MICROBIOLOGICAL QUALITY AND SAFETY OF RAW AND PASTEURIZED MILK MARKETED IN AND AROUND NAIROBI REGION

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ABSTRACT

The microbiological quality of raw and pasteurized milk marketed in Nairobi and its environs 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 analysed for titratable acidity, total viable count (TVC), *Staphylococcus aureus*, coliforms and Enterobacteriaceae. Titratable acidity was determined using titration method, while TVC, *S. aureus* and Enterobacteriaceae were determined by the spread plate methods and coliforms were determined by most probable number. Data collected were subjected to analysis of variance using Genstat statistical package. The mean acidity was 0.20% lactic acid (LA), while mean counts for TVC, *S. aureus,* coliforms and Enterobacteriaceae were 6.05, 3.46, 2.30, and 3.93 $\log_{10}$cfu/ml, respectively. The percentage of milk samples with acidity values greater than 0.18% LA, the upper limit set by Kenya Bureau of Standards (KEBS), was 52.8 %. Total viable count (TVC) greater than 10^6 cfu/ml, was detected in 95.2% and 21.4% of raw and pasteurized milk, respectively. Coliform counts greater than 4.70 and 1.0 $\log_{10}$cfu/ml for raw and pasteurized milk were detected in 77.8% and 4.8%, respectively of raw and pasteurized milk samples collected. Enterobacteriaceae and *S. aureus* were detected with mean counts ranging from 6.08-6.86 and 5.82-6.32 $\log_{10}$/ml, respectively. Highest mean acidity and counts were recorded from slum areas of Nairobi and there were significant differences between raw and pasteurized milk (P<0.05). The poor bacterial quality coupled with high acidity of raw milk, indicates poor hygienic practices and lack of temperature control during marketing. The incidence of high acidity and bacterial counts in pasteurized milk could indicate post process contamination and/or inappropriate storage of the milk. Most vendors of pasteurized milk were observed selling directly from the distributor crates without refrigerated storage. The rapid deterioration of raw and pasteurized milk marketed in Nairobi, at the time of this study, may be largely due to poor hygienic standards and non-adherence to temperature controls during handling, distribution and marketing. This requires urgent attention by the appropriate authorities, because the poor microbiological quality of raw milk and pasteurized milk may expose consumers to health risks associated with the consumption of contaminated milk.

Key words: Marketed milk, quality, acidity, total viable count, coliforms, enterobacteriaceae, *Staphylococcus aureus*

INTRODUCTION

Milk is a nutritious food for human beings, but it also serves as an ideal medium for the growth of various microorganisms. Milk is highly perishable making hygiene and sanitation during milking, transportation, storage, processing and distribution crucial for retention of quality. Storage of milk at ambient temperatures with poor hygienic standards favours bacterial growth and multiplication leading to deterioration [1]. The detection of Enterobacteriaceae, especially coliforms in milk indicates possible contamination with bacteria from the udder, milk utensils, water or the handler [2, 3]. Major sources of these microorganisms include the dairy cow, immediate environment where the animal is housed and milked, water, milking and handling containers and the handlers [2].

Freshly drawn milk from the udder of a healthy cow has a low bacterial load of less than 10^3 colony forming units per millilitre (cfu/ml). However, this low initial microbial load may increase up to 100 fold or more if milk is stored at ambient temperatures [4]. Refrigerated storage of milk in sterile containers immediately after milking may delay the multiplication of microorganisms [3, 5]. In Kenya, however, mixing of fresh morning milk with evening milk could be partly responsible for high microbial counts in raw milk [6]. In addition, high microbial load in bulk milk could also arise from contamination with mastitis milk [7].

More than 90 % of all reported cases of dairy related illness are of bacterial origin, with at least 21 milk-borne or potentially milk-borne diseases being recognized [8]. Pathogens that have been involved in food borne outbreaks associated with consumption of milk include *Listeria monocytogenes, Salmonella typhimurium, Campylobacter jejuni, Staphylococcus aureus, Bacillus cereus, E.coli* 0157:H7, *Coxiella burnetii, Mycobacterium tuberculosis, Mycobacterium bovis, Yersinia enterocolitica* and certain strains of *Staphylococcus aureus* [1, 9]. The illnesses caused include: brucellosis, tuberculosis, typhoid, paratyphoid and diphtheria [1]. The presence of these pathogens in milk is of public health concern especially with regard to wide consumption of milk across populations [10].

