East and Central African Journal of Pharmaceutical Sciences
Vol. 18 (2015) 23-31

Microbiological Quality of Pasteurized Milk Available in the Dar es Salaam Market, Tanzania

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Twenty packs of pasteurized milk were randomly purchased from various sources in three municipalities of Dar es Salaam City, Tanzania, with the aim of quantifying and identifying contaminant bacteria. A total of 24 bacterial strains were isolated following aerobic incubation at 37 ºC for 48 hours. The average aerobic count was 4.3×10^5 cfu/ml with 75 % of the milk samples found to be heavily contaminated. More than half (54 %) of the samples were contaminated with *Staphylococcus aureus*. The isolated bacteria were subjected to antibiotic susceptibility tests against 20 commonly used antibiotics using the Kirby-Bauer disk diffusion method. The inhibition zones obtained with the isolates were compared to those obtained when reference strains of microorganisms were similarly treated. Results were analyzed by SPSS software. About 29.2 % of the isolated bacteria were totally resistant to tested antibiotics. These findings advocate for more rigid sanitary measures during production and storage of pasteurized milk and other dairy products that are available in the market.

Key words: Pasteurized milk, bacterial contaminants, antibiotic resistance

INTRODUCTION

Milk and other dairy products are excellent high quality foodstuffs that provide both nutritional and gastronomical value. These products are consumed by people of almost all age groups. The fact that milk consists of a variety of nutrients such as proteins, fats, minerals, carbohydrates, vitamins and water makes it an excellent medium for microbial growth, and, subsequently, its spoilage [1]. For the same reason, milk can also serve as a carrier of pathogenic microorganisms [2, 3].

In several developing countries, particularly in urban areas such as Dar es Salaam, pasteurized milk is one of the most readily available and widely consumed foodstuffs. Milk is usually packaged in nylon and/or cardboard paper containers. Such products are not only available in supermarkets, mini-stores and cafeterias/restaurants, but are also sold by street vendors. A majority of consumers perceive packaged milk products as being safe for human consumption. However, infections due to milk consumption have been reported [4, 5] although such infections can successfully be minimized or prevented through pasteurization. The pasteurization process aims to increase milk safety for the consumer by destroying pathogenic microorganisms that may be present in milk. The process maintains quality of the products by getting rid of microorganisms and/or their enzymes that contribute to the reduced microbiological quality and shelf life of milk [6, 7].

Initial pasteurization conditions, known as flash pasteurization, involved heating the milk

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at 68.3 - 81 °C for an instant followed by cooling. The conditions were adjusted to heating at 61.7 °C for 30 minutes or 71.1 °C for 15 seconds in order to deactivate *Mycobacterium bovis*, the causative organism for tuberculosis. Enright *et al.* showed that these conditions were inadequate for the inactivation of *Coxiella burnetii* which causes Q fever in humans [8]. Therefore, currently, the following pasteurization conditions are used: high temperature short time (HTST) of 71.7 °C for 15 seconds, higher heat shorter time (HHST) of 88.3 °C for 1 second, ultra-high temperature pasteurization at 137.8 °C for 2 seconds and ultra-high temperature (UHT) sterilization at 138-150 °C for 1-2 seconds. The UHT treated milk may be stored for months without refrigeration.

Nevertheless contamination by dairy workers or handlers post-pasteurization, storage at inappropriate temperature (over 7 °C) as well as malfunction in processing equipment could lead to contamination of the milk. Similarly, when raw milk becomes heavily contaminated or when there is failure to maintain appropriate temperature during processing, storage or delivery, proliferation of pathogens may occur, thus affecting the quality of pasteurized milk [1, 9]. This study was aimed at assessing microbiological quality of pasteurized milk available in the Dar Es Salaam market and determining the antibiotic susceptibility profiles of the isolated microorganisms.

METHODOLOGY

Study area and design

This study was conducted in Dar es Salaam City, the most populous commercial city in Tanzania, with over 4 million inhabitants. The study involved all three of the city's municipalities namely Ilala, Kinondoni and Temeke. At least 4 localities from each district were covered.

