Original Research Article

Effects of fizzy drinks on blood sugar level, sputum and enteric bacterial populations in healthy human beings

ABSTRACT

Fizzy drinks are non-alcoholic beverage typically containing water, sweetener, carbon dioxide, acidulates, flavourings, colourings, chemical preservatives, antioxidants and/or foaming agents. They are usually characterized by high C/N ratio and low pH, which allows the growth of specific microbial groups while limiting others. Blood sugar level, sputum and faecal samples were collected from twenty healthy students (pre and post-fasting) and analysed microbiologically to determine the effect of the consumption of fizzy drinks on the body's enteric bacteria population.Samples were processed and cultured using pour plate and streak plate techniques in three selective media consisting of Maconkey agar, Salmonella Shigella agar and Eosin Methylene Blue agar. Differentiation and isolation of various isolates were based on gram-staining technique after culturing for 24 hours at 37ºC and biochemical reactions using MICROBACT 12A/12B and 24E identification kits. Following consumption of fizzy drinks 35cl for female and 60cl for male, results show there was no significant difference between pre and post fasting blood sugar level (84.2 and 85.7) and (90.7 and 118.1) mg/dl in males and females respectively. In females, post consumption blood sugar level was higher (86.6 to 82.0) mg/dl in Bigi Lemon group compared with lower post consumption blood sugar level (84.8 to 86.4) mg/dl in Bigi Orange group. Among males, post consumption blood sugar level was higher in both Coke and Fanta groups (93.6 to 123.8 and 87.8 to 112.4) mg/dl respectively.The average oral microbial load from sputum samples reduced in both males and females (1.90 to 0.93× 10^5) and (1.52 to 0.44× 10^5) cfu/g respectively post consumption. Post consumption, sixty-three bacterial isolates comprising eight organisms in sputum with the following percentageprevalence were detected, namely:*Micrococcus varians* (18.18%), *Staphylococcus saprophyticus*(3.030%), *Micrococcus acidophilus* (21.212%), *Bacillus macerans* (18.18%), *Bacillus cereus* (9.09%), *Staphylococcus aureus* (9.09%), *Streptococcus pyogenes* (3.030%) and *Bacillus subtilis* (18.18%), while in faecal samples with the following bacteria with the following percentage

prevalence were isolated: *Staphylococcus aureus* (7.94%), *Escherichia coli* (25.40%), *Klebsiella* (20.63%), *Shigella* (17.46%) and *Salmonella* (28.57%).

Keywords: Fizzy drinks, blood sugar, enteric bacteria, oral bacteria, faecal samples, Coke

INTRODUCTION

Fizzy drinks are enormously popular beverages consisting primarily of carbonated water, sugar, and flavourings. A soft drink is a non-alcoholic beverage typically containing water often carbonated and a flavouring agent. They are usually characterized by high C/N ratio and low pH, which allows the growth of specific microbial groups, such as acetic and lactic acid bacteria, moulds and yeast. Soft drinks typically contain water, sweetener (8-12%, w/v), carbon dioxide (0.3- 0.6% w/v), acidulates (0.05-0.3% w/v), flavourings (0.1-0.5% w/v), colourings (0-70 ppm), chemical preservatives (lawful limits), antioxidants (<100 ppm), and/or foaming agents (Example; saponins up to 200mg/ml) (Pranita*et al*., 2015). Fizzy drinks are of liquid containing bubbles and make a hissing sound. It is a non-alcoholic carbonated beverage. Fizzy drinks are also water into which Carbodioxide gas under pressure has been dissolved, a process that cause the water to become effervescent, and the process is called carbonation (Razia, 2013). Fizzy drinks are also called soft drinks. It contains carbonated water, a sweetener, and flavouring (natural or artificial). Nearly 200 nations enjoy the sweet, sparkling soda with an annual consumption of more than 34 billion gallons. Soft drinks rank as America's favourite beverage segment, representing 25% of the total beverage. In the early 1990s, per capital consumption of soft drinks in the United States was 49 gallons which was 15 gallons more than the next most popular beverage water. The sweetener may contain sugar; high-fructose corn syrup, fruit juice, sugar substitute or some combination of these. Soft drinks may also contain caffeine, colourings, preservatives and other ingredients. Soft drinks may contain small amount of alcohol but the alcohol may contain not less than 0.5% of the total volume. Soft drinks maybe served chilled, over ice cubes or at room temperature. In rare cases such as Pepsi can be served warm. Soft drinks can be served in various formats, including cans, glass bottles and plastic bottles (Emmins, 1991). The origin of soft drinks lies in the development of fruit-flavoured drinks. The term soft drink was originated to distinguish the flavoured drinks from hard liquor or distilled spirits. The first marketed soft drinks appeared in the 17th century as a mixture of water and

