Vol. 1 No. 2, December 2021

FUAM Journal of Pure and Applied Science

Available online at
www.fuamjpas.org.ng

An official Publication of
College of Science
Joseph Sarwuan Tarka University,
Makurdi.

FUAM Journal of Pure and Applied Science
© College of Science, Joseph Sarwuan Tarka University, Makurdi
https://fuamjpas.org.ng/
Vol. 1 No. 2 December 2021

Studies on Bacterial Contaminants on the Bottle Orifice of Opened Non-Alcoholic Drinks In Mkar-Gboko, Benue State

***[1,2]D.Yandev, *[2]A.U. Onwuka and [2]A. Msoo**
[1]Microbiology Department, University of Nigeria Nsukka, Enugu State, Nigeria
[2]Microbiology Department, University of Mkar, Mkar, Benue State, Nigeria
***Correspondence E-mails:** yandevdoowuese@gmail.com; angelaonwuka@gmail.com

Received: 26/08/2021 **Accepted**: 19/09/2021 **Published online**: 26/09/2021

Abstract

Drinking directly from the orifice of canned and or bottled drinks is a common health-risk practice. The aim of this work was to isolate and identify bacterial contaminants from the orifice of opened soft drink bottles The study was conducted at University of Mkar, Mkar, Gboko Local Government of Benue State. Fifty (50) samples were bought from shops around University of Mkar, Mkar and in the University cafeteria. Media preparation, inoculation and culture were carried out following standard practices. Morphologically different colonies were sub-cultured. Characterization and identification were carried out using cultural morphology variation, Gram's reaction and biochemical tests. Fanta bottle orifices had the highest bacteria load (2.87 x 10^3cfu/ml) followed by those of Pepsi orifices (2.83 x 10^3cfu/ml) and Coke (2.39 x 10^3cfu/ml). Bacterial load in Mountain Dew and Sprite orifices were 2.09 x 10^3 and 1.86 x 10^3 respectively. Three groups of bacteria were identified based on their reactions to the biochemical tests such as the Gram staining, coagulase test, catalase test, sugar utilization and hydrogen sulphide production. They were: *Staphylococcus aureus, Bacillus* spp and *Enterococcus* spp. *Staphylococcus aureus* had the highest percentage occurrence (50%) in Pepsi and Mountain dew samples. *Bacillus* spp had the highest percentage occurrence (40%) in Pepsi, Coke and Fanta samples. *Enterococcus* spp had the highest percentage occurrence (60%) in Sprite. Statistically, this difference was not significant (χ^2 = 0.08, P=0.961, P>0.05). No bottle orifice of any brand was associated with a particular bacterial type among the three identified species. The identified bacteria have clinical implications and they are of public health concern. This calls for appropriate measure to monitor and decontaminate orifice of soft drinks for the safety of consumers.

Keywords: Bacterial contaminants, Bottle orifice, Safety, Public health

Introduction

Soft drink is a general term for a non-alcoholic beverage, differentiating it from an alcoholic beverage. Soft drinks constitute a diverse group of beverages. They can be classified in several ways, for example based on the basis of sugar caloric/diet and fruit juice content, flavoring, carbonation level sparkling/ still, main non-water ingredient (fruit, malt, tea, soya, milk etc.) and functionality. Functional soft drinks are the trend of today. There is no official definition for a functional beverage in the European Union (EU). They can be considered to include enriched and fortified drinks such as juices and waters with added vitamins and minerals; sports drinks; energy drinks; wellness drinks and nutraceutical products with added ingredients targeted at specific medical or health benefits [1].

Most microorganisms in food products are intestinal in origin; however, some are found in nasal passages, in the throat, on hair and on skin. Thus, food handlers are often a main source of contamination and cross-contamination [2]. The ability of bacteria to adhere to food contact surfaces compromises the hygiene of those surfaces. Surface physiochemical properties of the bacterial cell as well as of the materials, such as hydrophobicity and roughness are determinants during the initial attachment phase (Ray, 2004). It has also been determined that even after adhering to typical and specific hygienic procedures, pathogenic microorganisms can survive in kitchens often for hours [3] Microorganisms such as *Flavobacterium* species and *Pseudomonas* could multiply in stored spring and mineral water at room temperature. Coliform bacteria have been known to occur in chlorinated drinking water supply [4]. There are a few published works on the public health effect of drinking soft drinks directly from the mouth or the orifice of glass bottles or canned drinks [3]. The objective of public health programs is to prevent illness and disease by intervention and actions such as health campaigns, advice/counselling especially behavioural and vaccinations. Changing health-risk behaviour has been a major focus of researchers. Drinking directly from the orifice of canned drinks is a common practice and it could be a great health risky habit.

