Evaluation of physicochemical properties and microbiological quality of UHT milk regularly introduced to resident patients in Mansoura University hospitals

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Abstract. *The quality of UHT milk is highly influenced by the microbiological characteristics of raw milk and the heat treatment applied. The current study aimed to check whether selected UHT milk brands meet the minimum legal Egyptian standard that is represented in their label manuscript to introduce it in complete nutritive values and hygienic state to resident patients in Mansoura University Hospital (MUH). A total of 80 UHT milk samples from four different brands (A, B, C, and D, 20 of each) were collected from MUH and directed to the physicochemical and microbiological evaluation. MCCW lactoscan® revealed that 100% of all brands were incompatible with the legal SNF% requirements and only brand A was compatible with all other legal standard requirements. The physicochemical investigation showed a significant increase in fat ($P < 0.05$), protein, TS, SNF, ash, density, and freezing point ($P < 0.01$) in A milk brand compared with the other three brands. Total bacterial count (CFU/mL) exceeded the legal standard in 10% of B, 10% of C, and 15% of D brands. Total coliform count (CFU/mL) was incompatible with the legal standards in 15% of B, 25% of C, and 40% of D brands. E.coli and Salmonella spp. were negative in all investigated brands. Pseudomonas was identified in 75% of B, 60% of C, and 75% of D brands. Klebsiella was detected in 25% of B, 40% of C, and 25% of D brands. Our findings indicate that there are somewhat inferior quality and potential risk hazards of consuming B, C, and D UHT milk brands.*

Introduction

Bovine milk is a rich source of fat, protein, carbohydrates, vitamins, and other miscellaneous constituents that play an important role as a diet in many countries for human beings, particularly children and adolescents for their intense growth and development as well as body support to reduce the incidence of chronic diseases such as type 2 diabetes, osteoporosis, hypertension and cancer (Salles et al., 2019). Moreover, milk provides a package of individuals' nutritional daily requirements (calcium and essential amino acids) that is difficult to obtain in other dairy-free diets (Awal et al., 2016).

Ultraheat treatment (UHT) of raw milk is usually applied to preserve its nutrient components and to kill or inactivate almost all pathogens which make it unsafe for human consumption (Ajmal et al., 2019). During the last few years, UHT milk has gained attention as a trustworthy product of must-have nutrients mainly due to its convenience to the Egyptian hot climate and long shelf-life extending from days to 6 months without refrigeration (Nassar et al., 2018). Despite this, UHT milk still liable to be contaminated with various microorganisms from different origins, either during production, processing, packaging, and handling which make it unsafe or even a dangerous source of infection among consumers constituting a potential health hazard (El-Leboudy et al., 2017). Hence, total bacterial count (TBC) and total coliform count (TCC) are the yardsticks among quality control tests applied on milk to evaluate its microbiological quality, the contamination of packaging material, and the low sanitation during manufacturing (Abdel Ghaffar et al., 2019).

Furthermore, organoleptic properties (taste and color) of UHT milk may deteriorate during extended storage especially if it is either contaminated with bacteria of the high proteolytic and lipolytic activity or prepared from raw milk that has previously encountered heat-stable proteolytic and lipolytic enzymes produced by gram-negative psychographs (GNS) (Zhang et al., 2020). Henceforth, raw milk destined for UHT processing and manufacturing should be stored refrigerated for no more than 36 hrs to prevent the growth of drug-resistant GNS such as *Pseudomonas* and *Klebsiella* that become a major contributor of undesirable flavors of UHT milk and a cause of worldwide serious health problem (Zhang et al., 2020).

The current study aimed to check whether milk brands regularly introduced to the resident patients in Mansoura University Hospitals (MUH) are free from microorganisms and meet the minimum legal Egyptian standard that is written in their label manuscript to offer it to patients in complete nutritive values and hygienic condition.