Sample collection and processing

A stratified random sampling technique was employed in selection of representative samples. From each district, 6 unexpired samples of different brands of packed pasteurized milk namely Azam Milk (Azam Group, Tanzania), Tanga Fresh Milk (Tanga Fresh Limited, Tanzania), Dar Fresh Milk (Dar es Salaam, Tanzania), First Choice Milk (Woodland Dairy, South Africa), Dairy Fresh Milk (Brookside, Uganda) and Asas Milk (Asas Dairy Limited, Tanzania) were purchased from various shops/stores. The purchased samples were deposited into clean cool boxes and transported to our laboratory. More than one sample was purchased provided it was not of the same brand. Each sample was processed by serial dilution and sub-cultured in appropriate culture media at least 2 hours after purchase.

Each milk sample was weighed and in order to prevent cross-contamination, the external surface of each milk pack was swabbed using 70% ethanol at the milk sample drawing point using a sterile syringe. Under aseptic conditions each milk sample was serially diluted by drawing 1 ml of the sample and adding to a sterile tube containing already calibrated volumes of sterile normal saline resulting in concentrations of 10^{-1} to 10^{-4} v/v. Subsequently, 1 ml of each sample was drawn and deposited onto a sterile nutrient agar (NA) plate, and incubated at 37 ºC for at least 48 h. An uninoculated NA agar plate served as the negative control. All plates were placed on a colony counter and the resultant bacterial colonies were recorded as colony forming unit per milliliter (cfu/ml).

Isolation of bacterial contaminants

Bacterial contaminants were isolated by sub-culturing in both selective and non-selective media and subsequently identified through observation of colony morphologies on differential media and other physiological/biochemical tests as per the Laboratory Manual [10]. Each of the identified bacteria was re-suspended in a freshly prepared Ringer's lactate solution for 2-4 hours at 37 ºC. Each bacterial suspension was compared to that of McFarland 0.5 turbidity standard prior to performing antibiotic sensitivity tests as per the Clinical Laboratory Standards Institute (CLSI) guidelines [11].

Antibiotic susceptibility profiling

The isolated and identified microbes were subjected to antibiotic susceptibility tests against 20 commercially available and widely used antibiotics namely gentamicin (GM10), ciprofloxacin (CIP5), tetracycline (T30), ceftriaxone (CR30), imipenem (IMI10), ticarcillin (TC75), vancomycin (AC30), amikacin (AK30), ampicillin (AP10), clindamycin (CD2) (Bioanalyse, Ankara, Turkey), erythromycin (E15), oxacillin (OX1), neomycin (NE30), chloramphenicol (C30) (Oxoid, Hampshire, UK) and co-trimoxazole (TS25), ceftazidime (CAZ30), cefalexin (CFX30), piperacillin (PRL100) and spectinomycin (SP100), amoxicillin clavulanate (AMC30) (Mast Group Limited, Merseyside, UK). The Kirby-Bauer disk diffusion method was employed for assessment of antibiotic sensitivity on Mueller-Hinton agar (Carl Roth GmbH, Karlsruhe, Germany) plates through determination of diameters of inhibition zones (IZ) for each bacterial isolate.

Statistical data analysis and interpretation of results

Quantitative data were compared among the different milk samples and with respect to the negative control and acceptable limits (standard). Similarly, analysis of variance for diameters of IZ (mm) produced by the bacterial isolates against the tested antibiotics was analyzed using IBM SPSS software version 20 (IBM Corp., Armonk, NY, USA). The Dunnett's test (two-sided) was used to compare the significance of any differences between each bacterial isolate with its reference strains. The results were interpreted as susceptible (S), intermediate (I) and resistant (R) according to the CLSI criteria [11]. Differences among the tested parameters were considered significant when $p < 0.05$.

RESULTS

Isolated bacterial species and levels of contamination

A total of 20 pasteurized milk samples from 6 different brands were purchased from various localities of the 3 municipalities of Dar es Salaam City. The tested brands comprised Azam Milk (Azam Group, Tanzania), Tanga Fresh Milk (Tanga Fresh Limited, Tanzania), Dar Fresh Milk (Dar Fresh, Tanzania), First Choice Milk (Woodland Dairy, South Africa), Dairy Fresh Milk (Brookside, Uganda) and Asas Milk (Asas Dairy Limited, Tanzania). The samples were randomly assigned codes A through F for safeguarding of commercial interests.