lemon juice with honey. In 1676, the *Compagnie des Limonadiers* was formed in Paris. Marketers carried tanks on their backs and dispensed cups of the soft drinks to thirsty Parisians (Mark *et al*., 2017).

Sugar is a simple, crystalline, edible, carbohydrate and comes in a variety of forms, all of them are sweet. Our bodies digest carbohydrates into glucose, a simple sugar that can easily convert to energy (Nazia, 2016). Blood sugar or blood glucose refers to sugar that is transported through the bloodstream to supply energy to all the cells in our bodies (Nazia, 2016). Blood sugar or blood glucose is the main sugar found in the body. It comes from the food you eat, and it's the body's main source of energy. The blood carries glucose to all of your body's cells to use for energy (United State National Library of Medicine *et al*., 2017).

Blood sugar concentration or Glucose level refers to the amount of glucose present in the blood of a human, in humans the blood glucose level is maintained at a reference range between about 3.6-5.8Mm. It is tightly regulated as a part of metabolic homeostasis (Vidyadhar, 2016). The blood sugar level, blood sugar concentration or blood glucose level is the amount of glucose present in the blood of humans and other animals. Glucose is a simple sugar and approximately 4 grams of glucose are present in the blood of humans at all times. The normal blood glucose level (tested while fasting) for non-diabetics, should be between 3.9 and 5.5mmol/L (70-100mg/dL). Blood sugar levels for those who are not fasting should be below 6.9mmol/L (125mg/dL) (Davidson et al, 2011). The American diabetes association's goal for blood sugar control in people with diabetes is 70-130mg/dL before meals and less than 180mg/dL after meals (Cassoobhoy, 2017).

Blood sugar or glucose is the main sugar found in the body. It comes from the food you eat, and it's the body's main source of energy. The blood carries glucose to all of your body's cells to use for energy (U.S National Library of Medicine *et al*., 2017).

Glucose is precious fuel for all cells in the body when it's present at normal levels, but it can behave like a slow-acting poison. High levels slowly erode the ability of cells in your pancreas to make insulin. The organ overcompensates and insulin stays too high. Over time the pancreas is permanently damaged. High levels of blood sugar can cause changes that lead to a hardening of

the blood vessels called atherosclerosis; almost any part of the body can be harmed by too much sugar.

Damaged blood vessels cause problems such as: Kidney failure requiring dialysis, Strokes, Heart attacks, Loss of vision or blindness, Weakened immune system, with a greater risk of infections, Nerve damage, also called Neurotherapy, that causes tingling, pain, less sensation in your feet, legs and hands, Poor circulation to the legs and feet, Slow wound healing and the amputation in rare cases (Cassoobhoy, 2017).

MATERIALS AND METHODS

The study area was carried out in Crawford University, Ogun state in south western Nigeria. The total population is 3,751,140 with a coordinate of 6.9075°N, 3.5813°E. Samples (faecal material, sputum and blood) were collected from Crawford University students.

SAMPLE COLLECTION

Twenty students (10 males and 10 females) from Crawford University made up the sample size. Blood, sputum and faecal material samples from students and taken to the laboratory for analysis.

