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Antibiogram of Opportunistic Pathogens isolated from the rims of commercially sold drinks in Calabar Metropolis, Cross River State, Nigeria

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ABSTRACT :

Microbiological Investigations carried out revealed the presence of Opportunistic Pathogens. Soft drinks are highly consumed beverages by majority of consumers in Calabar Metropolis. And in as much as bottle drink beverages are widely consumed by different age groups, both Children and adults, male and female alike by the Calabar residence. It is important to note that health associated problems, microbial spoilage and contamination of drinks have been reported by various researchers. This problem arises as a result of numerous factors such has poor raw materials, poor hygiene during production process, environmental contamination, improper regulation during formulations of this products, poor storage and other factors. All these factors contributed to the presence of Opportunistic Pathogens obtained from the rims of commercially sold drinks in Calabar Metropolis.

This research work identifies the different opportunistic pathogens associated with contamination and spoilage of drinks from their rims. Different Microbiological investigations was carried out to ascertain the microorganisms involve through isolation, characterization and identification. Furthermore, an antibiogram was conducted to proffer solutions to those that may fall ill as a result of ingesting the microorganisms via fecal-oral route. An antibiotic susceptibility testing (AST) was used to identify organisms susceptible to the various antibiotics introduced. This aims to reduce health risks, ensure safety of the consumer and as a tool for epidemiological research, control and analysis.

Samples were taken to the lab and stored at room temperature for microbiological analysis. A total of 18 samples were obtained from the Rims of commercially sold Bottled Drinks collected from Calabar Metropolis, Cross River State, Nigeria. Microbiological Tests carried out included total heterotrophic bacteria count (THBC), total fungi count (TFC), and total coliform count (TCC). Bacteria isolates were identified using cultural morphology, Gram staining, and biochemical tests (IMViC, Kliger's Agar, Simon citrate, etc.). The total Heterotrophic Bacteria Count (THBC) of Bottled Drinks ranged from (0.25-3.76 X10 ^{2} cfu/ml); with alcoholic drinks recording a significant growth of total fungi count (TFC) ranging from 0.05 X10^{2} cfu/ml -0.97 X10^{2} cfu/ml. The fungi isolated were *Saccharomyces spp, Candida spp, Aspergillus spp, Penicillium spp, Rhizopus spp, Trycophyton spp, mucor spp, Tricoderma spp and fusarium spp* indicating the possible presence of mycotoxins in bottled drinks.

Bacteria Isolates includes *Lactobacillus spp, Salmonella spp, Staphylococcus spp, Listeria spp, Proteus spp and Escherichia Coli* showing the presence of opportunistic pathogens in bottled drinks as a result of numerous environmental factors. The Total Coliform count (TCC) ranged from 0.09 – 13.1 X 10^{2} cfu/ml. Antibiogram carried out for both Gram- positive and Gram-negative Bacteria Isolates showed *Lactobacillus spp.* Susceptibility to most of the antibiotics. Good Manufacturing Practices (GMP) and HACCP guidelines during production, transport, and packaging. The study also recommended that regulatory agencies should conduct regular inspections of beverage industries. Also, NAFDAC should discourage re-use of bottles as they promote microbial growth and public health risks. Further epidemiological research was recommended to provide reference for outbreak situations.

KEYWORDS: Soft Drinks, Alcoholic Drinks, Microorganisms, Pathogens and Antibacterial Susceptibility.

