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Evaluation of the Bacteriological Quality of Milk Sold in Nnewi, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Contamination of milk products can result to severe intestinal and extra-intestinal diseases in man. This study was aimed at evaluating the bacteriological quality of various milk products sold in Nnewi.
Materials and Methods: Using the Cluster sampling technique, 30 milk samples (5 pasteurized skimmed milk, 5 powdered infant milk formulas, 5 powdered milk, 5 unsweetened evaporated milk, 5 branded soya milk and 5 unbranded soya milk) were purchased randomly from different shops and hawkers around Nnewi. Sample processing was done by serially diluting samples in sterile 1% peptone water before plating onto Mannitol Salt Agar, Violet Red Bile Glucose Agar, Blood Agar, MacConkey and Cysteine Lactose Electrolyte Deficient (CLED) agar using the Pour-plate technique. Bacterial count and identification were done using standard bacteriological as well as molecular techniques. The molecular techniques used were Polymerase Chain Reaction, Sanger Sequencing and BLAST analysis on the NCBI BLAST online.
Results: This showed that 15 (50%) out of 30 milk samples were contaminated to varying degrees with bacteria. Nine (9) samples showed the presence of *Escherichia coli* with 32.14% of all the milk samples tested. *E. coli* was present in skimmed milk (20%), evaporated milk (20%), branded soyamilk (40%) and unbranded soyamilk (100%) but was not isolated from Infant formula and Powder milk. *Klebsiella spp.* showed the second highest prevalence (28.57%) and was present in

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evaporated milk (20%), branded soyamilk (40%), unbranded soyamilk (100%). *Salmonella spp.* (3.57%) was isolated from unbranded soyamilk,*Enterobacter spp.* (14.29%) was isolated from powder milk and in unbranded soyamilk samples, *Staphylococcus aureus*(3.57%) and *Staphylococcus epidermidis* (3.57%) were isolated only in unbranded soyamilk, *Macrococcus caseolyticus* (3.57%) was isolated from unbranded soyamilk. Novel species such as *Aquitalea magnusonii* (3.57%), *Alishewanella fetalis* (3.57%) and *Lysinibacillus macroides* (3.57%) were identified by molecular analysis to be present in infant formula, evaporated milk and unbranded soyamilk respectively.

Conclusion: This research revealed that the bacteriological quality of some milk and milk products sold in Nnewi is not acceptable especially the unbranded soyamilk samples that showed gross contamination.

Keywords: Bacteriological quality; soyamilk; milk products.

1. INTRODUCTION

Milk and milk products constitute important nutritional components of all age groups. Good quality milk meets the nutritional needs of the body better than any single food as it contains all the essential food constituents. As a result of the presence of these nutrients, milk is an excellent medium for many kinds of microorganisms. The presence and multiplication of these microorganisms in milk brings about changes in the properties of milk thus reducing its quality [1]. Milk is pasteurized by heating at a temperature of about 63^0C (145^0F) for 30 minutes, rapidly cooling it, and then storing at a temperature below 10^0C (50^0F). Pasteurization kills most, but not all bacteria in milk. The combination of time and temperature used for heat treatment of milk are however, designed to kill all pathogenic microorganisms [2].

Milk is usually processed in a variety of ways. Condensed, evaporated and powdered milk are produced by evaporating some or all of the water in milk with the intention of extending the shelf life of the milk. The removal of about 50% water from whole milk, results in the production of a light brown milk product called unsweetened evaporated milk. However, with this amount of water, the milk is still susceptible to microbiological spoilage so the evaporated milk is packaged in cans and heat- processed under steam pressure in an attempt to destroy all the microorganisms present [3].The keeping quality of pasteurized milk depends both on the quality of heat treatment and on the extent of post-pasteurization contamination [4].

Soymilk (also called soyamilk, or soybean juice and sometimes referred to as soydrink/ beverage) is a beverage made from soybeans (*Glycine max*). It is the water extract of grounded soybeans or just dry grounded, roasted soy beans, which is also used to wean infants. The liquid drink is a stable white or creamy emulsion of water, oil, proteins and little carbohydrate. The drink has numerous health benefits including no lactose, lower fat, carbohydrate, calcium and phosphorus, more iron, similar protein as cow milk, and dietary fiber [5]. The medicinal properties of soy bean milk include lowering of serum cholesterol and low density lipoproteins, thus reducing the risk of heart disease [6].

Contamination of milk products could be due to contamination at the factory through the air, in the holding tanks, pipes or the pasteurization room. Contamination of milk products could also be incriminated to various roles performed by workers during milk processing [4]. Milk is widely consumed by Nigerians and it is imperative to determine its quality and to establish the presence or absence of pathogens in milk sold in Nnewi. This study evaluated the bacteriological quality of milk sold in Nnewi, Nigeria.

