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Evaluation of Bacterial Load in Four Commercially Sold Yoghurts in Warri, Delta State, Nigeria

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ABSTRACT: The major ingredient in ice-cream a popular children favourite snack, commonly sold to them at their school gate during the dry season is yoghurt. Therefore this paper investigates the bacteria load in these yoghurt ice-creams are important as they determine the health of the consumers. The bacteria load of four commercially sold yoghurt samples in Warri, Delta State, Nigeria using standard methods. Results reveal the presence of ***S****treptococcus thermophilus*, *Streptococcus salivarius* and *Lactobacillus bulgaricus* spp. The highest number of colonies were observed in CS1[NA, 232 and PC, 218] and CS2 [NA, 282 and PC, 91] while lower number of colonies were observed in HO [NA, 120 and PC, 90] and for NY [NA, 118 and PC, 112]. It was observed that Colony Forming Units for Nutrient Agar (NA) were consistently higher in all four brands of yoghurt than Colony Forming Units for Plate Count Agar. An average of 157 colonies were observed on both nutrient Agar and Plate count Agar. Colonies observed was similar in both Plate Count Agar and Nutrient Agar in the 4 brands of yoghurt. All the isolates were highly susceptible to Norfloxacin and resistant to Gentamicin, Drovid and *Clindamycin*. The results of the microbial quality indicate that the yoghurt are of acceptable food quality, with probiotic potential.

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Yoghurt is a cultured dairy product obtained from lactic acid fermentation of milk. It is one of the most popular fermented milk products in the world, produced commercially at home (Willey *et al.*, 2008). In its production, non-fat or low-fat milk is pasteurized and cooled to 43°C. Then inoculated with known cultures of microorganisms referred to as 'starter cultures'. This 'starter cultures' may be a pure culture of a particular species of *Lactobacillus* or a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a 1:1 ratio. *Streptococcus thermophilus* grows faster than *Lactobacillus bulgaricus* and it is primarily responsible for acid production, while the rod adds flavour and aroma. The growth of these Microorganisms causes the conversion of milk sugar: lactose into lactic acid. This process gives yoghurt its flavour. The associative growth of the two organisms results in the production of lactic acid at a rate that is faster than that produced individually. Yoghurt generally consists of a standardized mixture containing whole milk, partially defatted milk, condensed skim milk cream and non-fat dry milk, inoculated with beneficial microorganisms. While the microorganisms fermenting milk confers on it certain health benefits, inadequately pasteurized milk may contain microorganisms which are detrimental to human health (Boor and Murphy, 2002). The presence or absence of these harmful species of bacteria in milk is a determinant of the success or failure of good manufacturing practice

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(GMP); it is of economic importance in Africa where HIV/AIDS and cancer scourge has left the public who consume milk products immune suppressed and prone to bacterial and fungi infection. (Boor, 2001). Yoghurt may provide additional health benefits, for example, it may reduce cholesterol levels. Studies suggested that certain diseases of the gastrointestinal tract such as: lactose intolerance, diarrhea, colon cancer and other bacterial infection are traced to high consumption of poorly pasteurized yoghurt (Ojokoh, 2006). Health complications associated with consumption of inadequately pasteurized milk products include chronic infections that are difficult to treat with antibiotics. This raises a serious clinical concern, as pathogenic bacteria present in poorly pasteurized yoghurt can confer antibiotic resistance to other bacteria, if primary infection occur. Antibiotic resistant species of bacteria are difficult to manage, as a variety of drugs are ineffective in the treatment of infections caused by them (Gould, 1994). Heat treated yoghurt do not contain harmful bacteria, as these are killed during post fermentation. It is therefore important to evaluate the microbial load of milk products sold in Nigeria. Hence, the objective of this paper was to evaluate the bacteria load of four commercially sold yoghurt samples in Warri, Delta state, Nigeria.

MATERIALS AND METHOD

Study location: Warri is located between the coordinates 5^0 32'39.2280" N and5^0 45'36.9684" E. Due to its hot and wet climate over a large proportion of the year, usually between October and June, ice-cream had become a popular snack craved for by the masses on sunny days.

Sample collection: Three samples each of four different yoghurt brands were bought from manufacturing companies, preserved in ice and taken to University of Africa Microbiology laboratory.

Sample culturing: Serial dilution was carried out for yoghurt sample by adding 1 ml of the water sample to 9 ml of distilled water in a test tube. Using a different sterile pipette, 1ml from the first test tube was pipetted into the second test tube already containing 9ml of diluted normal saline, this continued following the same procedure till the last dilution. Using the spread plate method for all prepared media, 0.1ml of each sample unit from the test tubes was pipetted into the sterile Petri dishes containing sterilized and solidified growth media (Nutrient Agar, Plate count Agar). The plates were incubated at 37°C for 24 hours and subculturing was done from plates where bacterial growth was observed.

Bacterial isolate: Isolates were identified based on their morphological characteristics and biochemical profile following Gram staining, oxidase, catalase, coagulase, indole, citrate and sugar fermentation tests, according to Bergey's Classification Scheme detailed in Cheesbrough (2004).