1 Introduction

Commercially sold drinks are widely consumed by different regions of the world and these drinks are specifically and primarily consumed either due to their sweetness or to quench thirst. Bottled drinks can be classified into different groups such as Alcoholic bottled drinks beverages and Non-Alcoholic Bottled drinks beverages. Non-Alcoholic bottled drinks can be divided into soft drinks and Fruit drinks. Soft drinks are vastly popular beverages consisting basically of Carbonated water, sugar and flavors. The sweet sparkling soda is consumed by nearly 200 Nations, with about 34 gallons of it consumed annually[1]. Soft drinks are Popular beverages consumed widely by several category of people worldwide[2].The history of soft drinks could be dated back to around the ancient period. According to[3], it was discovered two thousand years ago by Romans and the Greeks after recognizing the medical value of mineral water and were using it to take their bath during relaxation, a practice that still holds till now. Soft drinks contain water, sugars, organic acids, vitamins and trace elements thus providing an ideal environment for spoilage by microorganisms; on other hand they generally have a lower PH <4.5[4].
The common feature of their potential spoilage agents is that they must be acid loving microorganisms. Yeasts are predominant because of their high acid tolerance and their ability of many of them to grow anaerobically e.g *Pichha membranifaciens, Candida maltosa, Candida sake, Sacchromyces balli, Sacchromyces bishopia, Sachromyces Cerevisae, Sacchromyces rouxii, Sacchromyces bayanes, Brettanomyces intermedius, Schizosacchromyces ponde, Torulsis Shomi, Hansenisporaguiller mondi, Schwaniomyces occidentals, Derkkera bruxcellensis*, etc[5].
Microorganisms are able to cause disease due to their ability to access their host, adhere to their hosts tissue, penetrate the host defenses and may eventually damage host tissue. Such organisms are able to overcome such harsh environmental conditions and are capable of causing disease in Man including *Bacillus spp, Staphylococcus spp, Enterococcus spp etc*[6]. The gram-negative bacteria also are indicative of probable fecal contamination and include the *Alkagenes, Psuedomonas, Escherichia Coli, Serratia and Proteus.* Soft drinks provide an ideal growth substrate for many microorganisms, given adequate supplies of the required nutrients. Apart from water, environmental necessity, typical requirements are sources of carbon(carbohydrates), Nitrogen (amino acids), phosphorus (phosphates), Calcium (mineral salts) etc.
[7]explained that microbes attach easily on the manufacturing surfaces (e,g processing pipes, feeding lines), forming biofilms, which are difficult to clean. The formation of biofilms starts from the initial attachment of the bacteria to a solid surface, formation of colonies, and differentiation of these colonies into a form of mature biofilm encased in exopolysaccharides[8].
Factors affecting microbial growth in drinks include the following;
PH and acidity: [9]had disclosed that pH and Acidity are the most important antimicrobial hurdles in bottle drinks. As a result, the risk of spoilage and growth of pathogen increases with increase in pH.
Carbonation and Oxygen: Carbonated soft drinks are generally less prone to microbial spoilage than non-carbonated bottled drinks. Carbonation may inhibit the growth of spoilage microbes by inhibition of cell division, inhibition of amino acids uptake, perturbation of cytoplasmic buffering, induction of sporulation and lowering cytoplasmic pH[10]. Other factors therefore include; Nutritional Status, Functional ingredients, ethanol, water activity and storage temperature[11-13].
Microbial health risks associated with drinks are;
Pathogenic bacteria: Maybe found due to poor hygiene and poor manufacturing process[14]. Bacteria pathogens can remain viable in carbonated soft drinks for higher periods[15]. *Pseudomonas aeruginosa and Salmonella* have been reported to have originated forward contaminated raw materials from water and surviving because of poor manufacturing process as stated by[16].
*[17]*in his works said records on outbreaks of illness caused by pathogenic microbes in alcoholic drinks have been known to be rare. E. coli 0157:H7, *Salmonella typhirum and Listeria monocytogenes* have been reported of rapidly been inactivated during fermentation of typical beer wort. Parasites and Viruses have also been associated with disease outbreaks from consumption of fruit juices. Contamination of food beverages occurs via the fecal oral routes even a few oocysts can lead to gastroenteritis with water been it most important Vehicle[18].
*[19]*opined that, viruses do not grow in foods as they need living cells for replication. However, only a few Virus particles may result in high probability of infection. Hepathitis A, Norovirus and Rotavirus could potentially transmit disease via improperly produced drinks.
Mycotoxins are fungal metabolites that cause sickness or death in people and other animals when ingested, inhaled or absorbed[20]. The relevant mycotoxins related to foods and beverages are produced by species in the genera Aspergillus, Penicillium, Fusarium, Alternaria, including; Aflatoxins, Ochratoxins A, Patulin and Fusarium toxins such has Trichothenes and Zerallenones. When present in high levels, Mycotoxins can have toxic effects ranging from acute (for example kidney or liver damages) to chronic symptoms like increased cancer risk and suppressed immune system[21]

Table 1: Health hazards associated with microorganisms in soft drinks (Juvonen *et al*. (2011).