From the 20 collected samples, a total of 24 bacterial isolates from 6 different species were identified. *Staphylococcus* species comprised a

majority of the 24 isolates (n= 15, 62.5%) while *Enterobacter aerogenes* and *Klebsiella pneumoniae* were the least commonly isolated species (n=1, 4.2% for both). The results are shown in Table 1.

The results revealed different types of microbial contaminants with varying bio-burdens (cfu/ml) as indicated in Table 1. Brands A and D were the most heavily contaminated (8.6×10^5 cfu/ml). This figure was 86 times greater than the acceptable level of 1.0×10^4 cfu/ml. Though *S. aureus* was the most frequently isolated bacterial species, *E. coli* accounted for the highest levels of contamination in majority of the samples. Only one brand, F, had an acceptable level of microbial content (Table 2).

Seven out of 20 samples (5 out of 6 brands) were contaminated with two species of bacteria. Brand A was found to harbor the highest number of bacterial species (Table 2). Statistically significant differences were observed among the samples with regard to levels of contamination ($p < 0.05$). Likewise, significant differences ($p = 0.001$) were observed when comparing the contamination levels in the pasteurized milk samples against the acceptable limits for both coliforms (10 cfu/ml) and other bacteria (10,000 cfu/ml).

Table 1: Isolated bacteria and frequency of isolation

Isolated bacteria	Frequency of isolation	Contaminated Brands	Ranges (cfu/ml)
Staphylococcus aureus	13	5	9.1×10^3 - 7.5×10^5
Escherichia coli	4	2	5.1×10^4 - 8.6×10^5
Staphylococcus saprophyticus	2	2	1.7×10^5 - 3.5×10^5
Klebsiella pneumoniae	1	1	2.1×10^5
Pseudomonas aeruginosa	3	2	5.1×10^4 - 4.3×10^5
Enterobacter aerogenes	1	1	8.5×10^5

Table 2: Levels of contamination (cfu/ml) of the tested brands

Brands	Isolated bacteria	cfu/ml (ranges)	No. of samples
A	*E. coli*	$8.9 \times 10^3 - 8.6 \times 10^5$	2
	S. saprophyticus	2.3×10^5	1
	S. aureus	2.8×10^5	1
	K. pneumoniae	2.1×10^5	1
B	*E. aerogenes*	8.5×10^5	1
	S. aureus	4.8×10^4 - 7.5×10^5	4
	P. aeruginosa	2.4×10^5 - 4.3×10^5	2
C	*S. aureus*	1.3×10^5 - 4.3×10^5	2
D	*P. aeruginosa*	2.4×10^5	1
	E. coli	5.1×10^4 - 8.6×10^5	2
	S. aureus	7.6×10^4 - 4.0×10^5	2
E	*S. aureus*	1.2×10^5 - 7.5×10^5	3
	S. saprophyticus	1.7×10^5	1
F	*S. aureus*	9.1×10^3	1

Antibiotic resistance profiles of the isolated bacteria

Of all 20 tested antibiotics, none was effective against all isolated bacteria (Table 3). A few bacterial isolates were not subjected to susceptibility tests against certain antibiotics on account of technical and clinical reasons [10, 11]. Nevertheless, significant differences in IZ (susceptibility to the tested antibiotics) between some isolates of bacterial contaminants and their reference strains were evident ($p < 0.05$). Seven (29.2 %) out of 24 bacterial isolates exhibited 100 % resistance to some tested antibiotics: *K. pneumoniae* (n=1) was completely resistant to ciprofloxacin, cephalexin, amikacin and amoxicillin clavulanate; *P. aeruginosa* (n=3) to cephalexin and ceftriaxone; *E. aerogenes* (n=1) to amoxicillin clavulanate, amikacin and spectinomycin while *S. saprophyticus* (n=2) were both resistant to cotrimoxazole.