Blood sugar test was done before the fizzy drinks (Bigi lemon, Bigi tropical, Coca-cola and Fanta) were taken at the early hours of the morning by 6:am for pre and 7:am for post and samples were taken from the patient as the pre and the same patient was given fizzy drinks (Bigi Lemon, Bigi Tropical, Coca-cola and Fanta) to drink and samples were collected an hour later as the post. Faecal material and sputum samples (Pre and Post) were collected from healthy patients within Crawford University and taken to the laboratory for analysis.

SAMPLE PROCESSING AND MICROBIAL ANALYSIS

For the microbiological analysis, the samples from the sterile swab stick were cultured in peptone water and incubated for 3hrs, at 37°C. After incubation, the growth was then sub-

cultured into three different agars, MacConkey Agar (MA), Eosin Methylene blue agar (EMBA), Salmonella Shigella Agar (SSA), for 24hrs at 37°C.

For the microbial analysis, samples from the sputum, serial dilution was carried out on the samples in ten-fold serial dilution and cultured on a nutrient agar plate for 24hrs at 37°C and sub-cultured into MacConkey Agar (MA), Eosin Methylene blue agar (EMBA), Salmonella Shigella Agar (SSA) for 24hrs at 37°C.

Gram-staining test was carried out using gram-staining technique for each isolate to determine if it was gram-positive or gram-negative.

Biochemical tests were done to confirm the specific organisms isolated.For female individuals 600mle of Bigi Lemon and Tropical was administered, while for the male 35cl of Coca-cola and Fanta was administered for the male.

RESULTS

A total number of 63 isolates from both sputum and faecal material were obtained from the samples (sputum and faecal material) of 20 individuals in Crawford University.

A few of the samples had no growth majorly on Salmonella Shigella agar and a little on MacConkey agar, Eosin Methylene Blue agar for the sputum samples. The rest of the samples grew well on each media for the faecal material sample.

Total result for blood sugar test

The blood sugar result for female (pre and post) showed that 4 student's blood sugar decreased after an hour of consuming the fizzy drink, while the others increased after consuming the fizzy drink. For the males, 4 student's blood sugar reduced after an hour of taking the drink, and the rest increased after an hour of consumption of fizzy drinks. The result showed that the male individual has the highest rate of blood sugar as shown in table 1.

Total plate count of colony for bacteria in sputum samples:

The microbial load of the sample varied with sex (male and female). The sputum sample collected from 20 students before drinking the fizzy drink shows that the microbial load ranging from $1.0 \times 10^4 – 5.00 \times 10^5$ and after taking the fizzy drinks shows microbial load ranging from $1.0 \times 10^4 – 1.40 \times 10^5$ and for the male before taking the fizzy drinks shows a microbial load ranging from$3.0 \times 10^4 – 5.00\times 10^5$ and after taking the fizzy drink shows a microbial load ranging from $2.0 \times 10^4 – 1.00 \times 10^5$. This result shows that the male has the lowest microbial load count and the female have the highest has the highest microbial load count as shown in table 2.

Appearance of organisms on different media:

The organism that grew on Eosin Methylene Blue agar was a green metallic sheen, purple black and dry pinkish coloration on MacConkey agar which is *Escherichia coli*. On MacConkey agar and is a smooth circular and mucoid with pink coloration is *klebsiella.* On *Salmonella-Shigella* agar is a black coloration all over the plate indicates *salmonella*. On MacConkey agar pale and yellowish colonies indicate *Shigella*. On MacConkey agar, Golden yellow indicates *Staphylococcus aureus*.

Biochemical characterization of isolated media:

Table 3, shows the result of different biochemical test used in identifying the isolates. A total of 5 organisms were identified from the isolates. *Salmonella* was found to have the highest and most occurring bacterium identified and *Escherichia coli, Klebsiella, Shigella* and *Staphylococcus* were the other bacterium identified. The biochemical test carried out on the organisms include: Kovac'sindole test, Simmon'scitrase test, Triple iron sugar test, Catalase test and gram staining.