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Materials and methods

A total number of 80 UHT milk samples of four different brands [A, B, C, and D, 20 of each) were randomly collected within 4 days to 2 weeks after production in clean, dry, and sterile containers from the food department in MUH. The collected samples were labeled and transferred in an ice tank to the microbiology laboratory, then analyzed immediately or kept at 4°C for future analysis at the Faculty of Veterinary Medicine, Mansoura University, Dakahlia Governorate, Egypt, from July to December 2019. Then, each collected sample was divided aseptically into 3 portions to be used for organoleptic, physicochemical, microbiological analysis.

Organoleptic investigation

The sensory evaluation of UHT milk samples was applied as recommended by the American Public Health Association (APHA, 1992) where all samples were examined at 20°C by trained panelists who can distinguish slight differences in taste, color, and flavor before being subjected to further investigations.

Physico-chemical investigation

Chemical examination

The percentage of fat, protein, lactose, total solids (TS), solid not fat (SNF), ash, moisture, density, and freezing point of all milk samples were measured by using an automatic milk analyzer device (MCCW lactoscan®, 8900 Nova Zagora, Bulgaria) (Musaad et al., 2013).

Determination of PH

As described by Hartman and La Grang (1985), pH values were determined by using a glass electrode PH meter (Adwa, AD 1000) with a temperature probe after calibrating with phosphate buffer solutions (pH = 7.4).

Determination of titratable acidity

As described by AOAC (2005), titratable acidity was determined by pouring 10 mL of the sample into a suitable porcelain dish along with 20 mL of CO_2 free ambient distilled water followed by titration against standard alkali (N/9 standard sodium hydroxide) using (1%) phenolphthalein alcoholic solution as indicator till reaching the endpoint that was estimated by observing persistent faint pink color. After all, the titratable acidity was calculated and recorded as lactic acid % as follows:

$$\text{Acidity \%} = R/10,$$

R = amount of N/9 NaOH used till reaches the endpoint.

Microbiological load analysis

Total bacterial count

It was determined by the pour plate method using standard plate count agar as mentioned by APHA (2000). Briefly, 1 mL samples were transferred into sterile separate Petri dishes, followed by pouring 10–15 mL of standard plate count agar (Oxoid, Basingstoke, UK), cooled to 45°C in each Petri dish, then mixed well by their rotation many times in various directions, then allowed to set and, finally, incubated at 37C° for 24 hours, after which all appeared colonies were counted.

Total coliform count

It was determined by the pour plate method using violet red bile agar (VRBA, Oxoid, Basingstoke, UK) in a duplicate manner as described by Hartman and La Grang (1985). Briefly, for each sample, one plate was used as a negative control without adding the sample for contamination judging, but the sample plates were dispensed with 1 mL of each sample followed by pouring 15 mL of cooled (45°C) VRBA to each plate, then carefully mixed and allowed to settle down for 10 min. After all, each plate was overlaid with a further 4–5mL of cooled VRBA and allowed to set again. And then, the plates were incubated at 37C° for 24 hours. The control plates should be completely clear, which indicates contamination absence, while the sample plates encountered no more than 250 purplish red colonies surrounded by a reddish zone (diameter of 0.5 mm or greater) carefully counted.

Isolation of Salmonella

Based on the method explained by Addis et al. (2011), each coliform positive sample was ten-fold diluted (10^{-1}) using buffered peptone water (Oxoid, Basingstoke, UK) and then incubated at 37°C for one day. Then, 1 mL of each diluted sample was transferred to 10 mL of Rappaport and Vassilidis enrichment broth followed by overnight incubation at 42°C. From each enrichment broth, one loopful was evenly streaked onto the surface of xylose lysine desoxycholate agar plate (XLD, Oxoid, Basingstoke, UK) and then aerobically incubated for 24 hours at 37°C.

Isolation of E. coli

In conformity with Vanderzant and Splittstoesser (1992), each coliform positive sample was ten-fold diluted (10^{-1}) using tryptone soya broth (Oxoid, Basingstoke, UK) and then incubated at 37°C for one day. From each diluted sample, one loopful was evenly speckled onto the surface of Macconkey agar (Oxoid, Basingstoke, UK). The inoculated plates were observed for the growth, color, form, elevation, margin, surface, and optical characters of colonies according to Eklund and Lankford (1967). These presumptive colonies were selected and subjected to gram staining and microscopic examination as described by Cowan and Steel (1985).