Table 3: Resistance profiles of isolated bacteria

Antibiotic	Resistant bacteria and % resistance rates
Gentamicin	*E. coli (50); S. aureus (31)*
Ciprofloxacin	*E. coli (50); S. aureus (23); K. pneumoniae (100)*
Tetracycline	*E. coli (25); P. aeruginosa (33.3)*
Chloramphenicol	*S. aureus (7.7)*
Imipenem	*S. aureus (23)*
Cefalexin	*E. coli (75); P. aeruginosa (100)*
Ticarcillin	*E. coli (25); P. aeruginosa (33.3); S. aureus (46)*
Amoxicillin/clavulanate	*E. coli (50);P. aeruginosa (66); K. pneumoniae (100); E. aerogenes (100)*
Vancomycin	*S. aureus (23)*
Piperacillin	*E. coli (50)*
Clindamycin	*S. aureus (46)*
Amikacin	*P. aeruginosa (66); K. pneumoniae (100); E. aerogenes (100)*
Ampicillin	*S. aureus (46)*
Erythromycin,	*E. coli (50); S. aureus (23); P. aeruginosa (66)*
Oxacillin	*S. aureus (23)*
Neomycin	*S. aureus (23)*
Cotrimoxazole	*S. saprophyticus (100)*
Ceftriaxone	*E. coli (50); P. aeruginosa (100)*
Spectinomycin	*E. coli (50);E. aerogenes(100)*
Ceftazidime	*E. coli (75); P. aeruginosa (33.3)*

DISCUSSION

Food-borne diseases are a growing health concern among consumers. Proper handling and preventive sanitary measures of fresh, processed, and ready-to-eat foodstuffs are considered to be the basis of prevention of food-borne disease outbreaks. However, it is well recognized that majority of food-borne illnesses are caused by hazards associated with improper post-production handling of such foods rather than from raw materials used or contamination derived from the processing premises [12,13].

Enteric bacteria such as *E. coli* are not uncommon in food manufacturing environments and may become part of the resident microflora of the premises especially when sanitary measures are not observed. It is also possible for *E. coli* to proliferate on some foods under refrigeration [14]. This makes it imperative for the pasteurization process to adequately eliminate any existing *E. coli* and other microorganisms. Pasteurization involves heating the milk to temperatures that may remove fastidious microorganisms, but not alter the food value and palatability of the milk. Usually such heat treatment processes do not sterilize the milk since *Lactobacillus* can survive the temperatures involved. However, the bacterial count is greatly reduced and most pathogens are killed [15, 16]. Nevertheless, even small amounts of *E. coli* ingested from milk or any dairy products can lead to severe gastrointestinal complications [17, 18].

The United Kingdom's Dairy Products (Hygiene) Regulations of 1995 recommend that the acceptable limits for total bacteria in pasteurized milk should be less than 2.0×10^4 cfu/ml while coliforms should not exceed 10 cfu/ml [19]. Our findings revealed that some of the samples had levels of bacterial contamination that significantly exceeded the acceptable limits (1000-fold for coliforms and 30-fold for other types of bacteria). The coliforms isolated from the pasteurized milk included *E. coli, K. pneumoniae* and *E. aerogenes*, which are microflora of the large intestine [2]. The presence of these microorganisms in milk may, therefore, indicate fecal contamination. *Escherichia coli* is an important food-borne infectious agent whose enteropathogenic serotype can cause diarrhea and other complications that occasionally result in fatalities [20]. Our findings concur with those of Kunda *et al.* who found that the total bacterial count of the pasteurized milk was relatively low ranging from 6.0×10^3 to 3.8×10^4 cfu/ml and that most of the bacterial contaminants was attributed to the presence of *Staphylococcus* spp, *Salmonella* spp and *Clostridium perfringens* [21].

Presence of bacteria in pasteurized milk could also be due to biofilm development on equipment surfaces as a result of inappropriate production and packaging processes. Such communities of bacteria develop when nutrients and water stick on surfaces during the cleaning process and reuse of the equipment. Bacteria in biofilms are more resistant to antimicrobial agents such as disinfectants than those in planktonic or suspension form [22, 23]. Disinfectants may be inactivated by biofilms allowing viable bacteria to be dislodged into the milk [24, 25].