Microbiological survey of non-alcoholic carbonated beverage has also been reported by Hassan *et al.* [4] where bacterial pathogens such as *Staphylococcus*, *Bacillus*, *Enterococcus*, *Micrococcus Proteus* and *Pseudomonas* species which are of public health significance were isolated. Most consumers believe that the soft drinks are safe for consumption in whatever form and that their quality is guaranteed. The aim of this work was to investigate the presence of bacterial contaminants from the orifice of opened soft drink bottles.

Materials and Methods

Study design

The study was conducted at University of Mkar, Mkar, Gboko Local Government of Benue State. The University is located at the foot of Mkar hills at the outskirts of the town.

Sample collection
Fifty samples (50) were bought from shops around University of Mkar, Mkar and in the University cafeteria. These fifty samples (50) were divided into five (5) batches, batch A, B, C, D and E, each batch comprised of ten samples (10). Each bottle of drink was carefully emptied of its content to avoid spoilage on the outer surface of the bottle. The bacterial isolates on the orifice and neck of the bottles were obtained by using sterile amies swab sticks ascetically soaked with normal saline to swab the orifice and neck of the bottles and immediately dipped into test tubes that contained 5ml of sterilized Mueller Hinton broth for pre-enrichment. They were incubated for 6 hours at 37˚C for the resuscitation of the dormant forms [5].

Media preparation and inoculation
The following media were prepared following standard microbiological practices as contained in Cheesbrough (2006). Mueller Hinton Broth, Nutrient Agar, MacConkey Agar and Klieger's Iron Agar (KIA). Inoculation of both NA (Nutrient agar) and MAC (MacConkey agar) plates were carried-out using the pre-enrichments. Exactly 0.1ml of each rinsate was pipetted into appropriate agar plates (with the use of syringes) and spread using a sterile glass-spreader. The plates were incubated at 37˚C for 24hours [5].

Identification of bacterial isolates
Morphologically different colonies were sub-cultured. Characterization and identification were carried out using cultural morphology variation, Gram's reaction and biochemical tests, according to the procedure for specific organisms [5].

Biochemical tests
Gram staining
Procedure described in Cheesbrough [5] was used. A sterile wire loop was used to make a smear from a culture plate on a clean slide. The smear was allowed to air dry on a staining rack. It was then fixed by gently passing the lower part of the slide through a Bunsen flame several times. The fixed smear was allowed to cool. It was flooded with crystal violet for 30-60 seconds. The stained was washed with clean water. The water was tipped off and the smear was flooded with lugol's iodine for 30-60 seconds. The iodine was drained off and the smear was washed with clean tap water. The water was tipped off. It was decolorized rapidly with acetone- alcohol and washed immediately with clean tap water. The water was tipped off. It was counter stained with Safranin stain for 2 minutes. The stain was washed off with clean water. It was on a draining rack for the smear to air dry. The smear was examined with the oil immersion objective lens (×100). The Gram reaction were noted and recorded.

Catalase test
Procedure described in Cheesbrough [5] was used. Two drops of hydrogen peroxide solution were placed on a glass slide. Then sterile wire loop was used to collect several colonies of the test organisms and inoculated into the hydrogen peroxide solution. It was observed for immediate active bubbling for positive reaction, whereas no bubble formation indicated negative reaction.

Coagulase test
Procedure described in Cheesbrough [5] was used. A drop of sterile distilled water was placed on each end of a sterile slide. Then a colony of the test organism was emulsified on each spot to make two tick suspensions and mixed gently. The slide was examined for clumping or clothing of the organisms within 10 seconds.

Hydrogen sulphide test
Procedure described in Cheesbrough [5] was used. A small quantity of bacterial colony was picked and inoculated into already prepared tubes of Klieger Iron Agar and incubated at 37˚C for 24 hours. After the incubation period the tubes were observed for hydrogen sulphide (black coloration) gas production.

Sugar fermentation
Procedure described in Cheesbrough [5] was used. Sugar fermentation test was carried out to determine the ability of organisms to ferment sugars with production of acid and alkaline. A small quantity of bacterial colony was picked and inoculated into already prepared tubes of Klieger Iron Agar and incubated at 37˚C for 24 hours. After the incubation period the tubes were observed. Yellow coloration indicates acid and red coloration indicates alkaline.