Percentage distribution of isolated bacteria:

This result shows the percentage of the presumed organism isolated and these include: *Salmonella* 28.57%, *Escherichia coli* 25.4%, *Klebsiella* 20.65%, *Shigella* 17.46%, *Staphylococcus aureus* 7.94%, as shown in table 3. After identification of the isolated bacteria, the following organisms were identified which includes: *Micrococcus varians* 18.18%, *Staphylococcus saprophyticus* 3.030%, *Micrococcus acidophilus* 21.212%, *Bacillus macerans*

18.18%, *Bacillus cereus* 9.09%, *Staphylococcus aureus* 9.09% *Streptococcus pyogenes* 3.030% and *Bacillus subtilis* 18.18% as shown in Fig 1

Identification and characterization of isolates using microbact identification system:

After the use of biochemical tests to detect implicated organisms in the samples, the confirmation of the isolates was carried out using the MICROBACT 12A/12B and 24E IDENTIFICATION as shown in Fig 2

Table 1: Blood sugar results of individual before and after taking the fizzy drinks.

Blood sugar (before) **Mg/Dl**	**Blood sugar (after)** **Mg/Dl**	**Type of fizzy drink**	**Sex (male/female)**
88	84	Bigi Orange	F
89	90	Bigi Orange	F
79	88	Bigi Orange	F
90	91	Bigi Orange	F
86	71	Bigi Orange	F
99	82	Bigi Lemon	F
80	75	Bigi Lemon	F
84	107	Bigi Lemon	F
84	77	Bigi Lemon	F
63	92	Bigi Lemon	F
94	112	Fanta	M
72	152	Fanta	M
67	82	Fanta	M
129	119	Fanta	M
77	97	Fanta	M
100	85	Coke	M
82	200	Coke	M
117	108	Coke	M
78	145	Coke	M
91	81	Coke	M

Table 2: Colony Count of Sputum from Samples

S/N	SPUTUM SAMPLES	MEDIA	MICROBIAL LOAD(Cfu/g) (BEFORE)	MICROBIAL LOAD(Cfu/g) (AFTER)
1.	FEMALE	NUTRIENT AGAR	$3.00 * 10^5$	$1.40 * 10^5$
2.	FEMALE	NUTRIENT AGAR	$5.00 * 10^5$	$1.00 * 10^4$
3.	FEMALE	NUTRIENT AGAR	$2.00 * 10^5$	$5.0 * 10^4$
4.	FEMALE	NUTRIENT AGAR	$2.00 * 10^5$	TNTC
5.	FEMALE	NUTRIENT AGAR	$s2 * 10^3$	$8.0 * 10^4$
6.	FEMALE	NUTRIENT AGAR	$3.00 * 10^5$	$8.6 * 10^4$
7.	FEMALE	NUTRIENT AGAR	$1.00 * 10^5$	$8.6 * 10^4$
8.	FEMALE	NUTRIENT AGAR	$2.00 * 10^5$	NG
9.	FEMALE	NUTRIENT AGAR	$1.00 * 10^5$	TNTC
10.	FEMALE	NUTRIENT AGAR	$1 * 10^3$	$2.0 * 10^5$
11.	MALE	NUTRIENT AGAR	TNTC	$1.0 * 10^5$
12.	MALE	NUTRIENT AGAR	$1.00 * 10^5$	$5.0 * 10^4$
13.	MALE	NUTRIENT AGAR	$1.00 * 10^5$	$5.0 * 10^4$
14.	MALE	NUTRIENT AGAR	$2.0 * 10^5$	$6.0 * 10^4$
15.	MALE	NUTRIENT AGAR	$1.50 * 10^5$	$2.0 * 10^4$
16.	MALE	NUTRIENT AGAR	$1.50 * 10^5$	$4.0 * 10^4$
17.	MALE	NUTRIENT AGAR	$6.0 * 10^4$	$3.0 * 10^4$
18.	MALE	NUTRIENT AGAR	$8.0 * 10^4$	$2.0 * 10^4$
19.	MALE	NUTRIENT AGAR	$3.0 * 10^4$	$5.0 * 10^4$
20.	MALE	NUTRIENT AGAR	$5.0 * 10^5$	$2.0 * 10^4$