The bacterial content of unexpired pasteurized milk in this study was unacceptably high as significant amounts of bacteria including coliforms were found in the milk samples of some brands. Of several coliforms isolated, *E. coli* was the most frequently isolated. Infections due to most of the isolated coliforms are common and do not clear out completely after antibiotic therapy. This may be due to development of drug resistance as a result of constant exposure of livestock to low doses of antibiotics in animal feeds that are not only used as growth promoters but also to improve the quality of meat by lowering fat content. Nevertheless, use of antibiotic growth-promoters imposes a selection pressure for bacteria leading to development of antibiotic resistance. Since these antibiotics may also be used in clinical or veterinary practice, the resultant reduced antibiotic susceptibility compromises the sustained practice of antibiotic chemotherapy [26].

It is worth stating that consumption of high bio-burden-containing milk or milk products by unhealthy individuals or those on antimicrobial chemotherapy may interfere with treatment regimens. This is because such individuals may become re-infected by more resistant microorganisms, which might lead to treatment failure. *Klebsiella pneumoniae*, *P. aeruginosa* and *E. coli* exhibited high resistant rates (75-100%) against widely used antibiotics. Usually these are the cheaper antibiotics that are affordable to majority of citizens and that are, therefore, employed in the prevention and control of a myriad of infectious diseases. Although *K. pneumoniae* is part of normal flora of the human intestine, it is also considered as a superbug attributable to a range of different illnesses [27, 28]. *Pseudomonas aeruginosa* exhibited high rates of antibiotics resistance, and it is one of the leading causes of nosocomial infections, particularly in immune-compromised individuals. Some of the most common nosocomial infections include septicemia, pneumonia, and infections following surgery, all of which can lead to severe illness and patient death. Moreover, *E. coli*, *E. aerogenes* and *S. aureus* are implicated in a number of infections including urinary tract and other soft tissue infections [29, 30]. Although fluoroquinolones (ciprofloxacin) and some cephalosporins (ceftriaxone, ceftazidime and cefalexin) are broad-spectrum antibiotics, they exhibited high rates of antibiotic resistance when tested against a number of the isolated bacteria in this study.

CONCLUSION

This study found that packaged milk samples are heavily contaminated. The commonest contaminants were *S. aureus* and *E. coli*. The overall rate of total antibacterial resistance was 29.2 %. Most of the bacterial isolates were resistant to commonly used antibiotics namely ciprofloxacin, cephalexin, amikacin, ceftriaxone and amoxicillin/clavulanate. Our findings call for close monitoring of the microbial quality of milk products circulating in our markets. Furthermore, we recommend that both consumers and suppliers/manufacturers should store milk at recommended temperatures in order to control the levels of microorganisms and to retard the rate of milk spoilage. Manufacturers, distributors/sellers and consumers should cautiously handle the pasteurized milk products despite the fact that the products are packaged in nylon and/or cardboard paper containers as the products are still subject to microbial contamination. Regulatory authorities and policy makers should institute more stringent measures for controlling easy availability of milk and milk products in our markets to safeguard the citizens' well-being. Also we advocate for periodic monitoring of milk quality before it is sold to consumers.

REFERENCES

[1] L.H. Ledenbach and R.T. Marshall. In: W.H. Sperber, M.P. Doyle (eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages*, Food Microbiology and Food Safety, Springer Science & Business Media, LLC 2009, p41-67.

[2] M. Anderson, P. Hinds, S. Hurditt, P. Miller, D. McGrowder and R. Alexander-Lindo. Asian. Pac. J. Trop. Biomed., 1(3), 2011, 205-211.

[3] T. S. Gunasekera, A. Sørensen, P. V. Attfield, S. J. Sørensen and D. A. Veal. Appl. Environ. Microbiol., 68(4), 2002, 1988-1993.

[4] M.T. Brady, C.L. Byington, H. D. Davies, K. M. Edwards, M. P. Glode, and M.A. Jackson. Pediatrics, 133(1), 2014, 175-179.