Statistical analysis
The data collected from this study was subjected to Chi square analysis using the Minitab software (version 16) at 95% confidence limit. Data were presented in tables and graphs.

Results and Discussion

Table 1-5 give the morphological descriptions of bacterial cultures (10 samples per drink brand type) and the respective bacterial counts from the bottle orifices. In Pepsi orifices (Table 1), cultures had wavy margin, white colour and flat elevation. Texture was either slimy or moist or dry or viscous or shiny. Culture assumed round or irregular or rhizoid shapes. Bacterial count in cfu/ml ranged between 1.0 $x10^2$cfu/ml and 5 $x10^3$ cfu/ml with an average count of 2.83 x 10^3cfu/ml in the ten sample plates. In Mountain dew orifices (Table 2), margin of culture plates varied (wavy or entire or smooth or lobate) mostly white in colour and flat elevation, Texture was round or filamentous or rhizoid. Bacterial count in cfu/g ranged between 1.0 $x10^2$ and 4. $x10^3$ with an average count of 2.09x 10^3 in the ten sample plates. In Coca-cola orifices (Table 3), margin of culture plates was wavy or undulate, all white in colour with flat elevation and round outline. Maximum bacterial count was 6.0 x 10^3 with an average of 2.39 x 10^3 in cfu) per culture plate.

In Fanta orifices (Table 4), culture plates had either wavy or entire margin; all white in colour; with flat elevation. Texture was either dry or moist slimy or shiny assuming round or irregular shape. Bacteria count had a minimum of 6.0 x 10^2cfu/ml and maximum of 5.4 x 10^3 cfu/ml. Average bacteria count was 2.87 x 10^3cfu/ml. In Sprite orifices (Table 5), margin of culture plates was wavy or smooth; white or opaque in colour; in colour; with flat elevation. Texture was either dry

or moist or slimy or shiny or viscous assuming round or rhizoid shape. Maximum bacteria count was 3.1 x 103 cfu/ml and average bacteria count was 1.86 x 10^3cfu/ml. Figure 1 compares the average bacterial counts from all samples. Fanta orifices had the highest bacteria load (2.87 x 10^3cfu/ml) followed by Pepsi orifices (2.83 x 10^3cfu/ml) and Coke (2.39 x 10^3cfu/ml) while Mountain Dew and Sprite had the least bacterial loads of 2.09 x 10^3 and 1.86 x 10^3 respectively

Table 1: Morphological Characterization and Bacterial Count from Pepsi Bottle Orifice from Mkar-Gboko, Benue State

Sample ID	Cultural characteristics	Total bacterial Count (cfu/ml)
Pepsi-1	Wavy, white, flat, slimy, round	2.5×10^3
Pepsi-2	Wavy, white, flat, moist, round	3.2×10^3
Pepsi-3	Wavy, white, flat, moist, round	2.3×10^3
Pepsi-4	Wavy, white, flat, dry, round	1.0×10^2
Pepsi-5	Wavy, white flat, dry, irregular	3.5×10^3
Pepsi-6	Wavy, white, flat, dry, irregular	5.0×10^3
Pepsi-7	Wavy, white, flat, viscous, round	1.5×10^3
Pepsi-8	Wavy, white, flat, shiny, round	3.8×10^3
Pepsi-9	Wavy, opaque, flat, translucent, round	5.0×10^3
Pepsi-10	Entire, white, flat, dry, rhizoid	1.4×10^3
Average count		2.83×10^3
S.E		5.01×10^2
Range		1×10^2 - 5×10^3

Table 2: Morphological Characterization and Bacterial Count from Mountain dew Bottle Orifice from Mkar-Gboko, Benue State

Sample ID	Cultural characteristics	Total bacterial Count (cfu/ml)
Mountain dew-1	Smooth, opaque, flat, viscous, round	1.0×10^3
Mountain dew-2	Smooth, white, flat, translucent, round	2.5×10^3
Mountain dew-3	Wavy, white, flat, slimy, Rhizoid	2.6×10^3
Mountain dew-4	Wavy, white, flat, dry, rhizoid	1.0×10^2
Mountain dew-5	Lobate, white, flat, moist, filamentous	1.7×10^3
Mountain dew-6	Entire, white, flat, dry round	1.0×10^2
Mountain dew-7	Wavy, white, flat, moist, round	3.3×10^3
Mountain dew-8	Wavy, white flat viscous, round	4.0×10^3
Mountain dew-9	Wavy white flat, shiny, round	3.8×10^3
Mountain dew-10	Wavy, white, flat, moist, round	1.8×10^3
Average count		2.09×10^3
S.E		4.45×10^2
Range		$1 \times 10^2 - 4 \times 10^3$

