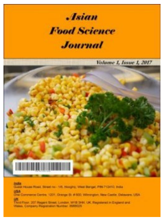

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Prevalence of Adulteration and Inhibitory Substances in Raw and Pasteurized Milk Marketed in Nairobi Region

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Authors' contributions

This work was carried out in collaboration between all authors. Author GWW designed the study, conducted literature review, conducted the experiments, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FMM, PMK and JMM interpreted the results and discussion of the study and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The prevalence of milk adulteration with water and inhibitory substances in Nairobi was determined. Milk samples were collected randomly at milk selling points from three market areas: rural (Kiambu/Ngong'), urban (East/West of Tom Mboya street) and slum (Kibera/Mathare). Samples were analyzed for specific gravity, hydrogen peroxide and presence of antimicrobials using lactometer reading, peroxide strips and the Copan test respectively. Data collected were subjected to analysis of variance using Genstat statistical package. The mean density was 1.029±0.000027 gm/L, while 16.5% and 21.1% of the milk sampled had residues of hydrogen peroxide and antimicrobials respectively. From 206 raw milk samples collected, 13.6% had densities lower than 1.026 gm/L while adulteration was detected in 2 of 98 samples of pasteurized milk. Approximately 18% and 25% of raw and pasteurized milk, respectively, had antimicrobials.

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The highest incidence of milk adulteration with water was in slum areas, while no adulteration was detected in pasteurized milk. The results indicate that pasteurized milk is of satisfactory quality with regard to adulteration with water, while raw milk marketed in Nairobi is likely to be adulterated. Pasteurized milk was found to be more likely to be adulterated with hydrogen peroxide than raw milk marketed in Nairobi. Therefore consumers may be exposed to residues of hydrogen peroxide on consumption of some pasteurized packaged milk. The highest level of antimicrobials in pasteurized milk was 33.3% in brands K.b and K.d while no residues were detected in brand K.c. The incidence of antimicrobials in marketed milk implies that consumers are likely to be exposed to possible drug residues. The incidence of antimicrobials in raw milk contributes to increased failures in controlled acidification of milk during the production of fermented milk products. High incidence of detection in pasteurized packaged milk is mostly contributed by residues of hydrogen peroxide and/or other sanitizer or sterilizer residues.

Keywords: Nairobi; market milk; adulteration; water; antimicrobials; hydrogen peroxide.

1. INTRODUCTION

Demand for milk in Nairobi is high due to the increasing population within the city and its environs. Most of the milk marketed in Nairobi is sourced from Kiambu District, where it constitutes the bulk of milk from small-scale farmers and sold to middlemen in the informal milk market. The unregulated informal market handles over 80% of marketed milk in most developing countries [1,2]. There are fears regarding the quality of this milk as after purchase from farmers, adulteration with water is done before eventual marketing [2]. Due to long distances coupled with lack of a cold chain, some milk handlers adulterate the milk with preservatives like hydrogen peroxide [2,3] to enhance marketing. Milk adulteration with water and/or hydrogen peroxide is alleged to predominate in the informal milk market in Nairobi, leading to inferior quality milk offered for sale.

Milk adulteration is illegal and a dangerous practice rendering it unsafe for human consumption as added substances could induce physical, chemical and biological hazards hence pose serious health problems to consumers. Constant exposure of consumers to chemical substances could lead to drug resistance and vulnerable groups like invalids, infants and the elderly are at higher risk. Adulteration with water could induce bacterial contaminants while rendering the milk unsafe for consumption as they could lead to bacterial disease transmission. Adulterated milk could lead to economic losses due to ill health, spoilage and possible rejection by consumers. The objective of this study was, therefore, to establish the presence and degree of adulteration of marketed milk, with water and hydrogen peroxide, in Nairobi and its environs.

Demand for milk in Kenya is ever increasing; however, the safety of milk from drug residues is still a major hindrance to the promotion of milk and dairy products. In dairy animals, drugs are administered for treatment of mastitis through intramammary or intravenous infusions [4] and drug therapy for other bovine illnesses. Antibiotic residues in milk result from failure to observe mandatory withdrawal periods after antibiotic administration to dairy animals [5,6]. Drug residues also occur due to illegal and/or extra use of drugs, incorrect dosages and some are more persistent in the animal body than discard time indicated on the label [7].

