Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.18, 2014

Microbial screening of fermented (yoghurt) milk samples sold in Makurdi metropolis and consumed in Federal University of Agriculture Makurdi, Benue State, Nigeria

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Abstract

Samples of commercially produced yoghurt samples (fermented milk) and locally produced milk samples commonly called kindrimu in common use in Federal University of Agriculture Makurdi, Benue State, Nigeria were microbiologically examined to certify their safety and quality for consumption. While *E.coli* was present in four of the samples A,B,D and E and *Pneumococcus* found only in sample D, *Klebsiella, Staphylococcus* and *Salmonella typhimurium* were not detected in any of the five samples A-E. In the locally prepared samples *Staphylococcus* was detected only in sample 3, while *Klebsiella* was not detected in samples 3 and 5. *Salmonella* was not detected in sample 2 just as *E.coli* was not dectected in sample 3 alone. *Pneumococcus* was detected in all the samples suggesting that the commercial samples are safer than the local Kindrimu.

Conclusion: Since these organisms like *Salmonella, Staphylococuss aureus* and*Escherichia coli* were isolated from both the commercially prepared and kindrimu yoghurts, there is need for proper handling of the production process to reduce contamination.

INTRODUCTION.

The quality of milk products continues to be a topic that attracts debate in the diary industry and in medical and public health sectors (Oliver et al., 2009). Production of the best quantities of
good quality milk and milk products is an important aspect of standard dairy practice. High-quality milk contains a low bacteria count and a low number of somatic cells and is free of human pathogens and antibiotic residues (Oliver *et al*., 2009). Different epidemiological studies have detected food borne pathogens in bulk tank milk (Umoh *et al*., 1990; Hassan *et al*., 2000; Jayaro and Henning, 2001; Waak *et al*., 2002; Murinda *et al*., 2002a, b, 2004; Muraoka *et al*.,
2003; Van Kessel *et al*., 2004; Oliver *et al*., 2005; Pangloli *et al*., 2008). Although the true incidence of milk borne disease in Makurdi, Nigeria is not known, there are reports that link the consumption of contaminated raw milk, inadequately pasteurized milk or consumption of dairy products adulterated with contaminated raw milk to incidence of human food borne diseases in some countries (Fleming *et al*., 1985; Fahey *et al*., 1995; Evans *et al*., 1996).

The dairy industry in Makurdi is still underdeveloped and crude with dietary supplementation with antioxidative nutrients not practiced. Makurdi is the state capital of Benue State, North Central Nigeria, with majority of the indigenes involved in farming and cattle rearing. It has an average daily temperature of between 35-40ºC during the peak heat period. In Nigeria and especially in the Northern part, fermented milk (yoghurt) of various types are produced and consumed as supplement to normal meals in homes and even for sale. Many farm families consume raw milk simply because it is a traditional practice and less expensive than buying pasteurized retailed milk (Hegarty *et al*., 2002).

Kindrimu refers to the local yoghurt in Hausa language that is produced without the use of the
conventional method of producing commercial yoghurt. Kindrimu is produced by milking the cow into a container. This is followed by the addition of small quantity of already fermented yoghurt or milk and is covered for 24 h to ferment. This product is vulnerable to contamination by microorganisms due to poor quality control. Because of its relatively lower price compared to other commercially produced yoghurts, the consumption of this brand of fermented milk has gained popularity around Makurdi metropolis despite the health implications. As part of our on-going comparability studies on the safety of some food beverages in different parts of Nigeria (Maduka et al., 2013 a,b; Maduka et al., 2014 a,b), we carried out the safety examination of the local brand

(kindrimu) and other commercially available yoghurt samples sold in shops in Makurdi and consumed in the Federal University of Agriculture Markurdi.

MATERIALS AND METHODS.

Collection of samples: A total of thirty (30) samples comprising six differently prepared yoghurts were purchased from shops in the University of Agriculture Makurdi. Samples A,B,C,D, and E were samples of kindrimu yoghurt while samples 1, 2, 3, 4, and 5 comprised fifteen different samples of commercially prepared yoghurts. They were stored in a refrigerator and used within 24 h of collection.

Media preparation: The following media were used in the study which includes sabouround dextrose agar, macconkey agar, nutrient agar, manitol salt agar, salinite feacal broth, salmonella shigella agar and blood agar. Different grams of each growth medium were added to 1 L of distilled water in a clean conical flask. They were each boiled to dissolve completely and then
sterilized by autoclaving at 121ºC for 15 min. Each was allowed to cool before being poured aseptically into sterile petri dishes and allowed to set.