Table 3: Morphological Characterization and Bacterial Count from Coke bottle Orifice from Mkar-Gboko, Benue State

Sample ID	Cultural characteristics	Total bacterial Count (cfu/ml)
Coca-Cola-1	Undulate, white, flat, moist, round	3.6×10^3
Coca-Cola-2	Wavy, white, flat, moist, round	4.0×10^3
Coca-Cola-3	Wavy, white, flat, dry, round	2.6×10^3
Coca-Cola-4	Wavy, white, flat, viscous, round	1.0×10^2
Coca-Cola-5	Wavy, white, flat, dry, round	3.7×10^3
Coca-Cola-6	Wavy, white, flat, shiny, round	1.0×10^2
Coca-Cola-7	Wavy, white, flat, translucent, round	3.0×10^3
Coca-Cola-8	Wavy, white, flat, moist, round	6.0×10^3
Coca-Cola-9	Wavy, white, flat, moist, round	7.0×10^2
Coca-Cola-10	Wavy, white, flat, moist, round	1.0×10^2
Average count		2.39×10^3
S.E		6.48×10^2
Range		$1.0 \times 10^2 – 6.0 \times 10^3$

Table 4: Morphological Characterization and Bacterial Count from Fanta Bottle Orifice from Mkar-Gboko, Benue State

Sample No.	**Cultural characteristics**	**Total bacterial Count (cfu/ml)**
Fanta-1	Wavy, white, flat, shiny, round	1.5×10^3
Fanta-2	Wavy, white, flat, dry, round	4.2×10^3
Fanta-3	Wavy, white, flat, moist, round	2.2×10^3
Fanta-4	Wavy, white, flat, dry, round	6.0×10^2
Fanta-5	Wavy, white flat, slimy, irregular	4.5×10^3
Fanta-6	Wavy, white, flat, dry, irregular	1.0×10^3
Fanta-7	Wavy, white, flat, dry, round	4.5×10^3
Fanta-8	Wavy, white, flat, shiny, round	1.8×10^3
Fanta-9	Wavy, white, flat, slimy, round	3.0×10^3
Fanta-10	Entire, white, flat, dry, round	5.4×10^3
Average count		2.87×10^3
S.E		5.34×10^2
Range		$6.0 \times 10^2 – 5.4 \times 10^3$

Table 5: Morphological Characterization and Bacterial Count from Sprite Bottle Orifice from Mkar-Gboko, Benue State

Sample No.	**Cultural characteristics**	**Total bacterial Count (cfu/ml)**
Sprite-1	Smooth, opaque, flat, slimy, round	1.7×10^3
Sprite-2	Smooth, white, flat, moist, round	2.0×10^3
Sprite-3	Wavy, white, flat, slimy, round	3.0×10^3
Sprite-4	Wavy, white, flat, dry, rhizoid	1.2×10^3
Sprite-5	Wavy, white, flat, moist, round	1.0×10^3
Sprite-6	Wavy, white, flat, dry, round	1.0×10^2
Sprite-7	Wavy, white, flat, moist, round	2.3×10^3
Sprite-8	Wavy, white flat viscous, round	1.4×10^3
Sprite-9	Wavy, white, flat, shiny, round	3.1×10^3
Sprite-10	Wavy, white, flat, moist, round	2.8×10^3
Average count		1.86×10^3
S.E		3.06×10^2
Range		$1.0 \times 10^2 – 3.1 \times 10^3$