Previous studies in Kenya have shown that animal products present in the market have unacceptably high levels of drug residues [8,9]. Exposure of consumers to antibiotics through food can result in allergic reactions in sensitive people, including toxicity and carcinogenic effects [10,6]. Frequent exposure of consumers to low doses of antibiotics can lead to the development of bacterial resistance to common antibiotics. Additionally, the presence of antibiotic residues in milk affects processing of fermented milk products, due to their inhibitory effects on starter cultures used [6]. East African Standard; EAS 69:2006 stipulate that unprocessed whole milk and processed milk should not contain antibiotic residues [11]. It is, therefore, very important to analyze raw and pasteurized milk marketed within Nairobi and it's environs for the presence of antimicrobials.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 206 raw milk samples were collected from three market areas in Nairobi region while

98 pasteurized milk samples were collected from the retail market (supermarkets and kiosks). The market areas were chosen based on consumption points within the study area, namely, rural set up (Kiambu and Ngong'), urban set up (east and west of Tom Mboya street) and slum set up (Mathare and Kibera), which were subgroups within which random sampling was done.

2.2 Sample Preparation

Approximately 250 ml, raw milk was aseptically sampled from plastic containers and/or aluminium cans from each milk trader into a sterile sampling bottle. Different brands of pasteurized milk, 500 ml sachets or packets were bought randomly from the retail chains. Milk samples were collected early in the morning up to mid-morning. A minimum of sixty-four milk samples were required to give a representative sample size for representing a category of sampled milk, raw or pasteurized [12,13].

Raw milk samples purchased from milk traders were transferred into sterile sampling bottles or packed in plastic sugar bags kept in cool boxes with ice cubes and transported to the laboratory within 45 minutes of purchase. Processed milk samples (500 ml) bought from kiosks and supermarkets, were immediately kept in a cool box and transported to the laboratory and stored at 4°C, unopened until the date for analysis. Sampling preparations and procedures were done according to AOAC methods [14]. For bulk milk, the milk was mixed (agitated) thoroughly and 250 ml milk sample drawn using a dipper.

2.3 Copan Test Kit

The Copan test kit (Chr. Hansen, Horsholm, Demark) is very sensitive to some antibiotics (Annex 1) such as β-lactams hence an effective "biological sensor" for detection of antibiotics in milk. Each kit contains 25 vials with *B. stearothermophyilus* var. *calidolactis* spores, nutrients and a pH indicator (Bromocresol purple). Also included are 25 patented "no drop" disposable pipettes and a colour card for comparison of results [6].

2.4 Determination of Specific Gravity

Specific gravity in gm/L was determined using a lactometer. The milk sample was mixed and poured gently into a measuring cylinder (250-500 ml). The lactometer was let to sink slowly into the milk. The last lactometer reading just above the surface of the milk was read and recorded in Lactometer degree (°L). Where the temperature of the milk was different from the calibration temperature of the lactometer, the temperature correction value was calculated. For each degree above the calibration temperature 0.2°L was added and for each degree below the calibration temperature, 0.2°L was subtracted from the recorded lactometer reading.

2.5 Determination of Hydrogen Peroxide

Hydrogen peroxide was detected using peroxide detection strips (Quantofix Marcherey-Nagel, Germany) according to a method described by Nicholette Marks [15]. The test strip was dipped in the sample for about one second and excess milk was drained off using a tissue paper. The reading was done after 15 seconds by comparing the test paper zone and the colour scale provided.

2.6 Determination of Antibiotic Residues

Antibiotic residues were determined using antibiotic test kits, the Copan test (Chr. Hansen, Germany) [6]. The foil cover was then carefully peeled back and 100 µL of the milk sample was added using the no drop pipettes provided with the kit. New pipettes were used for every replicate and new sample. The foil was replaced and sealed using adhesive tape and the vials were incubated in a water bath (using a foam rack) at 64.5°C for 2.30 – 3 hours. The control sample was freshly prepared yoghurt obtained from Department of Food Science and Technology, JKUAT. After incubation results were read by comparing the media colour with the colour chart provided [6].

All the analysis were done in triplicates and analyzed using Genstat statistical package [16].