Microbes	Hazards	Influence on human	Species	Disease Vehicle	Special Notes
Microbes	Hazards	Influence on human	Species	Disease Vehicle	Special notes
Moulds	Mycotoxins	Severe Chronic and toxicity	Penicillium, Aspergillus, Byssochamys	Fruit juices, cereals	Mycotoxins can end up in product even if no viable cells are left.
Bacteria	Food-borne infections and intoxications, allergic reactions	Variable	Salmonella, E. Coli 057: H7, L. monocytogenes	Fruit juices and concentrates, water	
Viruses	Human infections	Liver inflammation,	Hepatitis A, norovirus,	Fruit juices, water	

		gastroenteritis	rotavirus		
Protozoa	Human infections	Gastro intestinal illness	Crypto sporidium, Parvum Cr., Hominis Cyclospora, cayatenensis	Water, Fruit juices, and concentrates	
Yeasts	Fermentation products	Osmotic responses	Unknown	Fruit juices	Scientific proof missing

2. Materials and Methods

2.1 Sample collection

Duplicates samples of nine (9) different brands of bottled drinks (both alcoholic and non-alcoholic) where obtained from commercial bar shops in Calabar Metropolis, Calabar, Cross River - Nigeria. The samples were taken to the laboratory and stored at room temperature for microbiological analysis. Swabs sticks soaked in normal saline were used to swab and obtain samples from the rims and bottle cap of the bottle drinks. Antibiotics discs (maxi medical laboratory Nigeria Ltd) were obtained and found to be of analytical standards for susceptibility testing.

2.2 Microbiological analysis:

All media prepared were autoclaved at 121°C for 15 minutes.
The following media, nutrient agar (NA), MacConkey Agar (MA), normal saline, Sabouraud Dextrose Agar (SDA) and Muller Hinton where prepared and used according to manufacturer's directions.
Microbiological analysis carried out includes: Total Heterotrophic Bacteria Count (THBC), Total Fungi Count (TFC), Characterization and identification of bacteria isolates and Total m Count (TCC).

2.3 Enumeration of total heterotrophic bacterial count (THBC)

Nutrient agar was prepared and poured into 18 plates and allowed to solidify. The samples were inoculated into each plates using swab sticks, following spread plate methods. After which, the where incubated at room temperature for 24 hours. The THBC was enumerated and recorded.

2.4 Examination of total fungi

Eighteen (18) plates of Sabouraud Dextrose Agar (SDA), was prepared and used to examine the Total Fungi Count (TFC). Samples were inoculated using SDA using swab sticks. All the plates incubated at room temperature for 72 hours (3 days). Thereafter, isolates were examined for TFC and also, morphological and macroscopic examination of fungi was obtained.

2.5 Characterization and identification of bacteria isolates

Morphological different colonies of bacteria isolated were sub-cultured for characterization and identification. After sub-culturing, Characterization and identification was carried using cultural Morphological variation, Gram staining and biochemical tests.

2.6 Gram staining

This was carried out after sub-culturing in order to identify bacteria by their Gram reaction. After sub-culturing colonies of the suspected isolates were Gram stained. A smear of the test organism was made on a clean glass slide. After which it was emulsified with a drop of distilled water and passed over a flame to heat fix the smear. The slide was then flood with crystal violet and allowed for 60 seconds, after which it was rinsed off with distilled water. Iodine was then applied to act as a mordant for 60 seconds, and thereafter rinsed off. Acetone/ethanol was then added as a decolorizer and allowed for 10 seconds, and then rinsed off immediately with distilled water. The slide was then flooded with safranin, a counter stain, and then allowed for 60 seconds. After which it was rinsed off and allowed to air dry. The stained smear was then observed under the Microscope using X100 oil immersion lens magnification.

