



## Bacterial contaminants associated with soft drinks sold in Port Harcourt

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### ABSTRACT

Ten (10) brands of industrially manufactured Soft Drinks in Nigeria were randomly selected and subjected to bacteriological analysis by the pour plate count method. 1ml of each brand of the soft drinks were dispensed into different sterile Petri dishes while 12mls of molten Nutrient Agar and MacConkey Agar respectively were poured into the petri dishes at about 37°C, thoroughly mixed, allowed to set and incubated aerobically at a temperature of 37°C for 24 hours. The number of colonies was counted for viable cells per ml of the original sample. The colonies isolated from the samples include Ribena: *Staph aureus* 11(2.6%), *E.coli* 2(19%), *Klebsiella pneumonia* 2(3.2%); Pepsi *Staph aureus* 0(0%), *E.coli* 0(0%), *Klebsiella pneumonia* 0(0%). Coke: *Staph aureus* 0(0%), *E. coli* 29(27.8%), *klebsiella pneumonia* 0(0%). Schweppes: *Staph aureus* 57 (13.4%), *E.coli* 0(0%), *Klebsiella pneumonia* (0)0%. Lucozade boost: *Staph aureus* 3(0.7%), *E. coli* 0(0%), *Klebsiella pneumonia* 0(0%), Malta: *Staph aureus* 48 (11.3%), *E.coli* 41(39.4%), *klebsiella pneumonia* 0(0%). Sprite: *Staph aureus* 24 (5.6%), *E.coli* 32(30.7%), *Klebsiella pneumonia* 0(0%), orange juice: *Staph aureus* 0(0%), *klebsiella pneumonia* 0(0%). Malta Guinness: *Staph aureus* 92 (21.7%), *E. coli* 0(0%) *klebsiella pneumonia* 61 (98.8%) Fanta: *Staph aureus* 189 (44.6%), *E.coli* 0(0%), *klebsiella pneumonia* 0(0%). The highest number of colonies was found in fanta while lucozade boost yielded the lowest number of colony, pepsi and orange juice, showed no growth. *Staph aureus* had the highest prevalence while *klebsiella pneumonia* yielded the lowest number of organism. It is also recommended that regulatory agencies should ensure strict adherence to established standards, manufacturers should ensure that the soft drinks must be free from contaminants, and workers should regularly be screened.

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### Introduction

The term soft drink was originated to distinguish non-alcoholic beverage from hard liquor or spirits. Soft drink is non-alcoholic carbonated or non-carbonated beverages usually, containing a sweetening agent, edible acids, natural or artificial flavour. Soft drinks include cola beverages, fruit flavoured drink, and root beer (Riley, 1990).

The adjective “soft” specifies a “lack of alcohol by way of contrast to the term “hard drink”. The word drink, while nominally neutral, sometimes carries connotations of alcoholic content. Beverages like colas flavoured water, sparkling water, sweet tea, lemonade, squash and fruit punch are among the common types of soft drinks, while hot chocolate, hot tea, milk and milk shakes do not fall into this classification. Many carbonated soft drinks are optionally available in version sweetened with sugars or with non-calorie sweeteners. It should also be noted that carbonated soft drinks are commonly known as soda, soda pop, coke, cola or tonic in various parts of the world.

In May, 1886, coca cola was invented by Doctor John Pemberton, a pharmacist from Atlanta Georgia. John Pemberton concocted the coca cola formula in a three – legged brass little in his backyard. The name was a suggestion given by John Pemberton’s book- keeper Frank Robinson. Frank was the first man who scripted “coca cola” into letters which has become the famous logo of today. The soft drink was first sold to the public at the soda fountains in Jacobs’ Pharmacy in Atlanta May 8, 1886. Until 1905, the soft drink contained extracts of cocaine as caffeine rich kola nut (Rossant, 2005).

The beverage market is the main use of high potency sweetener after a fast growing between 1983 and 1990. The market for low calorie soft drink has recently shown signs of slowing down. However, diet sales showed 992% and 2.6% growth in 1990 and 1991, respectively, (Maxwell, 1992).