3. RESULTS AND DISCUSSION

Density measurements revealed a mean density of 1.028 gm/L, from 303 milk samples collected. According to the East African Standard (KS EAS) 67:2006, "density of unprocessed whole milk at 20°C shall be within the range of 1.026 – 1.032 gm/L", [11] therefore on average all milk samples analyzed had densities within acceptable limits. Raw milk sampled from all the market areas had mean densities within the acceptable limit (Table 1). This compares to a mean density of 1.029 gm/L in pasteurized milk (Table 2), which also is within the acceptable limit, but analysis of variance revealed a significant difference ($p=0.05$). This is probably attributed to deliberate addition of water and/or other substances to milk possibly by milkers and/or by immediate traders both at farm level and market. Some pasteurized milk samples (2) packed in sachets, however, were adultered with water. During packaging, good care should be taken as final ringer water can easily be filled together with pasteurized milk resulting in adulteration. Adulteration is a dangerous practice as the water used may contaminate the milk leading to microbial health hazards. This is especially critical if the water used is from questionable sources, including sewage, rivers and dams [2]. This can lead to spread of water borne diseases like cholera, typhoid and dysentery among consumers.

The level of raw milk adulteration with water was at 5.1%, 11.1% and 21.4% in rural, urban and slum areas, respectively (Table 1). This may imply that adulteration was done by some of the traders (milk transporters and/or milk vendors) because of increased incidence of adulteration from 5.1% in rural areas to a high of 21.4% in slum areas. Tendency to adulterate milk seemed to increase with decline in economic status, as 21.4% of raw milk marketed in slum areas was adultered with water as compared to 11.1% in urban Nairobi. Poor sanitary conditions exist in slum areas and the available water is of unsatisfactory quality therefore adulteration implies greater chances of disease transmission. Adulteration is almost double in slum areas as compared to urban areas hence more consumers in these areas are exposed to effects of contaminated milk. Though adulteration with water was low in rural areas, dangers associated with consuming contaminated milk are likely to occur. Food poisoning cases due to consumption of contaminated milk in Kisii in Kenya resulted in four deaths and many people hospitalized in 2005 [17]. These are preventable occurrences if practices like milk adulteration are stopped among milk traders through strict monitoring by government regulatory departments and increased consumer awareness. Considering containers used, 22.6% of milk conveyed in aluminium containers, was adultered with water as compared to 12.0% of raw milk marketed in plastic containers. Adulteration was, however, common among affluent transporters who also summed up as milk vendors in urban and slum areas. Majority of the small-scale farmers and traders, on the other hand, used plastic jerricans, where adulteration was practiced at lesser magnitudes. This can be explained by the need of these farmers to retain personal transactions with their customers hence they could not adulterate the milk. However, adulteration was fuelled by the increasing milk demand coupled with high prices of the commodity. In addition, the prevailing drought and famine conditions in most parts of the country at the time of the study led to shortages of water and animal feeds thereby affecting milk production. This reduction in milk production fuelled milk adulteration with water to increase volumes to satisfy target markets.

Deliberate adulteration of milk with chemical preservatives is dangerous as added substances may introduce chemical health hazards rendering milk unsafe for use [2]. Addition of H_2O_2 to raw milk can stimulate the antibacterial system in milk hence considerably extend its shelf life [18]. A previous study on the use of H_2O_2 to preserve raw milk marketed in Nairobi revealed that the substance was not being used to preserve milk [19]. However, results from the current study revealed a prevalence of 8.3% (Table 3) and 24.5% (Table 4) in raw and pasteurized milk, respectively. Deliberate use of H_2O_2 was at 8.8, 6.3 and 9.4% in rural, urban and slum areas, respectively (Table 3). Detection of H_2O_2 in raw milk in rural areas may occur due to deliberate addition to aid in eventual marketing due to lack of a cold chain. The lowest incidence was in urban Nairobi which can be attributed to fears by milk marketers to increased enlightenment of target consumers. High incidence of H_2O_2 in pasteurized milk can be attributed to the use of the substance in cleaning and disinfection of milk containers, holding and filling lines as well as packaging materials. Averagely 24.5% of the 98 pasteurized milk samples collected (Table 4) had peroxide residues. This exposes consumers to H_2O_2, which can disrupt activities of resident gut micro-flora leading to digestive problems. Invalids, the elderly, the sick and consumers with digestive problems are at higher risks, as milk is the commodity of choice to supplement their diets. However, heating milk to above 80°C or in the presence of catalysts like metals, acids or oxidizable organic materials, leads to decomposition of H_2O_2 into water and oxygen.