Commercially, marketing of milk in Kenya is handled through informal and formal channels. Currently, about 80% of the marketed milk is handled through informal trading, a trend that has caused public health concerns [11]. Unhygienic practices during milking, transportation and storage of fresh milk at room temperatures result in inferior quality milk for sale. Apparently these are common practices predominating raw milk being hawked in the Nairobi region. This study was, therefore, carried out to investigate the microbiological quality and safety of raw and pasteurized milk marketed in and around Nairobi.

METHODOLOGY

Sample collection and preparation
A total of 292 milk samples were collected from three market areas: Kiambu and Ngong, Nairobi central business district and Kibera and Mathare slums. Approximately 250 ml raw milk was aseptically drawn from plastic or aluminum containers from the milk traders and transferred into a sterile sampling bottle, kept in a cool box with ice cubes and transported to the laboratory within 45 minutes of purchase. Different brands of pasteurized milk, 500 ml in pouches or packets, were bought randomly from the retail chains (kiosks and supermarkets). These milk samples were immediately kept in a cool box, transported to the laboratory and stored at 4°C unopened until time for analysis. Sampling preparations and procedures were done according to AOAC methods [12]. For bulk milk, the milk was mixed (agitated) thoroughly and 250 ml milk sample drawn using a dipper. All the milk samples were collected from early morning up to mid-morning (from 6 to 10 am).

Determination of total acidity
Ten millilitres (10.0 ml) of milk sample was pipetted into 100.0 ml conical flask. Two drops of phenolphthalein indicator were added and titrated against 0.1N NaOH under continuous mixing, until a faint pink colour appeared [13].

Total viable count (TVC)
The spread plate method was used where serial decimal dilutions of milk samples were done using 0.1% tryptone water (Oxoid, England). Fifteen micro-litres (15.0µL) of the sample in triplicates were spread over the dry sterile plates of plate count agar (Himedia, India) using a sterile Conrad's glass rod. The plates were then covered, inverted and incubated at 37°C for 24 hours, after which the plates with colonies were counted using a colony counter and the results recorded [12].

Determination of Enterobacteriaceae
The spread plate method was used where serial decimal dilutions of milk samples were done using 0.1% tryptone water (X 4539 Oxoid, England). Fifteen micro-litres (15.0µL) of the sample in triplicates were spread over the dry sterile plates of Violet-Red Bile Glucose Agar (VRBGA) (Oxoid, England) using a sterile Conrad's glass rod. The plates were then covered, inverted and incubated at 37°C for 24 hours, after which the plates with colonies (those surrounded by a purple zone of growth) were counted as Enterobacteriaceae using a colony counter; the results obtained were recorded [12, 14].

Determination of coliforms
The Most Probable Number (MPN) method was used; 1.0 ml of the dilute sample was inoculated into three separate tubes of 9.0 ml lauryl sulphate broth (M 080 Himedia, India); with inverted Durham tubes. The tubes were then incubated at 37°C for 24-48 hours. After 24 hours, the tubes showing gas production were recorded as positive and a loop-full from each gas positive tube was transferred to a separate tube containing MacConkey's broth (X 4230 Oxoid, England) with Durham tubes and incubated at 44.5°C for 48 to 72 hours. Tubes showing gas production were noted and confirmed as

positive for coliforms. The MPN table was used to calculate the number of coliforms per ml [12, 15, 16].

Determination of *Staphylococcus aureus*
The spread plate method was used; serial decimal dilutions of milk samples were done using 0.1% tryptone water (X 4539 Oxoid, England). Fifteen micro-litres (15.0μL) of the sample in triplicates were spread over the dry sterile plates of Baird Parker medium (M 043 Himedia, India) with egg yolk tellurite (FD 046 Himedia, India) (using a sterile Conrad's glass rod in a backward and forward movement) while rotating the plate. The plates were then covered, inverted and incubated at 35-37°C for 24-48 hours after which the plates with colonies that were circular, smooth, convex, moist, 2-3 mm in diameter, grey-black to jet black were counted using a colony counter and the results recorded [16, 17, 18].