2.7 Biochemical tests

According to the procedures of [22], various biochemical tests such as IMVIC (indole, methyl red, Volges Praskeur and Simon citrate) reactions, catalase tests, KIA (Kliger Iron Agar) tests were carried out to ascertain the characteristic colonies of isolates sub-cultured, and to identify the suspected bacteria species involved.

2.8 Catalase Test:

Catalase test was carried out to identify organisms that are able to produce an enzyme called catalase. 4 mils of hydrogen peroxide (H2O2), was dropped into a sterile test tube and a colony of the isolates was dipped into the test tube using a wooden applicator. The emergence of bubbles after few seconds indicates a positive result.

2.10 An IMVIC test reaction (Indole, methyl red, Volges, Proskauer and Simmon Citrate test):

are usually used to identify Gram-negative bacteria, especially families of the Enterobacteriaceae example *E. coli, Klebsiella spp, S. typhi, Proteus spp, Pseudomonas spp, Enterobacter sp, etc.*

2.11 Indole Test:

Testing for indole is important in the identification of enterobacteria spp. This test occurs as indole positive organisms are able to breakdown the amino acids, tryptophan with the release of indole. An overnight peptone broth culture of isolates was prepared, while few drops of kovac's reagent was added. And indole production was indicated by a pink ring coloration on the surface of the broth.

2.12 Methyl red:

The test isolates were inoculated on glucose phosphate broth in test tubes. After incubating the tubes for 48 hours, drops of 0.04% methyl red indicator was added. Development of bright red color indicated positive result.

2.13 Volges proskauer:

The test isolates were inoculated on test tubes at room temperature for 48 hours. Then 3 drops of 40% KOH (Barritt Solution A) and 2 drops of 5% of alcoholic-a-naphthol (Barritt Solution B) were added to the test tubes. A pink coloration shows a positive result.

2.14 Simon, citrate:

Simon citrate test is the ability for isolates to be able to utilize citrate as their main carbon and energy source. With color change from green to blue indicating a positive result, while no change at all indicates a negative result. A saline suspension of the test organism is prepared from growth on a solid medium, using a bent rod or glass spreader. The medium is incubated at 28°C for 24 hours. The organism is able to transport citrate into the cells growing in the medium. Growth is observed on the plate medium, usually accompanied by a change from green to deep blue for a positive result, while no change in coloration or growth indicates a negative result.

2.15 Kliger Iron Agar (KIA):

Kliger Iron agar test are used mostly for differentiating certain members of the Enterobacteriaceae by the demonstration of hydrogen sulfide production, fermentation of dextrose and lactose. Fermenters usually show an acid slant/butt (yellow coloration), gas production and sometimes hydrogen sulfide production (H_2S), while non-fermenters of dextrose and lactose indicates an alkaline slant/butt (red coloration) after 24 -48 hours of inoculation of isolates into the tube slanted medium. Allow KIA to warm to room temperature prior to inoculation. A colony was picked from isolates using a wire loop and inoculated by stabbing the center of the butt and streaking the surface of the slant. Incubate aerobically at room temperature for 24 - 48 hours.

2.16 Identification of total coliforms

MacConkay agar was used for the identification of Total Coliforms. Isolates were inoculated using swabs obtained from sampling into the prepared plates and incubated at room temperature (28°C) for 24 hours. Identification of Total Coliform Count (TCC) was then obtained and observed after 24 hours.

2.17 Antibacterial susceptibility of bacteria isolates

Disc diffusion method was used to carry out sensitivity testing in accordance with the guidelines of clinical and laboratory standards institute[23]. Muller Hinton Agar was prepared into plates and allowed to solidify. After solidification, a spreader was used to Suspend and spread the sample on the agar plates. After ten minutes pair of sterile forceps was used to place the antibiotic disc, with Gram positive discs being place on isolates having Gram positive reactions and Gram-negative discs placed on isolates having Gram negative reactions.

