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Research Article

ISOLATION AND IDENTIFICATION OF COLIFORM BACTERIA *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* IN SOME COMMERCIALLY SOLD YOGHURTS WITHIN KANO METROPOLIS

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ABSTRACT

Yoghurt is a diary 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 some brands of yoghurt sold within Kano Metropolis. Ten brands of yoghurt which are sold in Kano Metropolis were bought and were designated as A, B, C, D, E, F, G, H, I and J respectively. Samples of these brands were bought from hawkers at 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 brands (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 (National Agency for Food and Drug Administration and control).

Keywords: *Escherichia coli*, *Staphylococcus aureus*, Yoghurt, Quality, Consumption, NAFDAC.

INTRODUCTION

Yoghurt is a diary product produced by lactic fermentation of milk (Hui, 1992). 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 cultures (Speck *et al.*, 2002). 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 tang (Davis, 1974).

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 acid (Heaton and Jones, 2008). 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 (Mbaeyi-Nwaoha *et al.,* 2012) had 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 sample. The principal components of milk are water, fat, protein and lactose (Adams and Moss, 1999).

Yoghurt contains all the protein, fat, calcium and vitamins of the original milk (Passmore and Eastwood, 1986) but contain a higher percentage of lactic acid than other fermented milk and it is rich in vitamin B complex. The high water activity of milk, moderate pH and ample supply of nutrients make it an excellent medium for microbial growth (Toder, 2007).

The present study aimed to isolate and identify coliform bacteria *Escherichia coli* and *Staphylococcus aureus* in some commercially sold yoghurts within Kano Metropolis.

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MATERIALS AND METHOD

Ten (10) 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.

Eosin methylene blue agar

Eosin Methylene Blue (EMB) agar was prepared according to the manufacturer's instruction. 37.5g of the powder was weighed and dispersed into 1L distilled water. It was allowed to soak for 10minutes, stirred to mix and sterilized by autoclaving at 121°C for 15minutes. The sterilized media was allowed to cool to 47°C. The sterile media was poured into sterile Petri dishes and allowed to solidify into gel.

Manitol salt agar

Manitol Salt Agar (MSA) was prepared according to the manufacturer's instruction. 37.5g of the powder was weighed and dispersed into 1L distilled water. It was allowed to soak for 10minutes, stirred to mix and sterilized by autoclaving at 121°C for 15 minutes. The sterilized media was allowed to cool to 47°C. The sterile media was poured into sterile Petri dishes 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 adopted.

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 9 ml 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 (Figure 1) containing each 9 ml (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

A loopful each from the gas produced tube (i.e. positive tube) was inoculated onto the surface of an Eosin Methylene Blue (EMB) agar plate (Figure 2) and then incubated at 37°C for 24 hours, for observation of colonies characteristics.

Figure 1. Tubes shows positive gas production after 24 hours of incubation in Mac Conkey Broth.

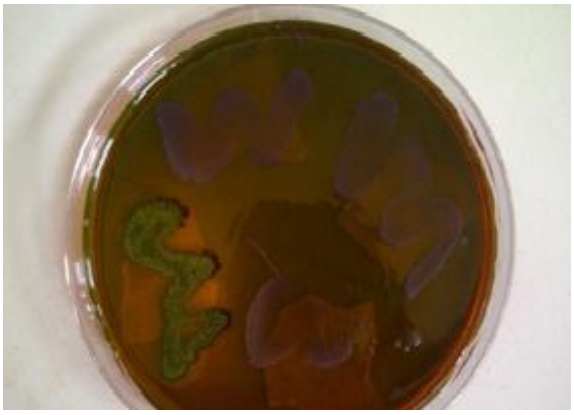

Figure 2. Samples of yoghurts a homogenous colony growth on an EMB agar.

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 confirmationand 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

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 (Figure

3). 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 >1100cfu/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 analyzed, 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 (10) 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 (Mbaeyi-Nwaoha *et al.*, 2012) had 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 sample.

The Table 1 represents the incidence of the *Staphylococcus aureus* and *Eshcerichia coli in* the yogourt sample. It revealed load of *Eshcerichia 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; *Eshcerichia coli* in brands A, B, C, E, H and I presents a health risk to the consumers of these brands of yoghurt. Some strains of this bacterial species are known to cause illness such as food poisoning, osteomyelitis, bronchopneumonia and septicemia, which are often very severe infections (Arora *et al.*, 2012).

Incidence of bacterial species in the yoghurt samples

Table 2 is a chi square table for statistical Analysis between the positive and negative *Eshcerichia coli* and *Staphylococcus aureus* 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 significance.

Biochemical test

Table 2 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 handling (Makwin *et al.*, 2014). *E. coli* on the other hand is an indicator of food and water contamination from fecal sources and its mere presence in a food renders the food unfit for human consumption (Makwin *et al.*, 2014).

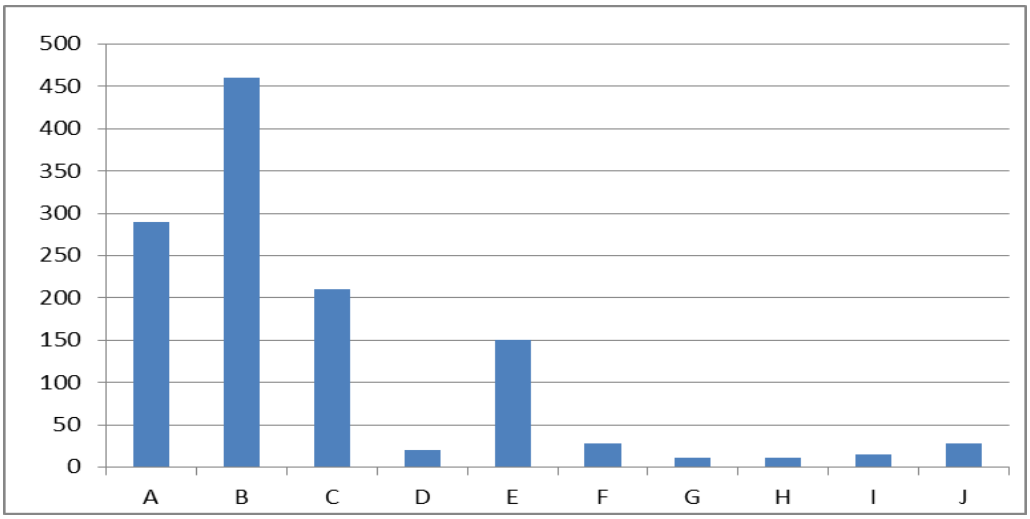

Figure 3. Coliform load (MPN/g) of some yoghurt brands sold in Kano.

Table 1. Incidence of bacterial species in the yoghurt samples.

Isolates	No. of total samples	No. of positive samples	% of occurrence	Positive samples
Escherichia coli	30	8	26.67	A_1, A_3, B, C, E, H, J_2 and J_3
Staphylococcus aureus	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).

Table 2. Results for biochemical tests.

Isolates	Indole test	MR-VP	Coagulase	Catalase
Escherichia coli	+	+	-	-
Staphylococcus aureus	-	-	+	+

(+ = positive.) (- = negative).

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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