[5] D.L. O'Connor, J.B. Ewaschuk and S. Unger. Curr. Opin. Clin. Nutr. Metab. Care, 18(3), 2015, 269-275.

[6] P. Sharma, P. Bremer, I. Oey and D.W. Everett. Int. Dairy J., *35*(1), 2014, 49-56.

[7] S. Nada, D. Ilija, T. Igor, M. Jelena and G. Ruzica. Food Control J., 25(2), 2012, 728-731.

[8] J.B. Enright, W.W. Sadler and E.C. Thomas. USA *Pub, Health Service Publication.* Pub. Health. Monograph No, 47(30), 1957.

[9] K. Girma, Z. Tilahun and D. Haimanot. World J. Dairy Food Sci., 9(2), 2014, 166-183.

[10] M. Cheesbrough. District Laboratory Practice in Tropical countries. Part 2: Cambridge University Press, Cambridge. 2006, p 132-143.

[11] Clinical and Laboratory Standards Institute. CLSI document M100-S22. Wayne, 2012.

[12] S. Rane. Indian. J. Microbiol., 51(1), 2011, 100-106.

[13] B M. Lund. Int. J. Environ. Res. Public Health, 12(8), 2015, 10117-10132.

[14] K.L. Perez, M.J. Alam, A. Castillo and T.M. Taylor. J. Food Protection, 76(1), 2013, 124-128.

[15] W.L. Claeys, S. Cardoen, G. Daube, J. De Block, K. Dewettinck, K. Dierick and L. Herman. Food Control, 31(1), 2013, 51-262.

[16] V. Ladero, E. Sánchez-Llana, M. Fernández and M. A. Alvarez. Int. J. Food Sci. Technol. 46(3), 2011, 516-521.

[17] M. Rivas, I. Chinen, E. Miliwebsky and M.A. Masana. Microbiol. Spectrum, 2(5), 2014, EHEC-0002-2013.

[18] A. Catford, V. Kouamé, A. Martinez-Perez, A. Gill, E. Buenaventura, H. Couture, and J.M Farber. Int. Food Risk Anal. J., 4, 2014

[19] H.M. Wehr and J.H. Frank. Standard Methods for the Examination of Dairy Products. 17th Ed. Amer. Public Health Assoc., Washington, DC. 2004.

[20] S.V. Sodha, K. Heiman, L.H. Gould, R. Bishop, M. Iwamoto, D.L. Swerdlow and P.M. Griffin. Epidemiol. Infect. J., 143(02), 2015, 267-273.

[21] B. Kunda, G. S. Pandey, C. Mubita, J.B. Muma and C. Mumba. Live. Res. Rural Develop., 27(143), 2015.

[22] H. Van Acker, P. Van Dijck and T. Coenye. Trends. Microbiol., 22(6), 2014, 326-333.

[23] R. Gabrani, G. Sharma, S. Dang and S. Gupta. Interplay Among Bacterial Resistance, Biofilm Formation and Oxidative Stress for Nosocomial Infections. In *Free Radicals in Human*

Health and Disease). Springer India. 2015, p 369-379.

[24] J.A. Otter, K.Vickery, J. T. Walker, E. deLancey Pulcini, P. Stoodley, S.D. Goldenberg and J.D. Edgeworth. J. Hospital. Infect., 89(1), 2015, 16-27.

[25] S. Kentish and H. Feng. Ann. Rev. Sci. Technol., 5, 2014, 263-284.

[26] D.I. Andersson and D. Hughes. Nat. Rev. Microbiol.,12(7), 2014, 465-478.

[27] J. Davies and D. Davies. Microbiol. Mol. Biol. Rev., 74(3), 2010, 417-433.

[28] R.M Caron. Public Health Lessons: Practicing and Teaching Public Health. In Preparing the Public Health Workforce. Springer International Publishing. 2015, p 67-93.

[29] M. Lu, Y. Shiau, J. Wong, R. Lin, H. Kravis, T. Blackmon and N.S. Wang. Food. Nutr. Sci., 4(07), 2013,113.

[30] X. Wang and D. Li. Omega, 40(6), 2012, 906-917.