



Isolation and Identification of Coliform Bacteria (*E.coli*) and *Staphylococcus aureus* in Some Commercially Sold Yoghurts within Kano Metropolis

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(Received 25 Dec, 2014; Accepted 30 Dec, 2014; Published 03 Jan, 2015)

ABSTRACT: Yoghurt is a dairy product produced by lactic fermentation of milk. Yoghurt is produced by the controlled fermentation of milk by lactic acid producing bacteria. Two species are commonly used in the commercial production, which are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. An investigation was carried out to determine the sanitary quality of the some brands of yoghurt sold within Kano metropolis. Ten brands of yoghurt were bought which are sold in Kano metropolis and these brands were designated A, B, C, D, E, F, G, H, I and J respectively. Samples of these brands were bought from hawkers different locations and were analyzed using standard microbiological methods in order to determine their respective sanitary quality. The results of this study demonstrate that four (A, B, C and E) out of the ten brands of yoghurts sold in Kano are hygienically poor in terms of sanitary quality because of their varying coliform count. Yoghurts G and H which had least coliform counts are also unfit for human consumption because they exceed the maximum limit as set by NAFDAC.

Keywords: Yoghurt; Quality; Consumption; NAFDAC (National Agency for Food and Drug Administration and control).

INTRODUCTION

Yoghurt is a dairy product produced by lactic fermentation of milk¹. Yoghurt is produced by the controlled fermentation of milk by lactic acid producing bacteria. Two species are commonly used in the commercial production, which are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These two species of bacteria have now been established as the yoghurt starter cultures². Any sort of milk may be used to make yoghurt, but modern production is dominated by cow milk. It is the fermentation of the milk sugar (lactose) into lactic acid that gives yoghurt its gel-like texture and characteristics tang³. Yoghurt is made by inoculating certain bacteria (starter culture), usually *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, into milk. After inoculation, the milk is incubated at approximately 110°F ± 5°F until firm; the milk is coagulated by bacteria-produced lactic acid⁴. The presence of coliforms in these yoghurt brands is of serious public concern because of its health implication on the consumers of these brands of yoghurts. It was reported based on the standard stipulated by the National Agency of Food and Drug Administration Control (NAFDAC) that *E. coli* and coliforms generally must not be detectable in any 100 ml of yoghurt sample⁵. The principal components of milk are water, fat, protein and lactose⁶. Yoghurt contains all the protein, fat, calcium and vitamins of the original milk⁷ but contain a higher percentage of lactic acid than other fermented milk and it is rich in vitamin B complex⁸. The high water activity of milk, moderate pH and ample supply of nutrients make it an excellent medium for microbial growth¹⁰. This demand high standard of hygiene in its production and processing. Milk as secreted in the mammary gland of the healthy cow is sterile, but it is not possible to obtain milk entirely free from bacteria even when the most elaborate precautions are taken to ensure cleanliness in milk¹¹. Yoghurt if prepared from well pasteurized milk is not hospitable medium for pathogens because of its high acidity and low pH, pathogens will not grow and will not survive well. Yoghurt is spoiled by aciduric organisms

such as yeast and molds. Advisory guidelines for microbiological quality suggested that yoghurt should not contain more than 10⁸ CFU/g of starter organisms, 1 coliform/g, <1 mold/g and <10 yeast/g organism such as *S. lactis* and *S. cremoris* together with *Lactobacillus* are harmless^{6&12}.

Indicator organisms are used to measure potential faecal contamination of environmental and food samples. The presence of coliform bacteria such as *E.coli* in food is a common indication of faecal contamination¹³. The coliform group of bacteria comprises all aerobic and facultative anaerobic, gram-negative nonspore forming rods, capable of fermenting lactose with the production of acid and gas at 90°F (32°C) within 48 hours. While the general source of these organisms is commonly accepted to be the intestinal tract of warm-blooded animals, it is emphasized that bacteria of both fecal and non-fecal origin are members of this group¹⁴.

Escherichia coli commonly abbreviated (*E. coli*) is a gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes are pathogenic and can cause serious food poisoning in humans, and are occasionally responsible for product recalls¹⁵. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K₂, and by preventing the establishment of pathogenic bacteria within the intestine¹⁶.