The growth was aided by factors including the increase in advertising activity, the introduction of new products and packaging and better economic conditions, the hot and humid weather experienced in Nigeria, for most of 2005, also boosted soft drinks sales. Many Nigerians consume about 2-8. Bottles a day and remain unaware of the health implications of their actions (Oluwande, 1997).

The benefits and demands for soft drinks in Nigeria have risen in recent years for dehydration and social activities. These coupled with water scarcity, especially in developing countries, due to drought, unavoidable hydrological factors, conflicts, ignorance and aggressive promotion mounted persistently by manufactures, often with enticing rewards are largely responsible for excessive consumption of

Soft drinks with little or no regards to the health implications associated with it (Escheat. 1980). Whereas the production of soft drinks and other beverages is governed by ethical codes and standard to ensure that potential hazard, especially those of bacterial origins are eliminated (Maunder, 2006). The overwhelming desire to maximize profit at all cost leads to sub-standard products which could be detrimental to the health of consumers (Oranusi et al, 1994).

Often recycled bottles may not be properly sterile and there could be collection of debris or sediment of finished products. In

some instances, whole or parts insects and other organisms have been recovered from of the soft drinks, causing serious psychological trauma and the possibility of food poisoning in consumers. In August 2006, after a controversial report about the presence of pesticides in soft drinks sold in India many state governments have issued a ban on the sale of soft drinks in schools (Majumder, 2006). Food borne diseases represents one of the most widespread and overwhelming "public health problems of the modern world (WHO, 2000).

This study was carried out to determine the bacterial load of contaminated soft drink sold in Port Harcourt, to isolate the contaminating bacteria in the soft drinks and to identify bacteria of medical importance in soft drinks sold in Port Harcourt.

## Materials and methods

### Study Area and Population

The study was carried out in Port Harcourt Municipal Council of Rivers State between the months of July, August and September 2013. Port Harcourt is located at the topical south south. (5.28800sq kilometers) Annual rainfall of 147.3meters. Port Harcourt has topical climate with two equal seasons, rainy season from March to October and dry season from November to March.

The area is dominated by Ikwerre with other ethnic scattered across the place and freedom of worship exists in the area, it is also a civil servant dominated capital city with exploration of oil and gas.

### Sample Collection

Ten brands of locally produced soft drinks named brands Ribena, Pepsi, Coke, Schweppes, Lucozade Boost, Maltina, Sprite, Orange Juice, Malta Guinness and Fanta, actual trade names protected were selected from a near-by shop and bought unrefrigerated.

Ten (10) bottles of each of the brands were collected and processed within a period of three months using the standard pour plate count method.

### Bacteriological Analysis of Soft Drinks

#### Processing and Cultivation

The methods adopted for the bacterial analysis of soft drinks (commercially bottled non-alcoholic drinks produced in Nigeria) was adopted from S.U Oranusi, L.I Ezogu and B.N. Okolo (Oranuso et al., 1994).

#### Determination of viable bacterial counts

The determination of total viable bacterial count of the ten brands was done using the standard pour plate count method.

One millimeter (1ml) aliquot of each brand of the soft drink were dispensed into sterile petri dishes with a 2mls sterile syringe and 12mls of molten Nutrient Agar was also poured into the petri dishes at about 37°C, the soft drink and molten Nutrient Agar was thoroughly mixed by rocking the plates on the working bench for about thirty (30) seconds for bacterial counts and allowed to set, the poured plate were immediately incubated aerobically at a temperature of 37°C for 24hours. The procedure was also done using MacConkey agar. After twenty four (24) hours the plates were brought out for bacteria growth and counted. The number of colonies that arose after a day incubation was counted to yield the number of viable cells per ml of the original sample.

#### Identification of bacterial isolates

Stock culture of the isolates with different cultural characteristics was made on nutrient agar slants. Gram staining was used to check for morphology and biochemical tests were performed to aid in identification.