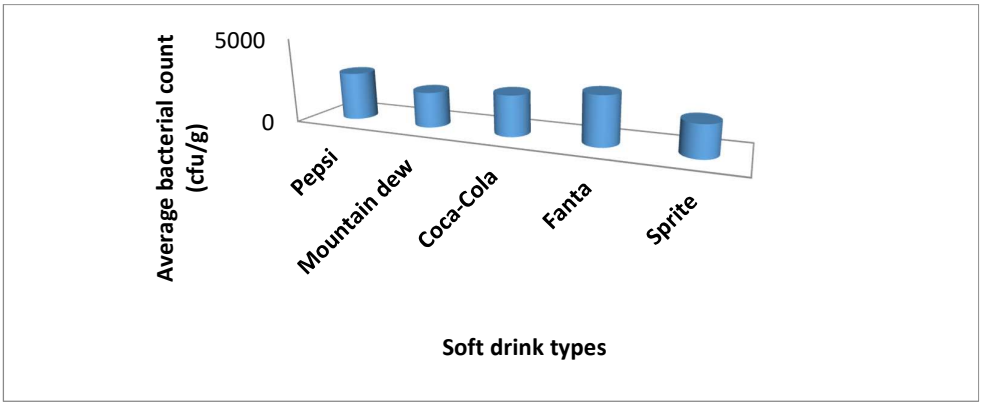

Figure 1: Average bacterial counts from bottle orifices of soft drinks
(χ^2 @ 4df= 329.435, P=0.000, P<0.05)

Tables 6-10 give the results of biochemical characterization of the isolated bacteria from the respective bottle orifices. Three groups of bacteria were identified based on their reactions to the biochemical tests such as the Gram staining, coagulase test, catalase test, sugar utilization and hydrogen sulphide production. They were: *Staphylococcus aureus, Bacillus* spp and *Enterococcus* spp. Among the 10 Pepsi samples (Table 6), there were 5 plates (50%) of *Staphylococcus aureus*; 4 plates (40%) of *Bacillus* spp and 1 (10%) plate of *Enterococcus* spp. In Mountain dew samples (Table 7), there were 5 plates (50%) of *Staphylococcus aureus*; 3 (30%) plates of *Bacillus* spp and 2 plates (20%) of *Enterococcus* spp. In Coke samples (Table 8), there were 2 (20%) plates of *Staphylococcus aureus*; 4 (40%) plates of *Bacillus* spp and 4 plates (40%) of *Enterococcus* spp. In Fanta samples (Table 9), there were 3 (30%) plates of *Staphylococcus aureus*; 4 plates (40%) of *Bacillus* spp and 3 plates (30%) of *Enterococcus* spp. Among the 10 Sprite samples (Table 10), there were 6 plates (60%) of *Enterococcus* spp and 2 plates (20%) of *Staphylococcus aureus* and *Bacillus* spp each

.

Table 6: Biochemical characterization of Bacteria Isolated from Pepsi Bottle Orifice from Mkar-Gboko, Benue State

Sample ID.	Gram's reaction	Coagulase test	Catalase test	Sugar fermentation (glucose and lactose)	Hydrogen sulphide test	Identification
Pepsi-1	+ve cocci	+	+	–ve red	–ve	*S. aureus*
Pepsi-2	+ve rods	–	+	–ve red	–ve	*Bacillus*spp
Pepsi-3	+ve cocci	+	+	–ve red	+ve	*S. aureus*
Pepsi-4	+ve cocci	+	-	–ve red	–ve	*S. aureus*
Pepsi-5	+ve rods	–	+	–ve red	–ve	*Bacillus*spp
Pepsi-6	+ve cocci	–	-	–ve red	–ve	*Enterococcus*spp
Pepsi-7	+ve rods	–	+	–ve red	–ve	*Bacillus*spp
Pepsi-8	+ve cocci	+	+	–ve red	–ve	*S. aureus*
Pepsi-9	+ve cocci	+	+	–ve red	–ve	*S. aureus*
Pepsi-10	+ve rods	–	+	–ve red	+ve	*Bacillus* spp

Key: "+" = positive, "-"= negative

Table 7: Biochemical characterization of Bacteria Isolated from Mountain dew Bottle Orifice from Mkar-Gboko, Benue State

Sample ID	Gram's reaction	Coagulase test	Catalase test	Sugar fermentation	Hydrogen sulphide test	Identification
Mountain dew-1	+ve cocci	+	+	+ve yellow	−ve	*S. aureus*
Mountain dew-2	+ve rods	−	+	−ve red	−ve	*Bacillus* spp
Mountain dew-3	+ve cocci	+	+	−ve red	+ve	*S. aureus*
Mountain dew-4	+ve cocci	−	+	−ve red	+ve	*Enterococcus* spp
Mountain dew-5	+ve rods	−	+	+ve red	−ve	*Bacillus* spp
Mountain dew-6	+ve rods	−	+	−ve red	−ve	*Bacillus* spp
Mountain dew-7	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Mountain dew-8	+ve cocci	+	+	−ve red	−ve	*S. aureus*
Mountain dew-9	+ve cocci	+	+	−ve red	−ve	*S. aureus*
Mountain dew-10	+ve cocci	+	+	−ve red	−ve	*S. aureus*