S. aureus is a genus of gram-positive cocci; organism is included in a number of microbiological criteria. The natural reservoir for *S. aureus* is human skin, hair and superficial mucous membranes (nose), while it is not a part of the normal flora on fish and fish product. Presence of large numbers indicates the possible presence of enterotoxin and/or faulty sanitary or production practice. Small numbers are to be expected in products handled by humans. It should be emphasized that *S. aureus* grows poorly in competition with large numbers of other microorganisms. For this reason, a test for *S. aureus* is only relevant for fish products which have received a bactericidal treatment, i.e. a heat treatment during processing¹⁷.

MATERIAL AND METHODS

Ten brands of packaged yoghurt were purchased randomly from hawkers sold within Kano metropolis Kano state, Nigeria. They were collected and designed as A, B, C, D, E, F, G, H, I and J. All the brands were packaged in cellophane nylon and were stored in a freezer until needed. The yoghurt samples were evaluated for sensory characteristics such as flavor, taste and smell, and the physical appearance were also recorded accordingly.

Preparation of Peptone Water: Peptone water was prepared according to the manufacturer's instruction, where 15g of powder was weighed and dispersed into 1L of distilled water. It was allowed to dissolve and mixed. The homogenous solution was pipette 9ml and dispersed into a cleaned test-tube with inverted Durham's tubes. The test-tubes were covered with cotton wool. They were sterilized by autoclaving at 121°C for 15minutes.

Eosin Methylene Blue Agar: Eosin Methylene Blue (EMB) agar was prepared according to the manufacturer's instruction, where 37.5g of the powder was weighed and dispersed into 1L distilled water. It was allowed to soak for 10minutes, swirled to mix and sterilized by autoclaving at 121°C for 15minutes. The sterilized media was allowed to cool down to 47°C and gently agitate to ensure even distribution of the precipitate. The sterile media was poured into sterile petridishes and allowed to solidify into gel.

Manitol Salt Agar: Manitol Salt Agar (MSA) was prepared according to the manufacturer's instruction, where 37.5g of the powder was weighed and dispersed into 1L distilled water. It was allowed to soak for 10minutes, swirled to mix and sterilized by autoclaving at 121°C for 15minutes. The sterilized media was allowed to cool down to 47°C and gently agitate to ensure even distribution of the precipitate. The sterile media was poured into sterile petridishes and allowed to solidify into gel.

Enumeration of Coliforms: The techniques as described by FAO/WHO, 1979 for the enumeration of coliform using Most Probable Number (MPN) were used.

Presumptive Test: In presumptive test, dilutions of the yoghurt samples were made using peptone water. 1ml of each yoghurt sample was pipetted into one sterile test-tube containing 9ml of peptone water,

making 10^{-1} 1:10 dilution. From this dilution, 1ml was transferred into the second test-tube making 10^{-2} 1:100 dilution. Then from the second dilution, another 1ml was transferred into the third test-tube making 10^{-3} 1:1000 dilution. From all the three dilutions, 1ml was transferred into already prepared Mac Conkey broth containing each 9ml (triplicate) with inverted positioned Durham's tubes. The tubes were covered with cotton wool and incubated at 37°C for 24 hours. They were observed for gas production which was recorded accordingly.

Confirmed Test: In the confirmed test, a loopful each from the gas produced tube (i.e. positive tube) was inoculated (strike) onto the surface of an Eosin Methylene Blue (EMB) agar plate and then incubated at 37°C for 24 hours, for observation of colonies characteristics.

Completed Test: Finally, gram's staining was carried out on the colonies to find the gram status of the colonies and it was recorded.

Biochemical Tests: After the isolation of pure culture from different agar media, the cultures were then preserved and were later subjected to various biochemical tests for the confirmation and identification of the isolates. The biochemical tests carried out were: Catalase Test, Coagulase test, Indole Test, and Methyl Red and Voges Proskauer (MR-VP) Test.