The Gram-positive antibiotic sensitivity disc contained the following antibiotics Rocephin-25µg, Ciprofloxacin-10µg, Streptomycin-30µg, Septrin-30µg, Erythromycin-19µg, Pefloxacin-10µg, Gentamycin-10µg, Ampiclox-30µg, Zinnacef-20µg and Amoxacillin-30µg, while that of Gram-negative disc contained Augmetin-30µg, Ciprofloxacin-10µg, Septrin-30µg, Streptomycin-30µg, Ampicillin-30µg, Ceporex-10µg, Tarivid-10µg, Nalidixic Acid-30µg, Gentamycin-10µg and Pefloxacin-10µg.

All plates were allowed to pre-diffuse for about 15 minutes, after which the where incubated at 35°C for 18-24 hours with the resultant zones of inhibition diameter measured and recorded.

3.0 Results and Discussion

Results presented in Table 2 showed that the Total Heterotrophic Bacteria Count (THBC) for both alcoholic and non-alcoholic drinks ranged from 0.25 - 3.76 $X10^2$ cfu /ml. With Guinness stout (sample B) representing the highest THBC of 3.76 $X10^2$ cfu / ml, while 33 export (sample A) records the lowest THBC of 0.25 $X10^2$ cfu/ml. Significant bacteria growth were observed amongst alcoholic beverages, while less significant growth occurred amongst soft drinks.

Total Fungal Count (TFC) recorded in Table 3 ranged from 0.05 $X10^2$ cfu /ml - 0.97 $X10^2$ cfu /ml Coke (sample A) posed the highest TFC of 0.97 $X10^2$ cfu /ml while Heineken (sample A) had the lowest TFC of 0.08 $X10^2$ cfu / ml among soft drinks, while the Heineken Represented the lowest TFC in beer drinks (0.05 $X10^2$ cfu / ml). whereas the highest TFC in soft drinks was obtained from Coke Sample A) (0.97 $X10^2$ cfu /ml) and that of beer drinks being 0.60 $X10^2$ cfu/ ml from Guinness stout (sample B). characterization and identification of fungi obtained in table 4 indicated that the following fungi isolates were suspected to include: *Penicillium spp, Candida spp, Aspergillus spp, Trichoderma spp, Trychophyton spp, Saccharomyces spp, Mucor spp, Rhizopus spp and Fusarium spp.*

Table 2: Data Samples and Analysis of Total Heterotrophic Bacterial Count

Samples	THBC (10^2 cfu/ml)
Non-Alcoholic Drinks	
Coke A	1.20
Coke B	0.90
Fanta A	0.40
Fanta B	0.26
Pepsi A	2.10
Pepsi B	2.07
Sprite A	0.65
Sprite B	0.47
Malta Guinness A	0.50
Malta Guinness B	0.69
Alcoholic Drinks	
Star A	1.47
Star B	0.40
33 Export A	0.25
33 Export B	0.36
Guinness Stout A	3.50
Guinness Stout B	3.76
Heineken A	2.50
Heineken B	2.00

Table 3: Data Samples and Analysis of Total Fungal count

Samples	TFC (10^2 cfu/ml)
Non-Alcoholic Drinks	
Coke A	0.97
Coke B	0.75
Fanta A	0.08
Fanta B	0.10
Pepsi A	0.42
Pepsi B	0.63
Sprite A	0.53
Sprite B	0.50
Malta Guinness A	0.36
Malta Guinness B	0.44
Alcoholic Drinks	
Star A	0.15
Star B	0.30
33 Export A	0.45
33 Export B	0.41
Guinness Stout A	0.39
Guinness Stout B	0.60