Key: "+" = positive, "-"= negative

Table 8: Biochemical characterization of Bacteria Isolated from Coke bottle Orifice from Mkar-Gboko, Benue State

Sample ID	Gram's reaction	Coagulase test	Catalase test	Sugar fermentation (glucose and lactose)	Hydrogen sulphide test	Identification
Coke-1	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Coke-2	+ve rods	−	+	−ve red	−ve	*Bacillus* spp
Coke-3	+ve cocci	+	+	−ve red	−ve	*S. aureus*
Coke-4	+ve rods	−	+	−ve red	−ve	*Bacillus* spp
Coke-5	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Coke-6	+ve rods	−	+	−ve red	−ve	*Bacillus* spp
Coke-7	+vecocci	−	+	−vered	−ve	*Enterococcus* spp
Coke-8	+vecocci	+	+	−vered	−ve	*S. aureus*
Coke-9	+vecocci	−	+	−vered	−ve	*Enterococcus* spp
Coke-10	+verods	−	+	−vered	−ve	*Bacillus* spp

Key: "+" = positive, "-"= negative

Table 9: Biochemical characterization of Bacteria from Fanta Bottle Orifice from Mkar-Gboko, Benue State

Sample ID	Gram's reaction	Coagulase test	Catalase test	Sugar fermentation (glucose and lactose)	Hydrogen sulphide test	Identification
Fanta-1	+vecocci	+	+	−vered	−ve	*S. aureus*
Fanta-2	+ve rods	−	+	−vered	−ve	*Bacillus* spp
Fanta-3	+ve cocci	+	+	−ve red	−ve	*S. aureus*
Fanta-4	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Fanta-5	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Fanta-6	+ve rods	−	+	−ve red	−ve	*Bacillus* spp
Fanta-7	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Fanta-8	+ve cocci	+	+	−ve red	−ve	*S. aureus*
Fanta-9	+ve rods	−	+	−ve red	−ve	*Bacillus* spp
Fanta-10	+ve rods	−	+	−ve red	−ve	*Bacillus* spp

Key: "+" = positive, "-"= negative

Table 10: Biochemical characterization of Bacteria from Sprite Bottle Orifice from Mkar-Gboko, Benue State

Sample ID	Gram's reaction	Coagulase test	Catalase test	Sugar fermentation (glucose and lactose)	Hydrogen sulphide test	Identification
Sprite-1	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Sprite-2	+verods	−	+	−ve red	−ve	*Bacillus* spp
Sprite-3	+ve cocci	+	+	−ve red	+ve	*S. aureus*
Sprite-4	+ve cocci	+	+	−ve red	+ve	*S. aureus*
Sprite-5	+ve rods	−	+	−ve red	−ve	*Bacillus* spp
Sprite-6	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Sprite-7	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Sprite-8	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Sprite-9	+ve cocci	−	+	−ve red	−ve	*Enterococcus* spp
Sprite-10	+ve cocci	−	+	−ve red	+ve	*Enterococcus* spp

Key: "+" = positive, "-"= negative

Figure 2 compares the percentage distribution of identified bacteria in all samples. *Staphylococcus aureus* had the highest percentage occurrence (50%) in Pepsi and Mountain dew samples. *Bacillus* spp had the highest percentage occurrence (40%) in Pepsi, Coke and Fanta samples. *Enterococcus* spp had the highest percentage occurrence (60%) in Sprite, followed

by Coca- cola samples (40%). The average percentage occurrence of each organism in all samples showed that *Staphylococcus aureus* and *Bacillus* spp had 34% each while *Enterococcus* spp had 32% presence in all samples. Statistically, this difference was not significant (χ^2 = 0.08, P=0.961, P>0.05). Thus, percentage occurrence of the three bacterial species was the same. The bottle orifice of each brand of non-alcoholic drink sampled was not associated with a particular bacterial species.