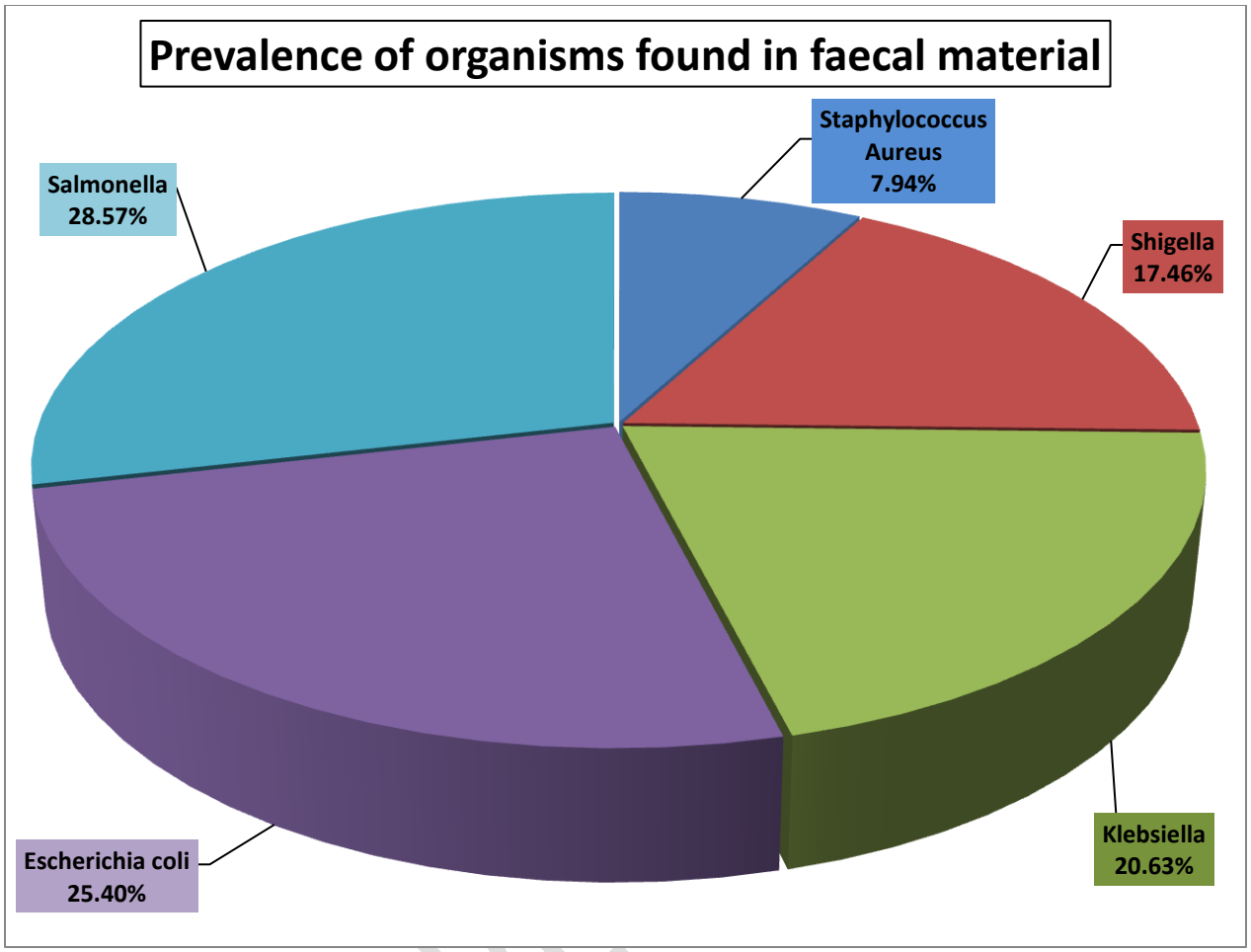

Figure 1: Presumptive Prevalence of organisms found in faecal material

Table 3: Biochemical test on suspected isolates

Organism	Catalase	Indole	Triple iron sugar test			Citrate test
			Sucrose	Lactose	Glucose	
Klebsiella	+	-	+	+	-	-
Escherichia coli	+	+	+	+	+	-
Salmonella	+	-	+	+	-	-
Shigella	+	-		NIL		-
Staphylococcus aureus	+	-		NIL		+