Confirmative biochemical tests for the isolates

As described by ISO 6579 (2002), the gathered

colonies from Macconkey agar were identified after subjection to some biochemical tests including; catalase test, oxidase test, nitrate reduction test, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, urease test, and triple sugar iron test.

Statistical analysis

With the aid of SPSS version 20.0 (IBM Corp., NY, USA), Shapiro-Wilk test revealed the normal distribution of physicochemical variables as well as TBC and TCC, which are expressed as mean ± standard error (SE). Then, the significant difference of these variables among different brands was estimated using one-way ANOVA with LSD. Besides, the prevalence frequencies of each bacterium were estimated using the chi-square test. The significance of differences between means was reported at $P \leqslant 0.05$.

Results and discussion

Ultraheat processing of raw milk in Egypt and worldwide by direct heat infusion at 143°C for 4–8 sec and homogenization at 200 Pa and then packing in tetra pack paper under aseptic conditions (Hamad et al., 2017; Ibrahim, 2018), usually modifies the physico-chemical composition, organoleptic, chemical, and microbiological characters of milk. Organoleptic features of the commercially available UHT milk brands have a great impact on their popularity and acceptance among consumers (Aldubhany et al., 2014). Examined brands in the current study exhibited the most acceptable organoleptic features despite being contaminated with *coliform*, *pseudomonas*, and *Klebsiella*, and this finding agrees with that of Richards et al. (2016) who stated that aroma, flavor, sensory quality, and texture did not deteriorate immediately in UHT milk tainted with bacteria but limited their shelf-life and stability if stored for a long period.

Milk is a nutritious healthy drink containing water and fat in great quantities. Milk fat content influences flavor, nutritional benefit, and quality parameters of all milk-based products (Wu et al., 2019). Data illustrated in Table 1 reported a significant increase ($P < 0.05$) in the mean value of fat percentage in A brand compared to other investigated brands. Furthermore, determined fat % were compatible with the current legal Egyptian standard (2005) in 20, 17, 15 and 13 samples of the examined A, B, C and D brands, respectively (Table 2).

Low-fat content in some samples of B, C, and D brands could be attributed to the partial withdrawal or over skimming of fat before processing or might be owned to using raw milk adulterated by adding water before manufacturing (Arafat et al., 2015).

Milk adulteration by addition of extraneous water not only deteriorates the nutritive value of milk but also may incorporate chemicals or pathogens of serious health hazard to consumers if added without any consideration to its purity (Kunda et al., 2015). The

Table 1. Physico-chemical properties of examined UHT milk samples

Variables	A brand (n = 20)			B brand (n = 20)			C brand (n = 20)			D brand (n = 20)		
	Min.	Max.	Mean ± SE									
Fat %	3.06	4.15	3.59 ± 0.20*	2.91	3.84	3.25 ± 0.14	2.87	3.45	3.13 ± 0.09	2.88	3.22	3.04 ±.0.05
Protein %	2.87	3.58	3.25 ± 0.12**	2.67	3.05	2.86 ± 0.06*	2.58	2.76	2.57 ± 0.03	2.28	2.66	2.46 ± 0.06
Lactose %	3.88	4.31	4.06 ± 0.06	3.83	4.17	4.00 ± 0.06	3.77	4.34	4.07 ± 0.08	3.88	4.33	4.09 ± 0.06
TS%	9.11	10.60	9.97 ± 0.24**	8.56	9.82	9.19 ± 0.21*	7.72	8.74	8.37 ± 0.15	7.71	8.32	8.00 ± 0.10
SNF %	6.05	6.84	6.38 ± 0.11**	5.36	6.52	5.94 ± 0.17*	4.85	5.61	5.24 ± 0.14	4.60	5.29	5.00 ± .0.10
Ash%	0.59	0.78	0.65 ± 0.03**	0.54	0.62	0.59 ± 0.01*	0.48	0.62	0.54 ± 0.02	0.44	0.55	0.51 ± 0.02
Moisture%	14.46	16.53	14.70 ± 0.32	15.38	17.19	16.23 ± 0.26*	16.46	18.26	17.13 ± 0.26**	16.38	18.38	17.46 ± 0.34**
Density%	22.59	25.97	24.22 ± 0.54**	21.89	23.36	22.80 ± 0.22*	21.00	22.42	21.73 ± 0.22	20.79	22.21	21.18 ± 0.21
Freezing point	–0.44	–0.49	–0.46 ± 0.007**	–0.36	–0.44	–0.40 ± 0.013*	–0.30	–0.43	–0.35 ± 0.019	–0.29	–0.36	–0.33 ± 0.011