Various tests performed and used in probable identification of isolates included the Gram staining procedure, Oxidase test,

Motility test, catalase test, Coagulase test, Indole test, Methyl red test, Voges- Proskauer and Citrate utilization test as described by Cheesbrough (2000).

## Results

The total number of colony in each soft drink include Ribena 15(2.5%), Pepsi 0(0%), Coke 29 (4.9%), Schweppes 57 (9.6%), Lucozade Boast 3 (0.5%), Maltina 89(15.1%), sprite 56(9.5%), orange juice 0(0%), Malta Guinness 153(25.8%) and Fanta 189 (31.9%) as shown in table 1 below. Also **no. of growth of organism (%)** in Ribena 3(23.1%), Pepsi 0(0%), Coke 1(7.7%), Schweppes 1(7.7%), Lucozade boast 1(7.7%), maltina 2(15.4%), Sprite 2(15.4%), orange juice 0(0%), malta Guinness 2(15.4%), Fanta 1(7.7%), Total 13(100%).

**Table 1: Total number of colony in each soft drink**

SOFT DRINKS	COLONIES (%)	no. of growth (%)
Ribena	15 (2.5%)	3(23.1%)
Pepsi	0(0%)	0(0%)
Coke	29 (4.9%)	1(7.7%)
Schweppes	57 (9.6%)	1(7.7%)
Lucozade	3 (0.5%)	1(7.7%)
Maltina	89 (15.1%)	2(15.4%)
Sprite	56 (9.5%)	2(15.4%)
Orange juice	(0%)	0(0%)
Malta Guinness	153 (25.8%)	2(15.4%)
Fanta	189 (31.9%)	1(7.7%)
<b>Total</b>	<b>591 (100%)</b>	<b>13(100%)</b>

The number of organism per colony in Ribena showed *Staph aureus* was 11 (2.6%), *E.coli* is 0(0%) and *Klebsiella pneumonia* is 0(0%). Coke *Staph aureus* is 0(0%). Schweppes *E.coli* 0(0%), *Staph aureus* is 57(13.4%). Lucozade boost *Staph aureus* is 3(0.7%), *E.coli* 0(0%), *Klebsiella pneumonia* is 0(0%). Malta *Staph aureus* is 48(11.3%), *E.coli* is 41(39.4%), *Klebsiella pneumonia* is 0(0%). *Staph aureus* is 24(5.6%), *E.coli* is 32 (30.7%), *Klebsiella pneumonia* is 0(0%), Orange Juice *Staph aureus* is 0(0%), *E.coli* is 0(0%), *Klebsiella pneumonia* is 0(0%). Malta Guinness *Staph aureus* is 92(21.7%), *E.coli* is 0(0%), *klebsiella pneumonia* is 61 (96.6%). Fanta *Staph aureus* is 189 (44.6%), *E.coli* is 0(0%), *Klebsiella pneumonia* 0(0%). *Staph aureus* produced the highest number while *klebsiella pneumonia* yielded the lowest number of organism as shown in table 2 below.

**Table 2: Number of organisms per colony**

Soft drinks	<i>Staph aureus</i>	<i>E .coli</i>	<i>Klebsiella pneumonia</i>
Ribena	11 (2.6%)	2(1.9%)	2(3.2%)
Pepsi	0(0%)	0(0%)	0(0%)
Coke	0(0%)	29(29.8%)	0(0%)
Schweppes	57(13.4%)	0(0%)	0(0%)
Lucozade boast	3(0.7%)	0(0%)	0(0%)
Maltina	48(11.3%)	41(34.4%)	0(0%)
Sprite	24(5.6%)	32(30.7%)	0(0%)
Orange juice	0(0%)	0(0%)	0(0%)
Malta guinness	92(21.7%)	0(0%)	61(96.8%)
Fanta	189 (44.6%)	0(0%)	0(0%)
<b>Total</b>	<b>424 (100%)</b>	<b>104 (100%)</b>	<b>63 (100%)</b>