RESULTS AND DISCUSSION

Coliform load of some yoghurt brands: Figure 1 represents the Most Probable Number (MPN) values per g or ml of sample for three sets of three tubes seeded with 10^{-1} , 10^{-2} and 10^{-3} ml of sample for ten (30) samples analyzed. The first three samples which include A, B and C yoghurts showed gas production in all three sets of three tubes seeded with 10^{-1} , 10^{-2} and 10^{-3} volumes of sample during the first week and therefore the MPN per g or ml as derived from the MPN table is $>1100\text{cfu/g}$. During the second week, the MPN values of samples A and B was found to be 34cfu/g and 36cfu/g respectively, while sample C revealed a value of 1100cfu/g . During the third week, sample A gave a value of 1100cfu/g while samples B and C were found to be 93cfu/g and 39cfu/g respectively. Sample D yoghurt produced a result with lower values of 64cfu/g , 28cfu/g and 7.2cfu/g for the first, second and third weeks respectively while the mean gave a value of 20cfu/g . Sample E yoghurt produced a result with MPN values of 460cfu/g , 290cfu/g and 15cfu/g for the three weeks respectively while the mean gave a value of 150cfu/g . Sample F yoghurt produced a result with MPN values of 1100cfu/g , 150cfu/g and 11cfu/g for the three weeks respectively while the mean turned out to be 28cfu/g . Sample G yoghurt also produced a result with MPN values of 120cfu/g , 7.2cfu/g and 9.3cfu/g for the first, second and third weeks respectively. The mean value was found to be 11cfu/g . Sample H yoghurt is another brand of yoghurt producing a similar result with Sample G yoghurt with a mean value of 11cfu/g and the MPN values for the first, second and third weeks were 3cfu/g , 20cfu/g and 210cfu/g respectively. Sample I yoghurt produced a result with MPN values of 11cfu/g , 11cfu/g and 35cfu/g during the first, second and third weeks respectively while the mean gave a value of 15cfu/g . Sample J being the last sample of yoghurt brand analysed, produced MPN values of 35cfu/g , 21cfu/g and 240cfu/g for the three weeks respectively with a mean value of 28cfu/g .

The results of this analysis revealed that out of the ten brands of yoghurts, four turned out to be heavily contaminated with coliforms. These samples are A, B, C and E. The other four brands had varying levels of bacterial contamination as indicated by their respective bacterial and coliform counts. The presence of coliforms in these yoghurt brands is of serious public concern because of its health implication on the consumers of these brands of yoghurts. It was reported that based on the standard stipulated by the National Agency of Food and Drug Administration Control (NAFDAC) that *E. coli* and coliforms generally must not be detectable in any 100 ml of yoghurt sample^{5&18}.

The Table 2 represents the incidence of the *Staphylococcus aureus* and *Eshcherichia coli* in the yoghurt sample. It revealed load of *Eshcherichia coli* sample A,B,C,E,H and J while *Staphylococcus aureus* load was observed in A, B, C, D, F, H and I. The presence of *Staphylococcus aureus* in brands A, B, C,D, F H and I ; *Eshcherichia coli* in brands A, B, C, E, H and I presents a health risk to the consumers of these brands of yoghurt¹⁹. Some strains of this bacterial species are known to cause illness such as food poisoning, osteomyelitis, bronchopneumonia and septicemia, which are often very severe infections²⁰.

Table 1: Coliform load of some yoghurt brands sold in Kano

S.No.	Sample	Week 1	Week2	Week 3	Mean (MPN/g)	WHO / NAFDACST Standard
1	A	>1100	34	1100	290	10
2	B	>1100	36	93	460	10
3	C	>1100	1100	39	210	10
4	D	64	28	72	20	10
5	E	460	290	15	150	10
6	F	1100	150	11	28	10
7	G	120	72	93	11	10
8	H	3	20	210	11	10
9	I	11	11	35	15	10
10	J	35	21	240	28	10