RESULTS

Titratable acidity
Raw milk collected from rural, urban and slum areas of Nairobi had high acidity with mean acidities of 0.19% LA, 0.20% LA and 0.22% LA, respectively while pasteurized milk packed in packets and pouches had mean acidities of 0.17% LA and 0.18% LA, respectively (Table 1).

Total Viable Count
Mean TVC in raw milk collected from rural, urban and slum areas of Nairobi were 7.57, 7.52 and 8.18 log_{10}cfu/ml, respectively. The pasteurized milk packed in packets and pouches had mean TVC of 3.59 and 3.19 log_{10}cfu/ml, respectively (Table 2).

Coliforms
Mean coliform counts in raw milk collected from rural, urban and slum areas of Nairobi were 4.56, 5.63 and 4.96 log_{10}cfu/ml, respectively (Table 2). The pasteurized milk had mean coliform count of 0.10 log_{10} cfu/ml (Table 3).

Enterobacteriaceae
Mean Enterobacteriaceae counts in raw milk collected from rural, urban and slum areas of Nairobi were 6.08, 6.86 and 6.30 log_{10}cfu/ml, respectively (Table 2), while pasteurized milk had mean count of 0.10 log_{10} cfu/ml (Table 3).

Staphylococcus aureus
Mean *S. aureus* counts were 5.83, 6.32 and 5.82 log_{10}cfu/ml in raw milk collected from rural, urban and slum areas, respectively while pasteurized milk had mean count of 0.10 log_{10}cfu/ml.

DISCUSSIONS

The overall mean acidity of the milk samples was greater than the acceptable limit of 0.18% LA set by Kenya Bureau of Standards (KEBS) [19]. This high acidity may

indicate lack of adherence to the cold chain in the distribution channels and the long duration taken from milking to marketing. High acidity levels detected in raw milk from rural areas could indicate rapid acidity development owing to high microbial load and activity at farm gate level. The acidity level usually increases when there is delay in chilling after milking and/or because of lipase activity and lack of pasteurization [20]. This may be one of the reasons why raw milk with high acidity is marketed in urban and slum areas of Nairobi. This could possibly be explained by non-compliance to using a cold chain during the collection and distribution of milk, which accelerates the deterioration of the milk.

The higher incidence of increased acidity of the milk in the slum areas followed by urban areas in Nairobi indicates poor hygienic practices. These unhygienic practices have been cited to predominate raw milk marketing in developing countries like Kenya and Mali [3, 21]. Unhygienic practices coupled with storage under improper temperature control [22], favours bacterial growth and development leading to undesirable changes in milk including high acidity [23]. Titratable acidity measurement is one of the sensitive indicators of small changes in acidity of milk as a measure of enzyme activity [13]. It should, therefore, be noted that most of the raw milk marketed in Nairobi and the environs may have high microbial and enzyme activity as indicated by high titratable acidity and lipolysis [22, 23, 24].

Based on acidity measurements alone, most of this milk was destined for rejection by prospective consumers as it could possibly clot on boiling. Milk rejection leads to economic loss to farmers as expenses incurred during production cannot be recovered from sale of the milk. Containers used in handling and conveying milk are important for quality management of milk. The ease of cleaning and sterilization are key factors to consider in the selection of milk handling and conveyance containers. In the three areas covered in this study, raw milk was conveyed in either plastic jerry cans or aluminium containers. High acidity above the acceptable limit was detected in 43.8% and 67.6% of milk samples conveyed in aluminium and plastic jerry cans, respectively (Table 1). The plastic jerry cans used were non-food grade and difficult to clean and sterilize [25], hence they could increase the incidence of milk contamination. The resultant effect is acid development at favourable temperatures due to high microbial load and enzyme activity [23, 24].

On average, the pasteurized milk samples analysed had acidity within the acceptable limits for good quality milk according to the Kenyan standards [19]. However, 27.9% and 56.1% (Table 1) of pasteurized milk packed in packets and pouches, respectively, had higher acidities beyond the limit as set by KEBS. Acid development in pasteurized milk could result from storage at ambient temperatures within the retail outlets. This was observed in most outlets where pasteurized milk was kept in the distributor crates and sold directly from the crates. The exposure of pasteurized milk to ambient temperatures may ultimately lead to product acidification. It could also be possible that post process contamination and the presence of active enzymes due to inadequate pasteurization may lead to acidification at room temperature. However, this fact was not examined during this study.