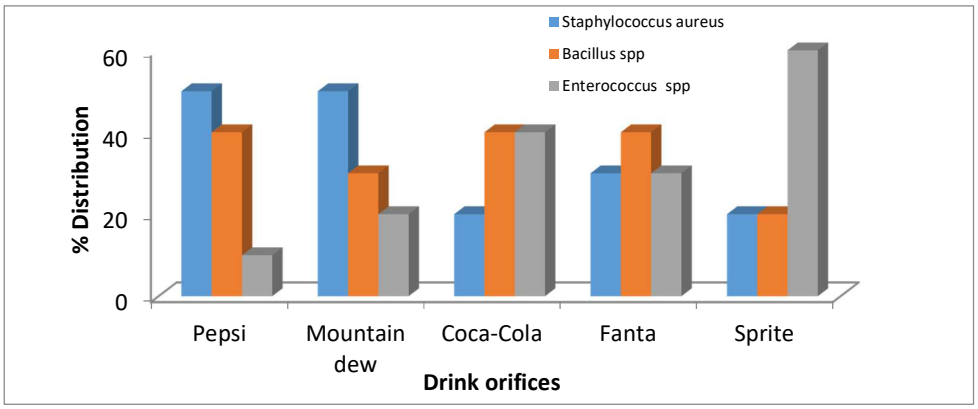

Figure 2: Comparative Distribution of bacteria in Bottle Orifices of Non-alcoholic Drinks in Mkar-Gboko, Benue State.

Results have shown that the orifices of soft drinks sold in the study area harbored some opportunistic pathogens on the surfaces, thereby posing health risk to the users. Across the drink types, some samples contained very low bacterial load (100 cfu/g), while other samples had higher bacterial load than the approved threshold for food containers. The wide variability might be due to the differences in physico-chemical features of packaging materials and problems arising from transportation, storage and hygiene. These views were upheld in other studies [6, 7]. The cell loads normally detected for mesophilic aerobic bacteria ranged between 10^3 and 10^6 cfu/cm^2 [8]. However, this study found that many samples irrespective of drinks type were heavily loaded with bacterial contaminants. For example, Fanta orifices had the highest average bacteria load (2.87 x 10^3cfu/ml) followed by Pepsi orifices (2.83 x 10^3cfu/ml) and Coke (2.39 x 10^3cfu/ml). The lowest average bacterial load observed was 1.86 x 10^3cfu/ml in Sprite. The safety of users of the affected orifices is not guaranteed, most especially in a situation whereby regulatory bodies do not enforce sanitary conditions in the handling and presentation of drinks and packaging materials. Furthermore, many vendors lack formal training on handling and storage of drinks containers which are kept indiscriminately under unhygienic storage condition.

This study therefore corroborates other views stating that the ability to control the permanence of microorganisms on surfaces, including packaging materials, is fundamental in reaching food safety standards [7, 9]. The occasional presence of pathogenic bacteria, parasites and viruses capable of causing human infections has been documented on surfaces of many materials [1, 2, 4].The adhesion and persistence of microorganisms to the surfaces can spread pathogens and spoilage microorganisms to foods, influencing their shelf-life and safety [7]. Several studies have shown 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 [10]. Additionally, if microorganisms remain on a given surface for a relatively long time, they can multiply and, eventually, form biofilms. Studies have shown that various foodborne pathogens can survive on utensils and equipment surfaces for hours or days [10].

Three groups of bacteria have been identified in this report as the main contaminants present in drinks orifices in the study area. They were *Staphylococcus aureus, Bacillus* spp and *Enterococcus* spp. Although previous studies on bacterial contamination of surfaces also reported these organisms in many surfaces, the number of bacterial species reported in the present work was low compared to other reports where diverse bacterial species were implicated in surface contaminations [3]. In previous studies, microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* spp *Klebsiella* spp, *Pseudomonas aeruginosa*, *Streptococcus* spp, *Salmonella* spp, *Lactobacillus* spp and *Proteus* spp had been reported [2, 4]. However, the present work aligns with many studies where *Staphylococcus aureus* was identified as the most frequently occurring species implicated in contamination of surfaces including food package materials. For example, *Staphylococcus aureus* had the highest percentage occurrence in all orifices followed by *Bacillus* species and *Enterococcus* species.

The worrisome outcome from this study is the likely occurrence of cross contamination. Bacterial 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 as defined by Erickson *et al.* [3]. Cross contamination of food borne pathogens is a major concern since it increases the health risk for humans due to the intake of contaminated food [3, 11, 12]. Therefore, some of the drink's orifices are not safe. The bacteria identified in this study are potential opportunistic pathogens that may become infectious when the situation arises. Opening the orifices of drinks with the

teeth therefore could favour cross contamination. The presence of these microorganisms and its high occurrence in samples is a serious health risk because of their ability to cause a wide variety of infection.