RESULTS AND DISCUSSION

Table 1 shows the morphological characteristic of the colonies observed on each plate and identification was done based on shape, Morphology, opacity, elevation, margin and size.

Table 1: Morphological characteristics of bacteria isolates in yoghurt samples

Media /Sample	Size	Morphology	Margin	Elevation	Shape	Opacity
NA CS1A	3mm	Yellowish, moist	Entire	Raised	Circular	Opaque
NA CS1B	6mm	Rough edges, fat	Entire	Flat	Circular	Translucent
NA CS1C	4mm	Bubble, cater layer raised	Entire	Raised	Circular	Opaque
PC CS1A	3mm	Yellowish, moist	Entire	Raised	Circular	Opaque
PC CSIB	6mm	Flat, cracks	Undulate	Flat	Irregular	Translucent
PC CS1C	3mm	Flat, dry	Entire	Raised	Circular	Transparent
PC CS2A	3mm	Yellowish, moist	Entire	Raised	Circular	Opaque
PC CS2B	4mm	Elevated, milkish	Entire	Raised	Circular	Opaque
PC CS2C	3mm	Milkish, Raised	Entire	Raised	Circular	Opaque
NA CS2A	4mm	Elevated, milkish	Entire	Raised	Circular	Opaque
NA CS2B	1mm	Pointed, Milkish, Elevated	Entire	Raised	Circular	Opaque
NA CS2C	3mm	Yellowish, moist	Entire	Raised	Circular	Opaque
PC NYA	4mm	Elevated, milkish	Entire	Raised	Circular	Opaque
PC NYB	3mm	Yellowish, moist	Entire	Raised	Circular	Opaque
NA NYA	4mm	Elevated, milkish	Entire	Raised	Circular	Opaque
NA NYB	1mm	Pointed, Milkish, Elevated	Entire	Raised	Circular	Opaque
NA NYC	5cm	Big, Rough, Irregular, undulate	Lobate	Raised	Irregular	Translucent
PC HOA	3mm	Yellow 13 n, elevated	Entire	Raised	Regular	Opaque
PC HOB	4mm	Elevated, milkish	Entire	Raised	Circular	Opaque
PC HOC	3mm	Yellowish, moist	Entire	Raised	Circular	Opaque
NA HOA	3mm	Milkish, Raised	Entire	Raised	Circular	Opaque
NA HOB	5mm	Raised, cover layer	Entire	Raised	Irregular	Translucent
NA HOC	3mm	Yellowish, moist	Entire	Raised	Circular	Opaque

Table 2: The identification of microorganisms isolated from yoghurt

Media/Sample	Oxidase	Catalase	Coagulase	Indole	Glucose	Lactose	Sucrose	H_2S	Gas	Citrate	Gram Reaction
NA CS1A	-	+	+	-	+	+	+	N	N	-	+
NA CS1B	+	+	+	-	+	-	-	N	N	+	+
NA CS1C	-	+	+	-	+	+	+	N	N	-	+
PC CS1A	-	+	+	-	+	+	+	N	N	-	+
PC CS1B	+	+	+	-	+	+	+	N	N	-	+
PC CS1C	+	+	-	-	+	+	+	N	N	-	+
NA CS2A	+	+	-	-	+	-	-	N	N	-	+
NA CS2B	+	+	+	-	+	-	-	N	N	-	+
NA CS2C	-	+	-	-	-	+	+	N	N	-	+
PC CS2A	+	+	-	-	+	+	+	N	N	-	+
PC CS2B	+	+	-	-	+	+	+	N	N	-	+
PC CS2C	+	+	+	-	+	+	+	N	N	-	+
NA NYA	+	+	+	-	+	+	+	N	N	-	+
NA NYB	+	+	-	-	+	+	+	N	N	-	+
NA NYC	+	+	-	-	+	+	+	N	N	-	+
PC NYA	+	+	-	-	+	-	-	N	N	-	+
PC NYB	+	+	-	-	+	+	+	N	N	-	+
PC NYC	+	+	-	-	+	+	+	N	N	-	+
NA HOA	+	+	+	-	+	+	+	N	N	-	+
NA HOB	+	+	+	-	+	+	+	N	N	-	+
NA HOC	-	+	-	-	+	+	+	N	N	-	+
PC HOA	+	+	-	-	+	-	-	N	N	-	+
PC HOB	+	+	-	-	+	-	-	N	N	-	+
PC HOC	+	+	+	-	+	+	+	N	N	-	+

Key: + = positive, - = negative

Table 3: Bacteria isolated from different yoghurt brands

Bacteria Isolated	**Yoghurt Brands**			
	NY	HO	CS1	CS2
Streptococcus thermophilus	+	+	-	+
Streptococcus salivaris	+	+	+	-
Lactobacillus bulgaricus sp.	+	+	-	-

Keys: *+ = Present;- = Absent*

Table 4: Antibiotic resistance pattern of bacteria isolated from yoghurt

Bacteria Isolated	**Inhibition zone (mm)**									
	D	CX	NB	GN	E	AC	AX	SXT	CIP	CD
Streptococcus thermophilus	0	2	6	0	0	9	2	2	0	0
Streptococcus salivarius	0	3	7	0	2	3	0	0	0	0
Lactobacillus bulgaricus sp.	0	4	10	0	0	6	0	3	2	0

Keys: *D - Drovid; NB - Norfloxacin ; CX – Cephalexin; AX – Amoxil; GN – Gentamycin; SXT – Septrin; E – Erytromycin; CIP – Ciprofloxacin; AC – Ampiclox; CD; - Clindamycin*

Colonies observed were similar in both Plate Count Agar and Nutrient Agar in the four yoghurt samples.