Bacterial count of milk indicates sanitary quality and most grading methods are based on estimating numbers of microorganisms present [26]. The grading of milk based on microbial quality can serve as an ideal incentive for pricing of raw milk to improve bacteriological quality of raw milk [27]. The situation in Kenya, however, is that once milk passes the platform test, the eventual price is dictated by supply and demand. Most of the raw milk marketed in Nairobi had total viable counts greater than 6.0 $\log_{10}$cfu/ml (Table 2), which is the limit set by KEBS [19]. The highest TVC was recorded from slums while the lowest count was detected in urban Nairobi. However, analysis of variance revealed that there was no significant difference in TVC with regards to sample areas (p>0.05). This implies that on average, raw milk marketed in Nairobi has unacceptable high TVCs. This finding compares to Riadh [28] in Jordan who detected a TVC of 6.70 $\log_{10}$cfu/ml, while Chye *et al.* [29] in Malaysia detected TVC of 7.08 $\log_{10}$cfu/ml. The current results show that raw milk in Nairobi is likely contaminated with microorganisms starting at farm level. Storage at ambient temperatures may favour microbes such as *E.coli, Pseudomonas aerogenes, Proteus mirabilis, Citrobacter* and *Klebsiella* to grow in unpasteurized milk [1]. The udders soiled with manure, mud, feeds or used bedding are critical factors in fresh milk production as they could harbour total counts often exceeding $10^8 – 10^{10}$ counts per gram [30, 31]. Though there was no significant difference with respect to sample areas (p>0.05) in the present study, quite a high percentage of raw milk marketed in Nairobi is of poor microbial quality.

Recent studies in Nairobi indicated that 86% and 85% of milk samples at household level had TVC above 6.30 $\log_{10}$cfu/ml in dry and wet seasons, respectively [21]. The high TVC counts in the current study indicates that milk of poor bacteriological quality is still marketed in Nairobi. If appropriate actions are not taken to reduce the current high microbial load in raw milk, further unfavourable conditions can induce bacterial toxin production in raw milk that may lead to major health risks in the community.

Hawked raw milk is often associated with the use of plastic unhygienic containers. Irrespective of the market area, there was no significant difference in TVC of raw milk conveyed in plastic containers and aluminium cans (p>0.05). Total viable count (TVC) greater than 6.0 $\log_{10}$cfu/ml was detected in 92% and 96% of raw milk conveyed in aluminium and plastic containers, respectively. It could be possible that the milk had high microbial load before being conveyed in the respective containers. Though direct sampling at farm level was not done, milk samples collected in the rural areas had equally high TVC. Aluminium containers were frequently used by affluent transporters who used vehicles for milk deliveries. Often the vehicles used lacked a top cover (roof) and had closed sides hence not ideal for transporting milk. This exposes the milk to direct heat from the sun, which favours acidification [22, 23]. Additionally, after milk delivery, the vehicles would be observed fetching animal feeds from the market like green maize cobs, cabbages, kales, banana peels and bean pods among other commodities. Therefore, these vehicles could contribute to milk contamination because of harboured microbes. Aluminium containers are easily cleanable and sterilizable, hence recommended for use over plastic containers. Plastic containers used were jerry cans, which are difficult to clean and sterilize [25] and they could harbour microbes especially at the top handles and rugged necks. Some of the jerry cans were observed to have been previously used to convey poisonous industrial chemicals as exhibited by the precaution labels. With

unsatisfactory sterilization, this may pose long-term exposure of consumers to chemical elements from these containers.

Mean TVC for pasteurized milk in packets and pouches, respectively, were 3.59 and 3.19 $\log_{10}$cfu/ml, (Table 3) which is acceptable good quality milk. However, 21.4% of pasteurized milk had TVC greater than the acceptable national limit [19]. High counts in pasteurized packed milk could possibly result from poor microbiological quality of the raw milk used and storage at elevated temperatures [32]. Poor microbiological quality of raw milk and storage at ambient temperatures has been observed to affect the shelf-life of the eventual pasteurized milk [33, 34]. This could be due to diverse chemical composition and enzyme activity that ultimately affects shelf-life of pasteurized milk.