Staphylococcus aureus is known to cause food poisoning, infections of the upper respiratory tract and skin abscesses [9, 12]. Toxin production is common to this microbe. An estimated 20% to 30% of the human population are long-term carriers of *S. aureus* which can be found as part of the normal skin flora, in the nostrilsand as a normal inhabitant of the lower reproductive tract of women [9]. *Bacillus* species are endospore-forming aerobic or facultatively anaerobic. The spores are resistant to heat, cold, radiation, desiccation, and disinfectants. *Bacillus* species are mostly associated with deadly food poisoning. Enterococci are part of the normal intestinal flora of humans and animals. They have been long recognized as important human pathogens [1].

Conclusion

The orifices of the bottles of soft drinks sold in the study area harbored some opportunistic pathogens on the surfaces, thereby posing health risk to the users. Across the drink types, some samples contained very low bacterial load (100 cfu/ml), while other samples had higher bacterial load than the approved threshold for food containers. Three groups of bacteria identified were *Staphylococcus aureus, Bacillus* spp and *Enterococcus* spp, the most frequently occurring in samples being *S. aureus*. The identified bacterial have clinical implications and they are of public health concern. This calls for appropriate measures to monitor and decontaminate orifice of soft drinks for the safety of consumers.

Declaration of conflicting interests

The authors declared no potential conflicts of interest

References

[1]. Abdullahi, I.O. and Abdulkareem, S., 2010. Bacteriological quality of some ready to eat fruits as retailed and consumed in SabonGari, Zaria Nigeria. Bayero.*Journal of Pure and Applied Science* 3, 173 175.

[2]. Adelana, F.O., 2004. Spoilage of fruits, vegetables and tuber crops. *Nigeria Food Journal* 2, 1-5.

[3]. Erickson, M. C., Liao, J., Cannon, J. L., and Ortega, Y. R., 2015. Contamination of knives and graters by bacterial foodborne pathogens during slicing and grating of produce. *Food Microbiology* 52, 138–145.

[4]. Hassan, A., Utku, O. and Koray, K., 2006. Determination of total aerobic and indication.Bacteria on some raw eaten vegetables and fruits from wholesalers in Ankara, turkey. *International Journal of Hygiene* and *Environmental Health* 209, 197-201.

[5]. Cheesbrough, M., 2006. District Laboratory Practice in tropical countries, part 2.Cambridge University Press, Cambridge, UK.137-150.

[6]. Alegre, I., Abadias, M., Anguera, M., Oliveira, M., and Vioas, I., 2010. Factors affecting growth of foodborne pathogens on minimally processed apples. *Food Microbiology* 27, 70–76.

[7]. Bae, Y. M., Baek, S. Y., and Lee, S. Y., 2012. Resistance of pathogenic bacteria on the surface of stainless steel depending on attachment form and efficacy of chemical sanitizers. International *Journal of Food Microbiology* 153, 465–473.

[8]. Bryan, V.C., Mandelon, F., Dean, O.C., 2006. Fruits consumed in Manhatan. *Public Health Reports* 118, 470 476.

[9]. Vilar, I., Garcia, M.L., Fontan, B.,Tornadijo, M.E. and Carballo, J., 2000. Studies on food contamination.*Journal of Applied Microbiology* 89, 1018-1026.

[10]. De Candia, S., Morea, M., and Baruzzi, F., 2015. Eradication of high viable loads of Listeria monocytogenes contaminating food-contact surfaces. *Frontier of Microbiology* 6, 733-738.

[11]. Ray, B., 2004. Fundamental Food Microbiology, 3rd Edition, .CRC Press, Pp 439-534.

[12]. Uzeh, R.E., Alade, F.A., Bankole, M., 2009. The bacteria quality of prepacked mixed fruits and vegetables in retailoutlets in Lagos, Nigeria. *African Journal of Food Science* 3, 270-275

Cite this article

Yandev D., Onwuka A.U. and Msoo A. (2021). Studies on Bacterial Contaminants on the Bottle Orifice of Opened Non-Alcoholic Drinks In Mkar-Gboko, Benue State. *.FUAM Journal of Pure and Applied Science*,**1**(**2**):20-28