Heineken A	0.05
Heineken B	0.12

Table 4: Identification and Character Morphology of Fungal Isolates

Colony Color	Pigments	Type Hyphae	Aerial Hyphae	Substrate	Asexual	Sporangiospore	Mycellum	*Suspected organisms*
Green	None	Septate	Septate/ branch	Septate	Many	None	Clear cottony	*Penicilluim spp*
White	Pale	Psuedo hyphae	None	None	Budding	Oval/ flat	Rough	*Candida spp*
Black	None	Septate	Powdery	Septate	Spore smooth	Flask Shape	Septate	*Aspergillus spp*
Green	None	Septate	Hyaline branch	Septate	Spore smooth	Flask Shape	White Colony	*Trichoderma spp*
Black	None	Septate	Spore smooth	Hyaline Septate	Septate	Waxy/ smooth	White cottony	*Trycophyton spp*
White	None	Septum	None	None	Budding	Spherical	Smooth	*Sacchromyces spp*
White	None	Non-Septate	Cottony	None	Colorless	Oval/ smooth	Rough	*Mucor spp*
White	None	Non-Septate	Cottony	Rhizoid	Colorless	Oval	Rough	*Rhizopus spp*
Orange	None	Sickle cell	None	None	Budding	None	Fluffy Psuedo	*Fusarium spp*

3.1 Characterization and Identification of bacterial isolates

Characterization and identification of bacterial isolates were reported in table 5. Organisms isolated includes; Lactobacillus spp, Salmonella spp, Staphylococcus spp, Listeria spp, Klebsiella spp, Citrobacter spp, Bacillus spp, Pseudomonas spp, Proteus spp and Escherichia coli. Table 5 showed that Lactobacillus spp, Listeria spp and Bacillus spp had the highest percentage of 16.7% occurrence. All Gram-negative bacteria species isolated indicated the least percentage occurrence of 5.6% except Klebsiella spp with 11.1% occurrence.

3.2 Identification of total coliforms

The Total Coliform Count (TCC) (Table 6) ranged from 0.09 X 10^2 cfu/ml - 13.1 X 10^2 cfu/ml. Malta Guinness (sample A) had the highest TCC, of 13.1 X 10^2 cfu/ml, while star (sample B) and sprite (sample B) co-incidentally having the lowest TCC of 0.09 X 10^2 cfu/ml. Guinness stout (sample A) contained the highest number of TCC (0.94 X 10^2 cfu/ml) in beer drinks, while Malta Guinness (sample A) contained the highest TCC of 13.1 X 10^2 cfu/ml for non-alcoholic drinks.

3.3 Antibacterial susceptibility of bacterial isolates

Many of the bacteria showed sensitive reactions to most of the antibiotics tested as shown in table 8. Gram-positive bacteria isolates were resistant to Erythromycin and Gentamycin. *Staphylococcus spp and Listeria spp* were highly sensitive to Pefloxacin and Rocephin antibiotics respectively. Although *Lactobacillus species* were susceptible to six (6) antibiotics (Rocephin, Ciprofloxacin, Streptomycin, Septrin, Zinnacef and Amoxacillin), therefore having the highest susceptibility of all the Gram-positive bacteria isolated. Meanwhile, the Gram-negative bacteria isolates was resistant to Nalidixic acid and Augmetin. Tarivid and Streptomycin showed high sensitivity to *Proteus and Citrobacter spp*. respectively. Whereas *Klebsiella spp* was observed to be highly sensitive to two antibiotics (Ceporex and Ciprofloxacin).

TABLE 5: Identification and Character Morphology of Bacterial Isolates

ISOLATES	GRAM RXN	MORPH.	XCAL TEST	XCAL TEST	XCAL TEST	XCAL TEST	XCAL TEST	XCAL TEST	XCAL TEST	XCAL TEST	SUSPECTED ORGANISMS
			CIT	CAT	1	MR	VP	KIA SLANT	KIA BUTT	KIA H2S	
1	+	Rods	-	-	-	-	-	NC	Y_G	+	*Lactobacillus spp*
2	-	Rods	-	-	-	+	-	NC	Y_G	+	*Salmonella spp*
3	+	Cocci	-	+	-	-	-	NC	NC	-	*Staphylococcus spp*
4	+	Rods	+	+	-	+	+	NC	NC	-	*Listeria spp*