Refrigerated storage at 4-7°C has been observed to have minimum bacterial growth in milk, but elevated temperatures of 15°C had increased activity by up to 15 times [35]. In the same study rapid microbial growth of up to 10^8 cfu/ml was observed in pasteurized milk stored at 25°C after 20-24 hours [35]. This indicates that spoilage of pasteurized milk may occur just within a day, particularly in tropical environments like Kenya where ambient temperatures average 25°C. There was a significant difference in total viable counts between hawked milk and pasteurized milk at ($p<0.05$) implying the importance of pasteurizing milk to assure safety. However, there were no significant differences in total viable count between pasteurized milk packed in packets and pouches ($p>0.05$). High TVC in milk could also be attributed to the health status of the udder due to mastitis.

Mastitis is a major problem affecting dairy herds in the tropical countries like Kenya [36], in particular, small-scale dairy farms [10]. The mixing of good quality milk with mastitis milk can increase viable counts in raw milk. Infected cows can shed counts in excess of 10^7 bacteria per ml [31], which ultimately increases the viable counts. A major causative pathogen is *S. aureus,* which can contaminate milk from sick cows or from handlers. Humans and sick dairy cows are the main carriers of *S. aureus,* presenting as mucosal or cutaneous lessions [37]. *Staphylococcus aureus* was detected in all the raw milk samples collected from the market areas. Pasteurized milk had mean counts of 0.10 $\log_{10}$cfu/ml, which was significantly different from raw milk ($p<0.05$). Detection of high *S. aureus* count in raw milk indicates the danger of food intoxication, as strains of *S. aureus* could produce enterotoxins A, B, C, D, and E [38] under favourable conditions. Though enterotoxin production was not examined, 22.1% of *S. aureus* in bulk milk in Norway produced enterotoxins as reported by Jorgensen *et al*. [39]. Riadh in Jordan detected *S. aureus* counts at 2.48 $\log_{10}$cfu/ml in raw milk and zero in ultra-high temperature (UHT) processed milk [28]; Bonfoh *et al*. in Mali detected 1.94 $\log_{10}$cfu/ml in raw milk from vendor's can [3], while 4.08 $\log_{10}$cfu/ml was detected in Malaysia [27]. Based on recent studies in Kenya [36], the danger of high *S. aureus* count in raw milk could expose the milk to production of toxins at favourable conditions. Research findings in Pakistan [28] attributed high *S. aureus* contamination in milk based sweet products to lack of compliance to observation of hygienic practices during preparation, handling and storage. This seems to be the hurdle for Kenya's dairy sector as hygienic standards are generally very low [40]. This is critical especially at farm level in Kenya, where the herders (shepherds) also perform the role of milking. Most of these farm workers are not

trained in hand milking and they do not observe personal hygiene, which could affect the quality and safety of milk.

High mean counts for Enterobacteriaceae, at 6.86 $\log_{10}$cfu/ml in urban Nairobi compares with 6.57 $\log_{10}$cfu/ml in vendors' cans in Bamako, Mali [3]. The results show significant difference ($p<0.05$) in the prevalence of enteric bacteria detected between raw and pasteurized milk, indicating the importance of pasteurization in destroying pathogenic organisms. Important members of this family responsible for illnesses in man through food and water include *Salmonella, Shigella,* and *E. Coli.* The prevalence of coliforms in this study compares to mean counts of 5.23 $\log_{10}$cfu/ml in Malaysia [29], 5.18 $\log_{10}$cfu/ml in Kiambu and Nairobi [21] and 2.78 $\log_{10}$cfu/ml in Karak, Jordan [28]. However, the prevalence in pasteurized milk was 0.10 $\log_{10}$cfu/ml (Table 3), which was within the acceptable limit of less than $1.0\log_{10}$cfu/ml [19]. No significant differences were detected with respect to sample areas, although significant differences between pasteurized milk and raw milk were detected ($p<0.05$).

