bacterial and percentages

Distribution of Isolates by Sample Source and study locations

Serratia rubidaea, *Citrobacter freundii* and *Salmonella typhi* are the bacterial that are statistically associated with surfaces of canned drinks ($P < 0.05$) in the study sites. While *Enterobacter cloacae*, *Pantoea agglomerans* and *Providencia stuartii* are significantly associated with the soft drink handlers in this study ($P < 0.05$) (Table 2).

Antimicrobial Susceptibility of Enterobacteriaceae Isolates from surfaces of Cans drinks and Handlers

The antimicrobial susceptibility test of the antibiotics had low antimicrobial activities against the Enterobacteriaceae isolates in this study. Most isolates had low antimicrobial activity to imipenem and ofloxacin of 66.7%, and 73.3% respectively. The antibiotics with the highest antimicrobial activity against the isolates are amikacin, and ciprofloxacin 24.4 and 2.8% (Figure 3).

Table 2: Distribution of Isolates by Sample Source and School

Organisms	Cans					Handlers				
	A.B.U	F.C.E	POLY	Chi square	P-value	A.B.U	F.C.E	POLY	Chi square	P-value
<i>Klebsiella ozaenae</i>	3	6	3	1.528	0.466	1	2	0	2.009	0.366
<i>Cronobacter sakazakii</i>	4	5	0	4.732	0.094	0	2	0	4.012	0.134
<i>Escherichia coli</i>	5	3	1	2.704	0.259	1	6	4	3.514	0.174
<i>Serratia rubidaea</i>	4	0	0	8.050	0.018	2	0	0	4.012	0.134
<i>Enterobacter cloacae</i>	0	0	0	NA	NA	3	0	0	6.028	0.049
<i>Citrobacter freundii</i>	0	9	10	9.868	0.007	3	5	3	0.740	0.691
<i>Serratia marcescens</i>	0	0	0	NA	NA	1	0	0	2.003	0.367
<i>Proteus mirabilis</i>	0	0	0	NA	NA	0	0	1	2.003	0.367
<i>Salmonella typhi</i>	0	3	0	6.028	0.049	0	0	0	NA	NA
<i>Pantoea agglomerans</i>	0	1	4	5.240	0.073	0	3	0	6.028	0.049
<i>Klebsiella pneumoniae</i>	3	0	3	3.028	0.220	2	0	1	2.009	0.366
<i>Providencia stuartii</i>	0	0	1	2.003	0.367	0	0	4	8.050	0.018
<i>Klebsiella oxytoca</i>	0	0	0	NA	NA	1	0	0	2.003	0.367
<i>Proteus vulgaris</i>	0	0	0	NA	NA	0	0	1	2.003	0.367
TOTAL	19 (16.7%)	27 (23.7%)	22 (19.3%)			14 (12.3%)	18 (15.8%)	14 (12.3%)

Figure 3. Antimicrobial Susceptibility of Enterobacteriaceae Isolates from Cans and Handlers

Discussion

Surfaces of canned drinks can be readily contaminated and might contribute to the risks of foodborne diseases if necessary hygienic measures are not observed or adhered to by the consumers (Alimi, 2016). In this study the total bacterial load of total viable count (TVC) revealed that the samples had counts ranged between 1.0×10^4 cfu/ml and 3.0×10^7 cfu/ml which is above the standard level of acceptable threshold of number of bacteria count of portable product for consumption ($< 10^4$ cfu/ml by National Agency for Food and Drug Administration and Control (NAFDAC)).

It was observed that swabs from the external surfaces of canned soft drinks had the highest isolation of enteric organisms, while samples obtained from distributors (samples in trays before removal of the shrink film) had non-contaminated with any of the enteric bacteria. This may be attributed to the poor hygienic

practices of the vendors as well as the surrounding conditions where these drinks where the drinks are sold in an unhygienic cooler with ice block which do not last long due to epileptic power supply this favours the survival and proliferation of the enteric bacterial isolated. These findings agree with Abraham *et al.* (2018) work on qualitative detection and isolation of bacteria from surfaces of canned drinks in Ugbor, Benin City, who observed 100% contamination of bacteria for analyzed samples from kiosks while all samples obtained from distributors were found to be non-contaminated. This is probably because the contaminations recorded in the analyzed samples were not from the manufacturers of canned soft drinks but rather from the vendors. The surface contamination observed is a clear indication of poor hygienic practices of vendors which is commonly associated with bacterial growth. Canned soft drinks in the study area are stored in ice slurry, which are potentially translocation of

bacteria between contaminated surfaces and the vendors (Dawson *et al.*, 2018). Abraham *et al.* (2018) also noted that surfaces of canned drinks were readily contaminated, irrespective of whether they are kept in the refrigerator or not.