Plating technique: The poor plate technique of Van Soestbergen and Ching (1969) was used. Samples were taken after thorough mixing of the parent stock to enhance homogeneity. Plating of the serially diluted samples was done on sterilized petri dishes.

Microbial analyses: The samples were analyzed by the streak plate method. One ml of each sample was inoculated unto each medium that was sufficiently dried. Each plate was labeled and inoculated at 30 °C for 24 h. The colonies were smeared on a slide and stained. The smear was spread evenly covering an area of about 15-20 mm diameter on a slide. The sample was spread thinly using a sterile wire loop. The flame sterilized loop was allowed to cool before it was usedfor the other ones. Colonies were emulsified in distilled water before being spread thinly. After drying, the smears were stained with crystal violet stain for 30-60 sec and then rapidly washed with distilled water. The water was then tipped off and the smears were treated with lugolsloding for 30-60 sec and were then washed off with distilled water. They were decolorized with acetone alcohol solution and washed immediately with distilled water. The smears were also treated withneutral red stain for 1 min, washed off and air dried. The smears were examined under the microscope for the presence of microorganisms (Zall, 1981).

RESULTS.

The results of the microbial screening of the commercially fermented milk samples are presented in Table one. The results show that *Klebesiella species, Staphylococcus aureus* and *Salmonella typhy* were not detected on any of the samples tested while *E.coli* was detected in all the samples except sample C. The result also shows that *Pneumococcus* was detected in sample D only.

Table 2 represents the result of the microbial screening of the locally fermented milk (kindrimu) samples. The result shows that all the tested organisms were present in samples 1 and 4. *Klebesiella species* was not detected in samples 3 and 5 while *Salmonella typhy* was also not detected in sample 2. *Salmonella typhy* and *Pneumococcus* were isolated in sample 3. The locally fermented samples showed more microbial contaminants than the commercially prepared samples suggesting caution in consumption.

DISCUSSION.

Preventing diseases and death associated with food borne pathogens continues to be a serious public health challenge especially in a developing country like Nigeria. Also, an increase in the movement of people and food products have made food safety a global issue and could lead to the introduction and establishment of new diseases in geographical areas that have not yet witnessed food borne pathogens (Oliver et al., 2005). Because of the regulation lapses in the production, sales and consumption of fermented milk and milk products in Nigeria, and as part of our on-going studies of fermented food products in Nigeria, we screened for the presence of some pathogenic microorganisms in yoghurt samples consumed in the University of Agriculture Makurdi, Benue State, Nigeria.

The microbial screening of the commercially fermented milk samples indicated the presence of *E. coli*(4.0%) in all the samples excluding samples C while *Pneumococcus*(4.0%) was present only in sample D. Unlike our previous results (Maduka et al., 2013), this study recorded the growth of *E.coli* in the samples of the commercially prepared yoghurt samples.

The results obtained from our previous study (Maduka et al., 2013) showed the absence of *E.coli* in commercially produced yoghurts sold in the University of Maiduguri, North east Nigeria. The results from this study showed the presence of *Salmonella typhy*(16%), *E.coli* (24%) and *Staphylococcus aureus* (16%) as in our previous report (Maduka et al., 2013) though to a lesser degree. Unlike our previous study, we isolated *Klebesiella species* from the samples of kindrimu yoghurts. The distribution of theses microorganisms in these kindrimu yoghurts sold in the University of Agriculture Makurdi are fairly on the high side to attract public

health attention.

The results obtained for percentage *E. coli* content was higher than that obtained from other studies (Okonkwo, 2011; Soomro *et al*., 2002; Ekici *et al*., 2004; Mohamed and El-Zubeir, 2007). The results are also in line with the reports of Schlegelova *et al*. (2002), Guta *et al*. (2002), Okpalugo *et al*. (2008), Adeleke *et al*. (2000) and Mahami *et al*. (2011) that found bulk cow milk in Czech Repulic, cow foremilk in Botswana, pasteurized milk in Nigeria and soy milk in Nigeria and cow milk in Ghana respectively to be contaminated with bacteria pathogens. Some studies have associated *E. coli* with the contamination of milk and milk products (Kulshrestha,1990; Asmahan and Warda, 2011; Okpalugo *et al*., 2008; Dadie *et al*., 2010). Study reports have also linked the presence of *E. coli* as an index organism indicative of the presence of other pathogenic organisms like *Klebsiella* and *Staphylococcus aureus* (Okpalugo *et al*., 2008; Adesiyun *et al*., 1995; Smooth and Pierson, 1997; Okonkwo, 2011). Recovery and counting of *E. coli* is used as an index of fecal contamination and shows the possible presence of enteropathogenic and/or toxigenic microorganisms which constitute public health hazard (Asmahan and Warda, 2011). Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al*., 2004; Asmahan and Warda, 2011).