Table 2 shows the biochemical properties of bacteria isolated from yoghurt samples. All isolates are Gram positive non H_2S and gas producers. Table 3: Shows the bacteria samples isolated, they include: *Streptococcus thermophilus, Streptococcus salivarius* and *Lactobacillus bulgaricus* sp. NY and HO contain three different species of probiotic bacteria: *Streptococcus thermophilus, Streptococcus salivaris* and *Lactobacillus bulgaricus* sp. *while* was CS1 and CS2 only contained a single species of probiotic bacteria each. Table 4 shows the antibiotic susceptibility of the bacteria isolate. Norfloxacin had the highest zone of inhibition, while Gentamicin, Drovid and *Clindamycin* the lowest inhibition zone. Table 5 shows number of colonies on cultured plates. The highest number of colonies were observed in CS1[NA 232 and PC 218] and CS2 [NA 282 and PC 91] While lower number of colonies were observed in HO [NA 120 and PC 90] and for NY [NA 118 and PC 112].

Table 5: Number of Colonies formed on Nutrient Aga r(NA) and Plate Count Agar (PC) plates.

Sample	**PC**	**NA**
CS1	218	*232*
CS2	*91*	*282*
HO	*90*	*120*
NY	*112*	*118*

The organisms isolated from the yoghurt samples are *Streptococcus thermophilus and Lactobacillus bulgaricus* sp., this is in agreement with the work of (Willey *et al.,* 2008). In the production of yoghurt, non-fat or low-fat milk is pasteurized, cooled to 43°c and then inoculated with known cultures of microorganisms referred to as starter cultures. This starter cultures may be a pure culture of a particular species of *Lactobacillus* or a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a 1: 1 ratio. *Streptococcus thermophilus* grows faster than the rod *Lactobacillus bulgaricus* and it is primarily responsible for acid production, while the rod adds flavor and aroma. The growth of these Microorganisms causes the conversion of milk sugar: lactose into lactic acid. No pathogenic bacteria were observed in all four brands of yoghurt and all bacteria strains isolate from the four brands were highly susceptible to majority of available antibiotics in circulation. Properly pasteurized yoghurts do not contain any form of pathogenic organisms. Health complications associated with consumption of inadequately pasteurized milk products include chronic infections that are difficult to treat with antibiotics. This raises a serious clinical concern, as organisms present in poorly pasteurized yoghurt can confer antibiotic resistance to other bacteria, if primary infection occur. Antibiotic resistant species of bacteria are difficult to manage, as a variety of drugs are ineffective in the treatment of infections caused by them (Gould., 1994; Anele *et al*., 2023). Heat treated yoghurt do not contain lactic acid bacteria, as these are killed during post fermentation. CS1, CS2, HO and NY all contained the right internationally recommended probiotic bacteria this is in agreement with the work of Oyedeke (2009). Dairy products such as yoghurt; contains probiotic cultures e.g., Lactobacilli which are currently among the best-known examples of "functional food" (Oyeleke, 2009). They improve freshness of breath and a healthy mouth. Consuming just 3.2 ounces of yoghurt twice a day not only lowers levels of hydrogen sulfide and other volatile sulfide compounds responsible for bad breath, it can also kill tongue-coating bacteria, reduce dental plaque formation, cavities in our teeth and reduce risk of gingivitis. According to the International Association for Dental Research: eating plain live yoghurt for six weeks can reduce levels of oral bacteria by up to 80%, so yoghurt is a traditional bad breath cure (Okpalugo *et al.,* 2008).

Yoghurt help prevent and treat Arthritis; Lactobacillus a probiotic (friendly) bacteria found in yoghurt offers "remarkable preventive and curative effects on arthritis, Yoghurt can decrease yeast infection: Gray., 2007 reported that taking about 8 ounces of yoghurt with live and active culture of probiotic bacteria daily reduces the amount of yeast colonies in the vagina and decreases the incidence of vaginal yeast infection.

*Conclusion***:** The study established that some yoghurt samples commercially sold in Warri, Delta State, Nigeria, contain probiotic bacteria such as *Streptococcus thermophilus, Streptococcus salivaris* and *Lactobacillus bulgaricus* sp. The isolates were highly susceptible to Norfloxacin and resistant to Gentamicin, Drovid and *Clindamycin*. No pathogenic bacteria was found in the yoghurts. Thus, the yoghurt are of good food quality.

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