Preservatives in raw milk can affect the processing of fermented milk products as these chemical substances can alter or inhibit the growth of live starter cultures. Given the fact that several types of H_2O_2 are present in the market, food grade, industrial, cosmetic and medical, it was not possible to identify which type was detected in both raw and pasteurized milk. Indeed, milk traders can access any of these types easily leading to indiscriminate use as unlike milk processing firms other traders lack the basic scientific concepts to aid in prudent use. Additionally, it has been reported that low LPs in yoghurt, only prevented post acidification hence the yoghurt had retained acceptable quality during storage and it was also observed to improve the quality of cheese [20]. According to the East Africa Standard (EAS 69:2006) [11], no preservatives and/or presence of any inhibitory substances should be present in milk. There is, therefore, urgent need to sensitize milk handlers to adopt the use of other friendly sanitizers and sterilizers like ozone as well as enlighten them on the proper use of H_2O_2 as a sanitizer. Therefore further research work needs to be done to establish trends of peroxide use among milk handlers and traders as well as determine the types of H_2O_2 commonly used.

Drug residues in milk and/or dairy products constitute a safety risk to potential consumers of milk or dairy products. The current research finding indicates a prevalence of 17.5% in hawked raw milk and 24.7% in pasteurized formally marketed milk (Table 5). Antimicrobials were highest in milk samples collected from rural and lowest in urban areas. Considering the zero residue limit set in Kenya; East African Standard; EAS 69:2016 [11], this indicates a very high percentage of detection in milk. Previous studies conducted in Nairobi and Kiambu showed prevalence of 4-16% and 8%, respectively [14]. Results from the current study show that incidence of antibiotic residues at rural level have increased from 8% [14] to 25% (Table 5) possibly due to an increased and more diverse study area (Kiambu and Ngong). In Nairobi however, prevalence is at 3.1% in urban areas and 22.5% in slum areas (Table 5), which compares to 4-16% according to the previous study [14]. High detection level in slums can be attributed the indiscriminate use of antibiotics and exploitation of consumers due to ignorance and their low economic status by traders. Low levels of antimicrobials (3.1%) (Table 5) detected in urban Nairobi is because traders know that potential consumers are knowledgeable and can detect the malpractice. The high percentage of antibiotic residue detection may be due to the lack of adherence to withdrawal period after antibiotic therapy [5,21]. This is attributed to the general lack of knowledge on discreet use of antibiotics [22], while harsh economic conditions push farmers to allow only a one-day withdrawal period after antibiotic therapy. At the time of the study, the prevailing drought and famine conditions led to shortages in milk production, therefore, this could have fuelled deliberate lack of adherence to withdrawal period after animal therapy. However, the possibilities of milk traders dosing raw milk with inhibitory substances to preserve the milk hence effect marketing cannot be ruled out. The high residue detection in pasteurized milk samples (Table 6) is alarming however, this can possibly due to sanitizer and sterilizer residue in filling lines and packaging materials.

Antibiotic residues affect safety of the milk hence consumer safety cannot be assured, because they cannot be removed from the milk. The general trend of boiling raw milk before use may result in chemical as well as structural changes of the drugs, with diverse adverse effects on consumption. As part of an ongoing study, most of the antimicrobials in pasteurized milk were H_2O_2 (Fig. 1), which is principally used in sanitizing and sterilization of packaging materials. However, low concentrations of hydrogen peroxide could not be detected according to the methodology used for antibiotic residues as observed for brand K.a (Fig. 1). Several brands K.b, K.e, and K.f had more antimicrobials than residues of hydrogen peroxide implying that other inhibitory substances could be present. However, brand K.c did not have residues of either hydrogen peroxide or antimicrobials (Fig. 1), implying that its possible to have safe milk in our market.

Table 1. Density of raw milk samples collected from different areas of Nairobi

Sample site	No. of samples	Mean density (gm/L)	% failed samples
Rural	59	1.028 ± 0.000026	5.1 % (3)
Urban	63	1.029 ± 0.000025	11.1 % (7)
Slum	84	1.028 ± 0.000020	21.4 % (18)

Values in brackets are the number of milk samples that had densities outside the normal range; (1.026 – 1.032 gm/L) (KEBS, 2003)

Table 2. Density of pasteurized milk in two different packages

Package type	No. of samples	Mean density (gm/L)	% failed samples
Packet	52	1.029 ± 0.000028	0 (0)
Sachet	45	1.029 ± 0.000029	4.4 % (2)