2. MATERIALS AND METHODS

The prevalence study was carried out in Nnewi in South East Nigeria. A total of 30 milk samples distributed as follows; 5 pasteurized skimmed milk, 5 powder infant milk formulas, 5 powder milk, 5 unsweetened evaporated milk, 5 branded soya milk and 5 unbranded soya milk, were used for the study. The samples were purchased in 3 batches on 3 different days from different sources which include supermarkets, shops, local markets and hawkers around the study area. The unbranded soyamilk samples were transported on ice to the laboratory and stored at 40°C pending when analysis is done.

Samples were serially diluted in sterile 1% peptone water and inoculated using pour plate

technique onto Mannitol Salt Agar, Violet Red Bile Glucose Agar, Blood Agar, MacConkey and CLED. The plates were incubated overnight at 37^0C. Bacterial and coliform plate counts were done using the single agar layer plate count technique [7]. Isolated colonies were also identified by standard bacteriological test.

Molecular analysis: Genomic DNA was extracted from the specimens and subjected to amplification by PCR using Fungal/Bacterial DNA MiniPrep™50 Preps.Model D6005 (Zymo Research, California, USA). The PCR reaction was performed on the extracted DNA samples using universal degenerate primers ITS1: 5´TCC GTA GGT GAA CCT TGC GG 3 and ITS4 5´TCC TCC GCT TAT TGA TAT GC 3´.

3. RESULTS

The study revealed that some of the milk samples were contaminated and in certain cases with pathogens of public health importance. The percentage occurrence of bacteria isolated from the milk samples is presented in Table 1.

Nine (9) milk samples showed the presence of *Escherichia coli* which had the highest prevalence with 32.14% of all the milk samples tested. *E. coli* was present in skimmed milk (20%), evaporated milk (20%), branded soya milk (60%) and unbranded soya milk (100%) but was not isolated from Infant formula and Powder milk. *Klebsiella spp.* showed the second highest prevalence (28.57%) and was present in evaporated milk (20%), branded Soyamilk (40%), unbranded soyamilk (100%). *Salmonella spp.* (3.57%) was isolated from unbranded soyamilk, *Enterobacter spp.* (14.29%) was isolated from powdered milk and in unbranded soyamilk samples, *Staphylococcus aureus* (3.57%) and *Staphylococcus epidermidis* (3.57%) were isolated only in unbranded soyamilk, *Macrococcus caseolyticus*(3.57%) was isolated from unbranded soyamilk. Novel species such as *Aquitalea magnusonii* (3.57%), *Alishewanella fetalis* (3.57%) and *Lysinibacillus macroides*(3.57%) were identified by molecular analysis to be present in infant formula, evaporated milk and unbranded soyamilk respectively. *Escherichia coli* and *Klebsiella spp*. were the most prevalent bacteria species and were isolated 100% in all unbranded, unregistered soya milk samples tested.

4. DISCUSSION

This study revealed that the bacteriological quality of some milk and milk products sold in Nnewi is not acceptable and some not fit for human consumption. All the categories of milk sampled were contaminated with unbranded soyamilk samples showing the highest prevalence of bacteria. The presence of *Klebsiella spp, E. coli, Salmonella spp.* and a host of other bacteria should attract public health attention as these drinks are consumed in large quantities by the public.

These findings are in agreement with Adeleke *et al.* [5], Schegelova *et al.*(2002), Guta *et al.* [8], Okpalugo *et al* .[9], Anderson *et al.* [10], Anagu *et al.* [6], who found bulked cow milk in Czech Republic, cow foremilk in Botswana, pasteurized milk in Nigeria, pasteurized milk in Jamaica, soyamilk in Nigeria to be contaminated with bacterial pathogens. Contrary to the findings of Mahami *et al.* [11] in Accra, this study realized growth from evaporated canned milk and infant formula.

The results show that *Escherichia coli* was present in all types of milk except infant formula and powdered milk. *E. coli* has been associated with the contamination of milk [5,9,12,11,10,6]. Existing researches have linked the presence of *E. coli* as an index organism indicative of the presence of other pathogenic organisms like *Klebsiella spp* and *Staphylococcus aureus* [9,13].

Novel bacteria species such as *Aquitalea magnusonii*, *Alishewanella fetalis* and *Lysinibacillus macroides* were identified by molecular analysis to be present in infant formula, evaporated milk and soyamilk (unbranded). *Macrococcus caseolyticus* was also isolated from unbranded soyamilk. *Staphylococcus epidermidis* was isolated only in unbranded soyamilk. These species have not been isolated from milk samples by previous researchers, probably due to the fact that molecular diagnosis is not commonly carried out to determine milk contamination.