Ribena *E.coli* is 1 (25%), *Staph aureus* is 1 (14.3%), *klebsiella pneumonia* is 1 (50%), Pepsi *E.coli* is 0(0%), *Staph aureus* is 0(0%), *klebsiella pneumonia* is 0 (0%), Coke *E.coli* is 1(25%), *Staph aureus* is 0(0%), *klebsiella pneumonia* 0(0%), Schweppes *E.coli* is 0(0%), *Staph aureus* is 1 (14.3%), *Klebsiella pneumonia* is 0(0%). Maltina *E.coli* is 1(25%), *Staph aureus* is 1 (14.4%), *Klebsiella* is 0(0%). Sprite *E.coli* is 1(25%), *Staph aureus* is 1 (14.3%), *Klebsiella pneumonia* is 0(0%). Malta Guinness *E.coli* is 0(0%), *Staph aureus* 1 (14.5%), *Klebsiella pneumonia* 1(50%), Fanta *E.coli* (0%), *Staph aureus*

is 1(14.3%) *Klebsiella pneumonia* is 0(0%). Total *E.coli* is 4(100%), *Staph aureus* is 7(100%) *klebsiella pneumonia* is 2(100%).

**Table 3: The occurrence of bacteria in the various brands**

Soft drinks	<i>Staph aureus</i>	<i>E .coli</i>	<i>Klebsiella pneumonia</i>
Ribena	1 (14.3%)	1 (25%),	1 (50%)
Pepsi	0(0%)	0(0%)	0(0%)
Coke	0(0%)	1(25%),	0(0%)
Schwepes	1(14.3%)	0(0%)	0(0%)
Lucozade boast	1 (14.3%)	0(0%)	0(0%)
Maltina	1 (14.3%)	1(25%)	0(0%)
Sprite	1 (14.3%)	1(25%)	0(0%)
Orange juice	0(0%)	0(0%)	0(0%)
Malta guiness	1 (14.3%)	0(0%)	1(50%)
Fanta	1 (14.3%)	0(0%)	0(0%)
<b>Total</b>	<b>7(100%)</b>	<b>4 (100%)</b>	<b>2 (100%)</b>

### Discussion

Industrially produced soft drink consists of two (2) major components. The concentrates and water components while the concentrates could easily be sterilized, the water component must pass through stringent measures of purification in compliance with the WHO (World Health Organization) guideline for drinking water that stipulates zero coliform count (cfu/ml). The result of this study has shown the presence of bacterial contaminants in some of the soft drinks assessed. This is similar to study of Majumder, (2006). Food borne diseases represents one of the most widespread and overwhelming "public health problems of the modern world (WHO, 2000).

Locally, these soft drinks are also required to meet the standard prescribed by the Institute of Public Analysis of Nigeria (IPAN) for soft drinks (beverages) and allied product of nil coliform bacteria. Non- alcoholic beverages are highly prone to microbial contamination. While high – level microbial contamination can cause economic loss through product spoilage and consumer rejection, lower and usually inconspicuous levels may, if in controlled, pose grave health problems of poor prognosis (Oranusi et al., 1994).

Furthermore, the contribution of human factors in the bacterial contamination of soft drinks investigated in this study is highlighted by the higher rate of contamination by *Escherichia coli* and *Bacillus* species and this could be probably traced to human beings working in these soft drink production factories as suggested by Baid Darker, (1980).

### Conclusion

Based on the findings, the soft drinks contained bacteria and can cause food poisoning. It is recommended the National

Agency for Food and Drug Administration and Control (NAFDAC), Standard Organization of Nigeria (SON) and similar law enforcement agencies should ensure that both local and international standards for drinks production are satisfactorily met while the public should be enlightened about the dangers of the consumption of soft drinks contaminated with bacterial pathogens while workers directly involved in production should maintain good personal hygiene and should be well protected.

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