Values in brackets are the number of milk samples that had densities outside the normal range; (1.026 – 1.032 gm/L) (KEBS, 2003)

Table 3. Prevalence of use of hydrogen peroxide in raw milk

Sample site	No. of samples	% of samples with Hydrogen peroxide
Rural	57	8.8 % (5)
Urban	64	6.3 % (4)
Slum	85	9.4 % (8)
Total	206	8.3 % (17)

Values in brackets are number of milk samples containing hydrogen peroxide

Table 4. Incidences of the use of hydrogen peroxide in pasteurized milk

Brand code	No. of samples	% of samples with hydrogen peroxide
K.a	29	55.2 % (16)
K.b	15	6.7 % (1)
K.c	11	0 % (0)
K.d	12	33.3 % (4)
K.e	13	0 % (0)
K.f	10	0 % (0)
K.g	8	37.5 % (3)
Total	98	24.5 % (24)

Values in brackets are the number of milk samples containing hydrogen peroxide

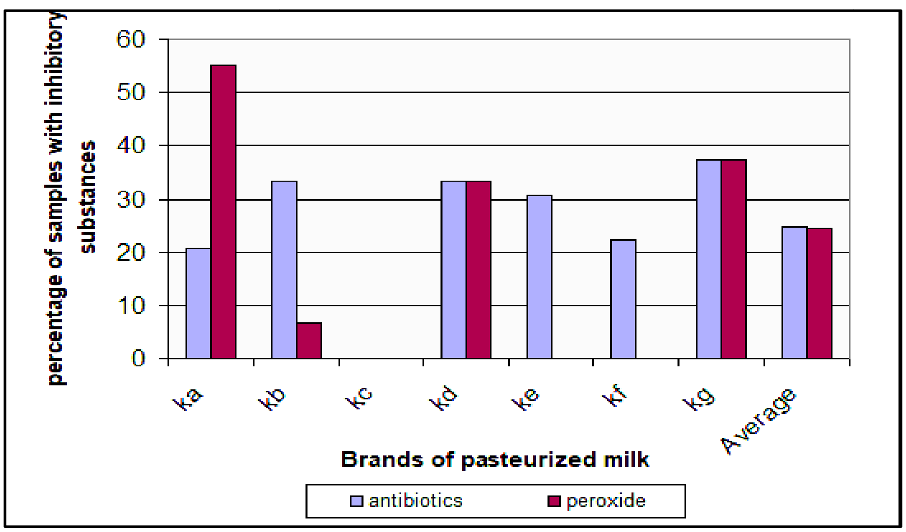

Fig. 1. Incidence of antibiotics and hydrogen peroxide in different brands of pasteurized milk

Due to lack of efficient and rapid methodology at farm gate, raw milk with antibiotic residues can easily be accepted together with good quality milk. Deliberate dosing of raw milk with inhibitory substances by milk suppliers (traders) could also be responsible for the high antimicrobials in market milk. In addition, an elaborative control strategy for antimicrobials must be deployed and evaluated periodically to gauge effectiveness. Without such interventions alongside farmer trainings and consumer enlightenment, the problem may persist.

Table 5. Incidence of antibiotic residues in raw milk samples collected

Sampling area	No. of samples	% of samples with inhibitory substances
Rural	59	25.4 % (15)
Urban	64	3.1 % (2)
Slum	89	22.5 % (20)
Total	212	17.5 % (17)

Each value is the percentage of raw milk sample collected containing inhibitory substances.
Values in brackets are the number of milk samples containing inhibitory substances

Table 6. Incidence of antibiotic residues in different brands of pasteurized milk

Brand code	No. of samples	% of samples with inhibitory substances
K.a	29	20.7 % (6)
K.b	15	33.3 % (5)
K.c	11	0 % (0)
K.d	12	33.3 % (4)
K.e	13	30.8 % (4)
K.f	9	22.2 % (2)
K.g	8	37.5 % (3)
Total	97	24.7 % (24)

Each value is the percentage of raw milk sample collected containing inhibitory substances.
Values in brackets are the number of milk samples containing inhibitory substances

4. CONCLUSION

In conclusion, the levels of adulteration and antimicrobials in market milk were quite high, hence strict controls through monitoring by government regulatory departments should be enhanced and consumers of dairy products should be enlightened more on dangers of consuming unsafe products. Additionally, sensitization on prudent use of H_2O_2 and/or other sanitizers by milk handlers should be considered a priority intervention measure.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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