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A total of fourteen microorganisms belonging to ten different genera was isolated from the external surfaces of canned soft drinks and handlers. The genera are *Klebsiella*, *Cronobacter*, *Escherichia*, *Serratia*, *Enterobacter*, *Citrobacter*, *Proteus*, *Salmonella*, *Pantoea* and *Providencia*. According to Abdulkadir and Mugadi (2012), the presence of *most of these* species can be attributed to the fact that they are commonly found in the environment and have been reported to be common contaminants. Some of these identified pathogens can survive on hands, external surfaces of packaging materials and other objects (Kilic *et al.*, 2009) for several days and weeks after contact (Dantas *et al.*, 2006). However, their presences in some of the commercial samples are of public health concern since some of the organisms are associated with some form of disease conditions such as gastroenteritis (Ogodo *et al.*, 2008). The predominant species observed on canned soft drinks were *Citrobacter freundii*, *Klebsiella ozaenae*, *Escherichia coli* and *Cronobacter sakazakii* while that of its handlers were *Citrobacter freundii* and *Escherichia coli*. A study by Abraham *et al.* (2018) identified some enteric bacterial such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus* species and *Escherichia coli*. The bacteria isolated in his work were fewer compared to the present study that recorded enteric bacteria on the surfaces of the canned soft drink examined in the study areas. Kusumaningrum *et al.* (2002) revealed that some bacteria, such as *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* may either directly or indirectly contaminate surfaces through aerosolized droplets. The isolation of these bacteria shows that canned surfaces and

hands could act as a medium for the spread of both pathogenic and opportunistic microbes.

The antimicrobial susceptibility test revealed that most of the antibiotics had low antimicrobial activities against the Enterobacteriaceae isolates across the schools. In this study, Imipenem and ampicillin had the highest antimicrobial activity (58.6% each) whereas ciprofloxacin (29.6%) was the least active antibiotic against the isolated bacteria from the samples analyzed. This finding also concurs with the study of Igwe *et al.* (2016) in Zaria, who reported amoxicillin and ofloxacin to have low antimicrobial activities and amikacin was found to have high antimicrobial activity against *E. coli* although differed from ciprofloxacin which had low antimicrobial activity as against the high antimicrobial activity obtained in this study. Another report by Olayinka *et al.* (2009) in Zaria also reported ofloxacin to have low antimicrobial activity and ciprofloxacin and amikacin to have high antimicrobial activities. This varying ranges is notable to the fact that Ella *et al.* (2008) observed that antimicrobial susceptibility pattern differs in different studies as well as at different times in the same region because of emergence of resistant strains as a result of abuse of antibiotics.

In this study the total number of antibiotics to which the organisms were exposed showed a minimum of 96.9% isolates in MAR index ≥ 0.2 . The MAR indices obtained is a possible indication that high proportion of the bacteria isolates have been exposed to several antibiotics as also reported by Adeshina *et al.* (2012).

Conclusion

A total of 48.1% of the surfaces of canned soft drinks investigated are contaminated with enteric bacterial. *Citrobacter freundii* (16.7%) and *Klebsiella ozaenae* (10.5%) have the highest frequency in canned soft drinks while for handlers *Citrobacter freundii* and *Escherichia*

coli have similar frequency of 9.6%. Antibiotics such as ciprofloxacin, pefloxacin, amikacin are sensitive against the isolates while ofloxacin, amoxicillin, imipenen, ampicillin have low antimicrobial activities to the isolates. This poses serious health implications, hence, incorporation of routine check of canned drinks in the health system is required.

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Competing Interest

No conflict of Interest exists.

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