5	-	Rods	+	-	+	+	+	NC	Y_G	-	*Klebseilla spp*
6	-	Rods	+	-	-	-	+	NC	Y_G	+	*Citrobacter spp*
7	+	Rods	-	+	-	-	-	NC	NC	-	*Bacillus spp*
8	-	Rods	+	+	-	-	-	NC	NC	-	*Pseudomonas spp*
9	-	Rods	+	-	+	+	-	NC	Y_G	+	*Proteus spp*
10	-	Rods	-	-	+	+	-	Y_G	Y_G	+	*Escherichia Collii*

KEY:

RXN= REACTION, MORPH= MORPHOLOGY, XCAL= CHEMICAL, CIT= CITRATE, CAT= CATALASE, I= INDOLE, MR=METHYL RED, KIA= KLIGER IRON AGAR, H2S= HYDROGEN SULPHIDE, $Y_{G=}$ ACID/GAS PRODUCED, NC= NO CHANGE/ALKALINE PRODUCED.

Table 6: Data Samples and Analysis of Total Coliforms obtained

Samples	TCC (10^2 cfu/ml)
Non-Alcoholic Drinks	
Coke A	0.25
Coke B	0.21
Fanta A	0.56
Fanta B	0.50
Pepsi A	0.31
Pepsi B	0.30
Sprite A	0.11
Sprite B	0.009
Malta Guinness A	13.1
Malta Guinness B	12.5
Alcoholic Drinks	
Star A	0.11
Star B	0.09
33 Export A	0.63
33 Export B	0.59
Guinness Stout A	0.94
Guinness Stout B	0.90
Heineken A	0.71
Heineken B	0.58

Table 7: Analysis of Bacterial Isolates Discovered

SUSPECTED ORGANISMS	TOTAL ISOLATES (n)	Incidence (I)	Percentage (100%) (1/n X 100/1)
Lactobacillus spp	18	3	16.7
Salmonella spp	18	1	5.6
Staphylococcus spp	18	2	11.1
Listeria spp	18	3	16.7
Klebsiella spp	18	2	11.1
Citrobacter spp	18	1	5.6
Bacillus spp	18	3	16.7
Pseudomonas spp	18	1	5.6
Proteus spp	18	1	5.6
Escherichia Coli	18	1	5.6

KEY: Percentage= Percentage Frequency

Table 8: Shows the different bacterial Isolates zone of Inhibition resistance or Sensitive to the Antibiotics introduce.

ISOLATES	ANTIBIOTICS										
	PN	CEP	OFX	NA	CN	AU	CPX	SXT	S	PEF	% Sensitivity (S/R X 100/1)
Gram Negative											
Salmonella spp	12(R)	10(R)	6(R)	8(R)	0(R)	14 (R)	17 (S)	19 (S)	O (R)	O (R)	20

Citrobacter spp	21(S)	6(R)	13(R)	19 (R)	15(S)	0 (R)	10 (R)	8(R)	25 (S)	16 (S)	50
Pseudomonas spp	17(R)	13(R)	20(S)	20 (S)	10 (R)	8(R)	21 (S)	13 (R)	12 (R)	15 (S)	50
Proteus spp	6(R)	15(S)	25(S)	6(R)	0(R)	6(R)	6(R)	6(R)	18 (S)	11 (R)	30
Escherichia Coli	0(R)	8(R)	6(R)	0(R)	0(R)	16 (R)	17 (S)	25 (S)	0 (R)	9 (R)	20
Klebsiella spp	18(S)	30(S)	6(R)	9(R)	8(R)	0 (R)	25 (S)	0 (R)	19 (S)	15 (S)	50
Gram Positive	R	CPX	S	SXT	E	PEF	CN	APX	Z	AM	
Lactobacillus spp	19 (S)	22 (S)	25 (S)	2S (S)	10 (R)	6 (R)		6 (R)	21 (S)	20 (S)	60
Staphylococcus spp	19 (S)	12 (R)	13 (R)	21 (S)	10 (R)	27 (S)	6 (R)	9 (R)	10 (R))	12 (R)	30
Listeria spp	30 (S)	8 (R)	21 (S)	7 (S)	0 (R)	19 (S)	0 (R)	0 (R)	6 (R)	20 (S)	40
Bacillus spp	0 (R)	6 (R)	6 (R)	18 (S)	13 (R)	22 (S)	0 (R)	21 (S)	0 (R)	6 (R)	30