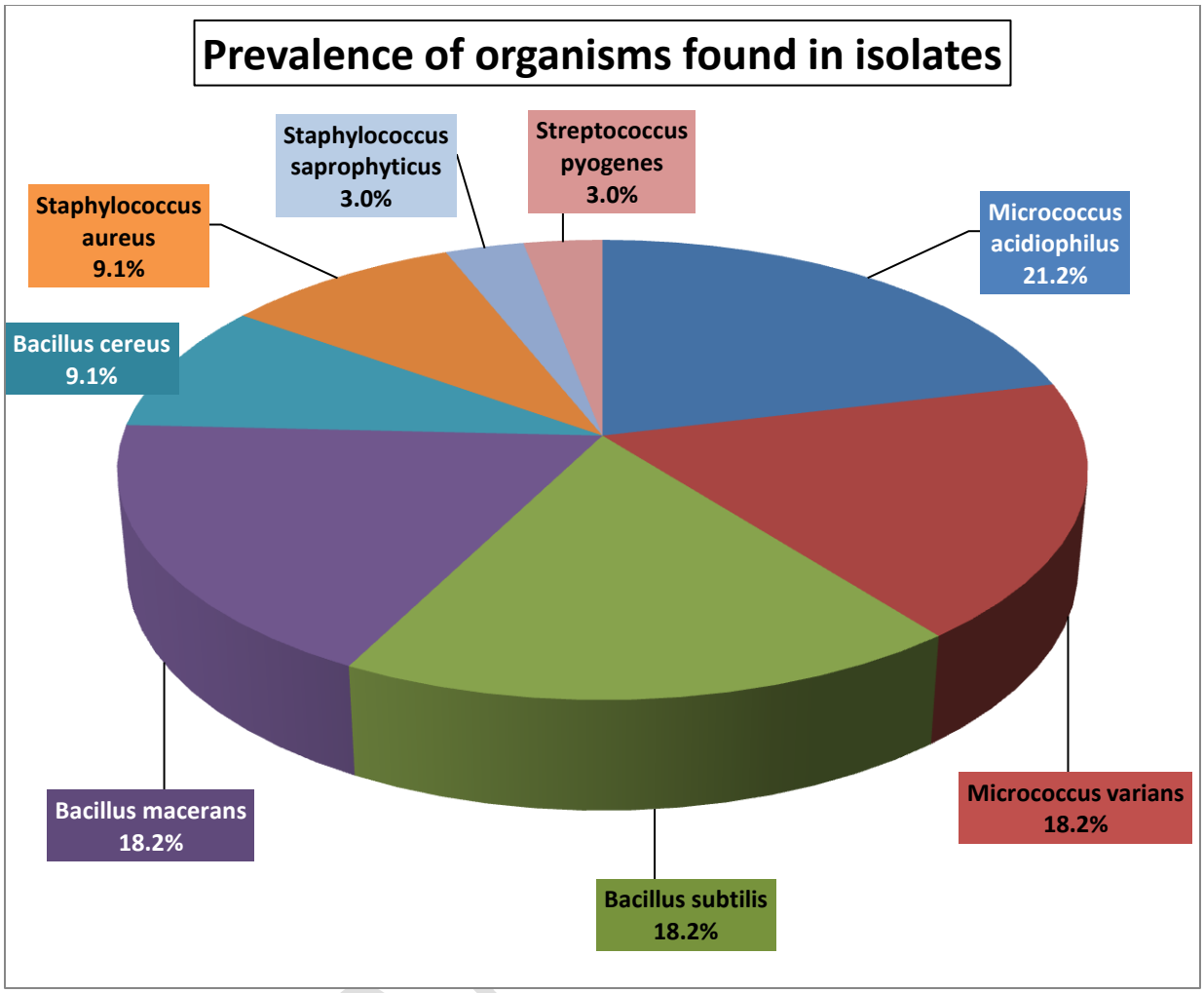

Figure 2: Prevalence of organisms found in isolates

DISCUSSION

Different microbes were isolated from different student's sputum and faecal materials and their blood sugar was also tested using Accucheck blood sugar reader of students in Crawford University, Ogun state. During the course of this work, it was observed that the blood sugar of few of the students before the consumption of the fizzy drink was low and after consuming the drink was high and vice-versa. For the sputum and faecal material, it was observed that not only enteric bacteria grew but other bacteria grew which is as a result of the drink taken by the individual. Different student's body exhibited different result in the effect of the fizzy drink in the blood sugar test, sputum and faecal material samples.

Since ancient times, the consumption of fizzy drinks has been known to be of high prevalence especially among youths. In recent research, fizzy drinks have shown a high rate of side effect when consumed regularly and has its health implications on the human body. Not all individuals have the ability to degrade sugar fast or have a fast metabolism and in turn do not favour the enteric bacteria organism present in the body especially the digestive system which digest every food and drink taken into the body and also affect the blood of individuals as well. The knowledge of blood sugar, bacteria and fizzy drinks has developed along with the evolution of scientific research and social progress. Bacteria found in the body which aids digestion and provides shelter for such organism and when the condition at which they thrive in changes, their role wherever they find themselves changes and they try to adapt to the environment as in the case of enteric bacteria in the body.

The enteric bacteria were not really affected by the consumption of fizzy drinks at the end of the research because the normal enteric bacteria found at the beginning and end of digestion were present but a few species of organisms were present in places that is not their niche due to the fizzy drink.