Coliform counts above 4.50 $\log_{10}$cfu/ml were detected in 62.5%, 79.5% and 83.8% of raw milk in rural, urban and slum area. Previous studies showed that 46% of milk bought at household level had coliform counts greater than 4.50 $\log_{10}$cfu/ml in Nairobi and Kiambu [21]. Findings from the current study indicate that there has been no appreciable improvement with regard to changes in bacteriological quality of raw milk marketed in Nairobi. Detection of coliforms and pathogens in milk indicates possible contamination of the milk with bacteria either from the udder, milk utensils, water or possible post process contamination [2, 3]. The coliform counts were quite high, signifying poor hygienic standards during transportation and in distribution channels in Nairobi.

CONCLUSION

The bacteriological quality of raw milk marketed in Nairobi at the time of this study does not satisfy the requirements set by the Kenya Bureau of Standards (KEBS) for the quality of milk. The high acidity, TVC, coliforms, Enterobacteriaceae and *S. aureus* in raw milk should be of concern to the appropriate authorities; thus, the need for appropriate actions to be taken to assure consumer safety as well as ensure further processing of the milk into value added products. The handling of milk under poor hygienic conditions and at ambient temperatures increases the bacterial loads and acidity which may be harmful to consumers. The observed poor storage of pasteurized milk may lead to rapid deterioration of fresh milk in distribution channels. Therefore, more awareness creation and training of milk handlers are needed at primary production levels and marketing to enhance quality and safety of the milk for consumers.

Table 1: Titratable acidity of raw and pasteurized milk collected from rural, urban and slum areas in Nairobi

Milk type	Market area	No. of samples (n = 208)	Mean acidity	% (n) failed samples
Raw milk	Rural	64	0.19 ± 0.0049	37.5% (24)
	Urban	64	0.20 ± 0.0049	68.8% (44)
	Slum	80	0.22 ± 0.0044	81.3% (65)
Raw milk	Container type	No. of samples (n = 208)	Mean acidity	% (n) failed samples
	Aluminium	32	0.19 ± 0.0069	43.8% (14)
	Plastic	176	0.21 ± 0.0029	67.6% (119)
Pasteurized	Packaging type	No. of samples (n = 84)	Mean acidity	% (n) failed samples
	Packet	43	0.17 ± 0.0059	27.9% (12)
	Pouch	41	0.18 ± 0.0061	56.1% (23)

Each value is the mean acidity in % lactic acid (% LA), of raw or pasteurized milk samples analysed Values in brackets (n) are the number of milk samples with acidities > 0.18 % LA upper limit [19]

Table 2: Microbial counts of raw milk samples collected from rural, urban and slum areas in Nairobi

Market area	Parameter	No. of Obs.	Mean (log $_{10}$/ml)	% (n) samples with counts > national standards
Rural	TVC	25	7.57	96% (24)
	Coliform	25	4.56	62.5 % (15)
	Enterobacteriaceae	25	6.08	
	S. aureus	25	5.83	
Urban	TVC	39	7.52	94.9% (37)
	Coliform	39	5.63	79.5% (31)
	Enterobacteriaceae	39	6.86	
	S. *aureus*	39	6.32	
Slums	TVC	62	8.18	96.7% (59)
	Coliform	62	4.96	83.8% (52)
	Enterobacteriaceae	62	6.30	
	S. aureus	62	5.82	
Containers				
Aluminium cans	Total viable count	25	8.11	92% (23)
	Coliform	25	4.96	52% (13)
	Enterobacteriaceae	25	6.20	
	S. *aureus*	25	5.43	
Plastic cans	Total viable count	101	7.88	96% (97)
	Coliform	101	4.96	75.2% (76)
	Enterobacteriaceae	101	6.70	
	S. *aureus*	101	6.08	

Values in brackets (n) are the number of milk samples with mean counts >the national standards, for TVC and coliform counts [19]

TVC- Total viable count

Table 3: Microbial counts of pasteurized milk packed in different packaging materials

Package type	Parameter	No. of Obs.	Mean (log $_{10}$ /ml)	% (n) of samples with counts > national standards
Packets	Total viable count	43	3.59	27.9% (12)
	Coliform	43	0.10	4.6% (2)
	Enterobacteriaceae	43	0.10	
	S. *aureus*	43	0.10	
Pouch	Total viable count	41	3.19	14.6% (6)
	Coliform	41	0.10	4.8% (2)
	Enterobacteriaceae	41	0.10	
	S. *aureus*	41	0.10	

Values in brackets (n) are the number of milk samples with mean counts >national standards for TVC and coliform counts [19]

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