KEY: R= Resistant, S= Sensitive

Microbial growth in bottle drinks is one that should however, is never to be over looked, as they are aided by different environmental factors such as raw materials, storage, packaging, factory environment and production processes. This work therefore agrees with other studies[24-27] concerning growth of microorganisms in drinks.High fungal presence in soft drinks indicated the ability of yeast isolates to be able to survive despite high carbonation. This is possible due to its ability to utilize carbondioxide (CO_2) for its fermentative processes ([28-29]supports these notion). Pathogenic yeasts and moulds as *Sacchoromyces spp, Candida spp, Aspergillus spp, Penicillium spp, Rhizopus spp, Trycophyton spp, Mucor spp Trichoderma spp and Fusarium spp* were detected, corresponding with the studies of [30-31]. Also, toxins have been reportedly produced from *Aspergillus spp, Fusarium spp and Penicillium spp* etc.[32]. And as a result, these mycotoxins may cause health damages such as kidney failure, liver damages or even unto increased chronic situations such as cancer risk and immune suppression.

The different bacteria suspected in this research is in line with the researches of [33-35] regarding the presence of opportunistic pathogens in bottled drinks, thereby posing a significant threat to the microbiological health safety of the consumers. [36] Also reported that *Eschericha coli, Salmonella spp, Listeria Monocytogenes*, were not killed in coca-cola during high acidity of the drink (pH 2.7).

The presence of Coliform bacteria indicates contamination from both environmental and faecal coliforms in bottle drinks which may be as a result of biofilm formation in plant environments [37].

This work is supported by the work of [38-39] *that Escherichia coli* is sensitive to ciprofloxacin; This work also agrees with work of [40-41] that *E. coli* is resistant to streptomycin. *Bacillus spp* was sensitive to both Pefloxacin, Septrin and Ampliclox, disagreeing with the result of [42] stating that *Bacillus aureus* was resistant to Ampiciox. Although this study also agrees with [43] that *Bacillus* was resistant to Erythromycin. It disagrees with the report that *Staphylococcus aureus* was resistant to pefloxacin but susceptible to streptomycin and ciprofloxacin [44]. Whereas this research showed that *staphylococcus spp* is sensitive to pefloxacin and septrin. There is therefore need for good manufacturing practices and good storage of bottle drinks to be encouraged [46].

4.0 Conclusion

Presence of opportunistic pathogens associated with bottled drinks in recent times has become a thing of necessity to be look unto. This may be due to the global emergence of resistant strains of certain microorganisms, leading to the adverse risks of health associated issues caused by opportunistic pathogenic microorganisms found in bottled drinks. Therefore, despite various researches that has been carried out in recent times, continuous research still needs to be encouraged, so as to offer lasting solutions. And to aid sustain human health and its environments.

An antibiogram was being carried out in this research so as to proffer solutions, especially during adverse and epidemiological situations. As a result, the antibiotics that were susceptible to the different pathogenic microorganisms was well noted.

I am recommending that good quality manufacturing practices and microbial safety should be observed during production, transport and packaging of drinks according to the Hazard analysis and critical control points guidelines (HACCP). Secondly agencies should ensure periodic visitation and assessments of drinking bottle industries and companies. NAFDAC should also help discourage totally the use of re-use bottled drinks as these usually act as a medium for microbial growth and public health risks in drinks, but rather an alternative and healthy means of packaging should be employed.

Finally, this research should be recommended for further epidemiological studies, so as to act as a standby tool in epidemiological situations.

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