The most common bacteria isolates identified in all the types of milk except powdered milk were *E. coli*, *Klebsiella spp., Enterobacter spp.* all of which belong to the coliform group. According to Witte [14], Catry *et al.* [15] and Mahami *et al.* [11], the presence of *E. coli* is an indication of faecal contamination which may have resulted from faeces of milked animals, hands of milk collectors or from human source. Environmental microbes *Klebsiella spp.* and *Enterobacter spp.* may have entered milk samples from the environment, the water used in preparation of the

Table 1. Isolated/Identified bacteria isolates in the milk samples

Type of milk sample	Number of samples	Number positive	Bacteria identified (%)	Acceptable Standard
Pasteurized skimmed milk	5	1	*E.coli (20%),*	*0%*
			Klebsiellaspp (20%	*0%*
Powdered milk	5	3	*Enterobacterspp (60%)*	*0%*
Powdered Infant milk formula	5	1	*Aquitaleamagnusonii (20%)*	*0%*
Evaporated milk	5	1	*E. coli(20%),*	*0%*
			Alishewanellafetalis (20%)	*0%*
Branded Soya milk	5	3	*E. coli (60%),*	*0%*
			Klebiellaspp (60%)	*0%*
Unbranded Soya milk	5	5	*E.coli (100%),*	*0%*
			Klebsiellaspp (100%),	*0%*
			Lysinibacillusmacroides (20%),	*0%*
			Macrococcuscaseolyticus (20%),	*0%*
			Staphylococcus epidermidis (20%),	*0%*
			Staphylococcus aureus (20%),	*0%*
			Salmonella spp (20%),	*0%*
			Enterobacter cloacae (20%)	*0%*

milk, dust, contaminated utensils or the hand of the handlers. *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella spp*. were also isolated from the milk samples tested. The prevalence of these food-borne pathogens in milk may be influenced by many factors such as farm size; number of animals on the farm; hygiene; farm management practices; variation in sampling and types of samples evaluated; differences in detection methodologies used; geographic location and season [16]. The presence of *Staphylococcus aureus* and *Staphylocccus epidermidis* is similar to results of Okpalugo *et al.* [9], Okonkwo [13], Tormo *et al.* [17] and Mahami *et al.* [11]. *Staphylococcus aureus* is heat labile and is destroyed by nearly all sterilizing agents. Its presence in milk is an indication of poor sanitation or post-pasteurization contamination [9,11]. Results show that *Staphylococci spp.* is one of the causative agents of mastitis in dairy animals and may have contaminated milk from the udder of infected animals [18]. Additionally, the nasopharyngeal cavity of human beings is the reservoir of *Staphylococci* from where these bacteria get localized on the skin, especially on human hands [18]. *Staphylococci* contamination of milk could therefore also been through the hands of the milk handlers [11].

Unbranded soyamilk also indicated the presence of *Klebsiella quasipneumoniae subsp. similipneumoniae* strain ATCC 700603 while unbranded soya milk purchased in the microbiology unit of the Nnamdi Azikiwe University Teaching Hospital, Nnewi, contained Multiple-drug resistant *Klebsiella pneumoniae subsp. pneumoniae* HS11286. Other samples that showed the presence of *Klebsiella pneumoniae* are branded soya milk and evaporated milk. *Klebsella pneumoniae* is a common opportunistic pathogen that can infect both plants and animals [19]. It is widely recognized as an urgent threat to human health because of the emergence of multidrug-resistant and hypervirulent strains associated with hospital and community-acquired infections [20]. *Klebsiella quasipneumoniae subsp. similipneumoniae* strain ATCC 700603, formerly known as *Klebsiella pneumonia* K6, is known for producing extended-spectrum β-lactamase (ESBL) enzymes that can hydrolyze oxyimino -β-lactams (e.g. ceftazidime), resulting in resistance to these drugs [21].