This study has revealed the presence of 63 bacterial isolates comprising of Staphylococcus aureus, Escherichia coli, Klebsiella, Shigella, Salmonella and their prevalence respectively: 25.4%, 17.46%, 28.57%, 7.94%, and 20.65%. The organism with the highest prevalence is salmonella with 28.57% and the least prevalent is Staphylococcus aureus with 7.94%. The five

different species of organisms isolated, four are gram negative and one is gram positive which is Staphylococcus aureus.

CONCLUSION

Enteric bacteria are regarded as normal intestinal microflora which aids digestion and can only cause harm if their environmental condition has changed due to different factors like excessive consumption of drinks.

The rate at which the blood sugar increase due to consumption of fizzy drinks or any sweetened drink with high sugar content leads to diabetes which is among the factors contributing to high blood sugar but this is not applicable to every individual.

It also revealed that fizzy drinks don't affect the enteric bacteria population but just changes the environmental condition of the organisms and shows a significant difference in the the blood sugar of individuals.

RECOMMENDATION

The world needs to be re-orientated about the health implications of not regularly checking their blood sugar and the effect of consuming drinks excessively and how it can affect the body as a whole, most especially internally which could be a major threat to the well-being of an individual most especially the youths as they advance in age. The way fizzy drinks are consumed should be cautioned to reduce high blood pressure that can lead to other health hazards. The general public should be enlightened on the dangers of excessive consumption of fizzy drinks on an individual's ealth and can lead to diabetes especially for adults. individuals are advised to go check their health status twice a month and carry out test like sugar test, urinalysis test and other test related to the digestive system and general body check-up.

It is advised that consumption of fizzy drinks should be taken minimally like twice a week to avoid drastic increase in blood sugar which can have its health implications and cause harm to the micro flora present in the intestinal tract. Blood sugar test is advised to be done often to prevent any health implications and to know how the consumption of drinks is affecting one's health.

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