The prevalence of food borne pathogens in fermented milk products is influenced by many factors including the size and number of animals on the farm, hygiene, farm management practices, variation in sampling and types of samples evaluated, differences in detection strategies used, geographic location and season (Oliver *et al*., 2005). These show that milk can be a major source of food borne pathogens of human health significance. Introduction of raw milk contaminated with food borne pathogens into processing plants and their persistence in biofilms represents an important risk of post-pasteurization contamination that could lead to exposure of the consumer to pathogenic bacteria (Arizcun *et al*., 1998; Roberts and Weidmann, 2003; Wong, 1998). The outbreak of human Salmonellesis had been associated with *Salmonella* (De Buyer *et al*., 2001). Results show that *Salmonella spp.* is one of the most etiologic agents responsible for several outbreaks associated with the consumption of raw milk (De Buyer *et al*., 2001). Our results showed 24% contamination of the locally produced yoghurt (Kindrimu) and 4% of the commercially fermented yoghurt respectively. Human infections of *Salmonella* come from different sources including fecal contamination of food products and water; consumption of unpasteurized milk and dairy products, particularly by farm families etc (Wells *et al*., 2001, Pangloli *et al*., 2008, Headrick *et al*., 1998; Lejeune and Rajala-Schultz, 2009). Because cull dairy cows and cattle are primary reservoirs and source of *Salmonella*, effective control of the pathogen on farm through management strategies may prevent or lower contamination of foods in the food chain. The presence of *Staphylococcus aureus* is in line with the results of Okpalugo *et al*. (2008), Okonkwo (2011) and Tormo *et al*. (2011). *Staphylococcus aureu* has been associated with gastroenteritis as it produces enterotoxins, boils and skin infections. Because *Staphylococcus aureus* is highly liable to destruction by heat treatment and nearly all sterilizing agents, its presence in pasteurized yoghurt is an indication of poor sanitation or post-pasteurization contamination (Okpalugo *et al*., 2008). Several studies reported that *Staphylococci spp* (Kaplan, 2005) and *Salmonellatyphi* (Fontaine *et al*., 1980) are agents that cause mastitis in dairy animals and may have contaminated milk from udder of infected animals.

CONCLUSION.

Milk can harbor a number of microorganisms and can be a good source of food borne diseases. The results obtained in this study suggest that the locally fermented milk (kindrimu) available to consumers in Makurdi was contaminated with food borne pathogens unlike the commercially prepared yoghurts. Since these organisms like *Salmonella, Staphylococuss aureus* and*Escherichia coli* were isolated from both the commercially prepared yoghurts and the kindrimu yoghurts, there is need for proper handling of the production process to reduce contamination. It is possible to have pathogen-free yoghurts through high and strict preventive measures.

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Table 1. Microbial screening of the commercially fermented milk (yoghurt) samples.

Isolated organism	Sample A	Sample B	Sample C	Sample D	Sample E
Klebesiella species.	ND	ND	ND	ND	ND
Staphylococcus aureus.	ND	ND	ND	ND	ND
Salmonella typhy.	ND	ND	ND	ND	ND
E. coli.	+ (4.0%)	+(4.0%)	ND	+(4.0%)	+(8.0%)
Pneumococcus.	ND	ND	ND	+(4.0%)	ND

+ = Present, ( )= number of positive samples, ND = not detected.

Table 2. Microbial screening of the locally fermented kindrimu milk (yoghurt) samples.

Isolated organism	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Klebesiella species.	+ (16.0%)	+ (16.0%)	ND	+ (16.0%)	ND
Staphylococcus aureus.	+ (16.0%)	+ (16.0%)	ND	+ (16.0%)	+ (8.0%)
Salmonella typhy.	+(8.0%)	ND	+ (6.0%)	+ (8.0%)	+ (8.0%)
E. coli.	+ (24.0%)	+ (24.0%)	ND	+ (15.0%)	+(24.0%)
Pneumococcus.	+ (12.0%)	+ (12.0%)	+ (12.0%)	+ (12.0%)	+ (12.0%)

+ = Present, ( )= number of positive samples, ND = not detected.