Branded soyamilk tested indicated the presence of *E. coli* and *Klebsiella app.* contained in 3 products all produced by the same company, though all APCs were within the acceptable limit stipulated by the Standard Methods for the Examination of Dairy Products but had unacceptable coliform counts. Unbranded Soyamilk samples had the highest bacteria count with all samples tested having unacceptable APCs and Coliform count. Soyamilk C, had the highest aerobic plate count with a value of 9.4 X 10^5cfu/ml while Soyamilk D had the lowest APC of 2.3X10^4.The Coliform counts were also exceedingly high with Soyamilk A leading the pack with a coliform count of 2.1 X 10^5, followed closely by Soyamilk C with APC of 2.0 X 10^5. Soyamilk E had the least Coliform count with 2.3 X 10^4. All the unbranded soyamilk samples had Coliform counts above the acceptable standard of 10cfu/ml. Further testing identified a host of bacteria species which include *E. coli, Klebsiella spp., Staphylococcus aureus, Staphylococcus epidermidis, Macrococcus caseolyticus, Lysinibacillus macroides, Salmonella spp., and Enterobacter cloacae.* Soyamilk has a pH of around 7 and this pH favours bacteria growth [6].

Salmonella enterica subsp. entericaserovar Uganda and *Salmonella enterica subsp. enteric serovar infantis* were also isolated from unbranded soya milk samples. *Salmonella enteric subspecies enteric* is an important food-borne pathogen responsible for disease in animals and humans. It has been the leading cause of many outbreaks and infections around the world and is considered as one of the major causes of human gastroenteritis worldwide [22]. *S. enterica* subsp. *Enteric serovar infantis* is a nontyphoidal emerging serovar showing increased morbidity in humans worldwide [23]. Studies by Aviv *et al.,* [24] have shown that some emergent *S. infantis* isolates carry a self-transmissible megaplasmid that confers stress tolerance and promotes pathogenicity, which can be horizontally transferred to the gut microbiota by conjugation. Animals have been implicated as important sources of *Salmonella*-contaminated food products that are responsible for human salmonellosis [25]. The clinical outcome of *Salmonella* infection in humans presents in two broad features; first, it manifests as a serious systemic infection (enteric fever) caused mainly by *Salmonella enteric serovar typhi* (typhoid fever) and the second usually takes the form of a self-limiting food poisoning (gastroenteritis) and is caused by a large number of nontyphoidal *Salmonella* serovars [26]. Non-typhoidal *Salmonella* serovars (NTS) are one of the most important etiological agents of

foodborne diarrheal diseases in humans worldwide and cause an estimated 80.3 million foodborne illnesses a year [27]. Although most cases are self-limiting episodes of gastro-enteritis, severe cases of infection, including bacteremia and meningitis require antimicrobial treatment. In Sub-Saharan Africa, nontyphoidal Salmonella are emerging as a prominent cause of invasive disease in infants and young children [26].

Lysinibacillus macroides is a large Gram-positive and Gram-negative motile rods. They are strictly aerobic [28]. Formerly known as *Lineola longa* then *Bacillus macroides, L. macroides* was isolated in 1947 from cow dung *Lysinibacillus macroides* is associated with infections such as periodontitis and has previously been isolated from butterfly larvae [28].

Macrococcus caseolyticus is a large Gram-positive cocci belonging to the family *Staphylococcaceae*. *M. caseolyticus* isolated in this study was revealed by molecular studies to be cellulolytic bacteria from cow rumen. This organism may have been introduced into the SoyamilkA from contaminated powdered milk which was added during processing. *M. caseolyticus* has a probable primordial form of a *Macrococcus* methicillin resistance gene complex, mecIRAm on one of its plasmids. *M. caseolyticus* is considered to reflect the genome of ancestral bacteria before the speciation of *Staphylococcal* species and may be closely associated with the origin of the methicillin resistance gene complex of the notorious human pathogen methicillin-resistant *S. aureus*. Unlike *Staphylococcal* species, *M. caselyticus* do not cause human or animal diseases [29]. It is important to note that *Klebsiellaspp* (100%) and *Escherichia coli* (100%) were the most prevalent bacteria in unbranded soyamilk samples as it was present in all soyamilk samples tested.

5. CONCLUSION

In conclusion, milk sold in Nnewihave been proven by this study to be contaminated with a host of bacterial species that should raise public health concerns. The bacteriological quality of these milk products are sub-optimal as significant amounts of bacteria including coliforms were found in both pasteurized, branded milk and unbranded milk. Since some of the milk products, especially infant formula, were devoid of pathogens, it is possible to have pathogen-free milk through strict preventive and hygienic measures. The key to preventing spoilage and prolonging the shelf-life of milk products is to prevent post-pasteurization contamination through well-designed quality assurance. It is also the responsibility of both consumers and suppliers to adequately store milk at suitable temperatures in order to control the levels of microorganisms and to slow the rate of milk spoilage. Effective measures to ensure safe milk for human consumption should be routinely performed on each batch of milk processed by dairy companies and medical examination of milk handlers should also be done to reduce milk contamination by infected handlers. It is therefore very important that high aseptic conditions be maintained in the Nigerian milk product industry.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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