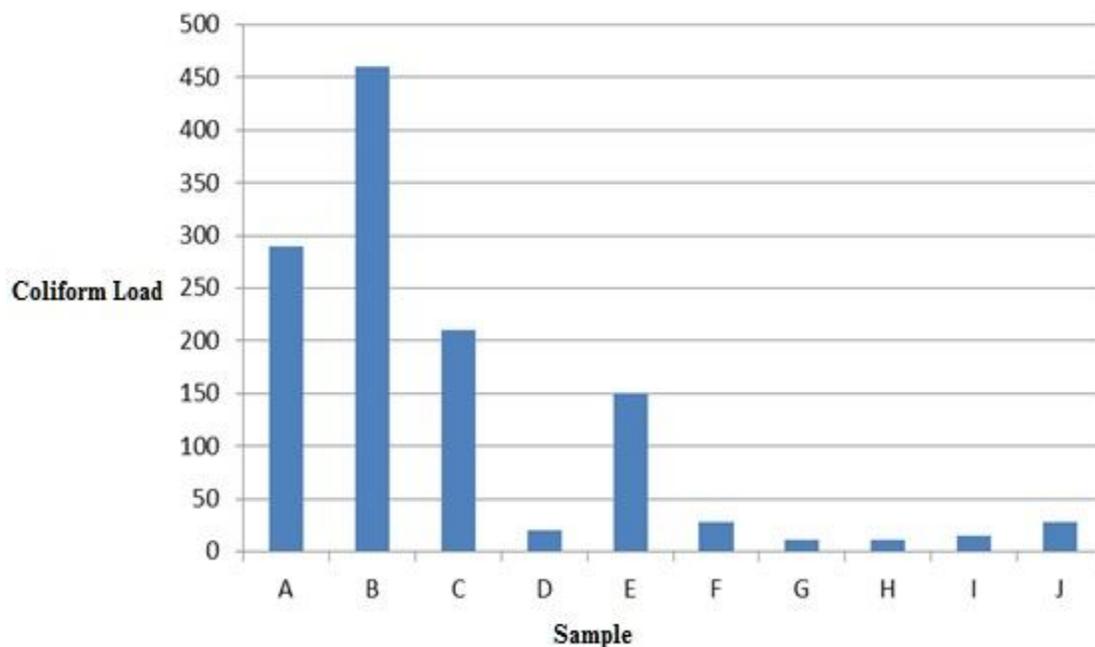


Figure 1: Coliform load of some yoghurt brands sold in Kano

Incidence of bacterial species in the yoghurt samples: Table 2 is a chi square table for statistical analysis between the positive and negative *Eshcherichia coli* and *Staphylococcus aureusin* in the sample. From the result of the analysis, it showed that the difference between the two isolates is not significant at 5% level of significant.

Table 2: Incidence of bacterial species in the yoghurt samples

Isolates	No. of Total Samples	No. of Positive Samples	% of Occurrence	Positive Samples
<i>Escherichia coli</i>	30	8	26.67	A ₁ , A ₃ , B,C,E,H,J ₂ and J ₃
<i>Staphylococcus aureus</i>	30	9	30	A, B ₂ ,B ₃ ,C,D,F ₂ ,F ₃ , H and I

The significant difference between the two isolates as calculated using chi-square is (0.36)

Biochemical test: Table 3 revealed a biochemical test of the *Eshcerichia coli* and *Staphylococcus aureus* respectively. *Eshcerichia coli* gave a positive result to indole and MR-VP and negative to catalase and coagulase, while in other hand the *Staphylococcus aureus* gave negative result to indole and MR-VP and positive to catalase and coagulase. The presence of *Staphylococcus aureus* in any food Particle is an index of its contamination from personnel sharing in production and handling²¹. *E.coli* on the other hand is an indicator of food and water contamination from feecal sources and its mere presence in a food renders the food unfit for human consumption²¹.

Table 3: Results for biochemical tests

Isolates	Indole Test	MR-VP	Coagulase	Catalase
<i>Escherichia coli</i>	+	+	-	-
<i>Staphylococcus aureus</i>	-	-	+	+

Key (+ = positive) (- = negative)



Figure 2: Tubes shows positive gas production after 24 hours of incubation in Mac Conkey Broth

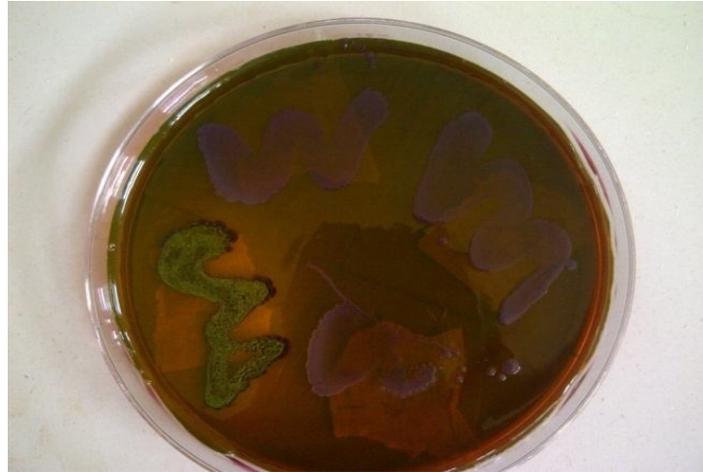


Figure 3: Plate showing homogeneous colony growth on EMB Agar.

CONCLUSION

The result of this study demonstrate that out of the ten brands of yoghurts analyzed within Kano metropolis, four brands were found to be heavily contaminated with coliform bacteria which is unsafe for human consumption. The results are thus significant to the health of the public, especially consumers of these brands of yoghurt.

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