*Significance at $P < 0.05$. **Significance at $P < 0.01$.
Min – minimum; Max – maximum.

Table 2. Chemical and microbial results of examined UHT milk brands compared with their label manuscript and the current Egyptian standard (2005)

Variables	Label manuscript of each brand and Egyptian Standards (EOSQC)	A brand (n = 20)		B brand (n = 20)		C brand (n = 20)		D brand (n = 20)	
		Compatible samples No. (%)	Incompatible samples No. (%)	Compatible samples No. (%)	Incompatible samples No. (%)	Compatible samples No. (%)	Incompatible samples No. (%)	Compatible samples No. (%)	Incompatible samples No. (%)
Fat%	⩾ 3 (%)	20 (100)	0 (0)	17 (85)	3 (15)	15 (75)	5 (25)	13 (65)	7 (35)
SNF %	⩾ 8.5 (%)	0 (0)	20 (100)	0 (0)	20 (100)	0 (0)	20 (100)	0 (0)	20 (100)
Acidity%	⩽ 0.17 (%)	20 (100)	0 (0)	18 (90)	2 (10)	18 (90)	2 (10)	17 (85)	3 (15)
TBC	⩽ 10 (cfu/mL)	20 (100)	0 (0)	18 (90)	2 (10)	16 (80)	4 (20)	15 (75)	5 (25)
TCC	Nil (cfu/mL)	20 (100)	0 (0)	17 (85)	3 (15)	15 (75)	5 (25)	12 (60)	8 (40)

TBC – total bacterial count; TCC – total coliform count.

freezing point of milk is usually estimated to explore for possible milk adulteration with extraneous water. As milk is further diluted, the freezing point of water adulterated milk potentially decreases to become closer to the freezing point of pure water which equals 0°C (Zagorska and Ciprovica, 2013). Notably, 100% of examined samples of all brands were incompatible with the legal SNF % standard requirements but Table 1 showed that the percentage of protein, TS, SNF, ash, density, and freezing point more significantly elevated ($P < 0.01$) in A brand compared with other estimated UHT milk brands and also less significantly increased in B brand ($P < 0.05$) compared with C and D brands. This finding is attributed to using raw milk either of low solid components or adulterated by water that leads to inferior UHT milk quality especially in B, C, and D brands (Hamad et al., 2017). Accordingly, moisture percentage was significantly ($P < 0.05$) elevated in adulterated B ($P < 0.05$), C ($P < 0.01$), and D ($P < 0.01$) UHT milk brands in comparison with the A UHT milk brand.

Notably, the amount of acids in UHT milk depends on the cleanliness, freshness of milk during the production process, and the temperature at which the milk is preserved (Hossain et al., 2011). Henceforth, for milk quality judging, we investigated the acid in milk and the result in Table 3 revealed that mean pH values and titratable acidity % of examined brands insignificantly differed in between each other. Moreover, all examined samples of A brand proved their better quality and freshness as they were compatible with the current available Egyptian standard (2005) which reported that high-quality milk should have less than 0.17% titratable acidity % (Table 2). On the contrary, 2 (10%), 2 (10%), and 3 (15%) samples of investigated B, C, and D UHT milk brands, respectively, were incompatible with the Egyptian standard indicating high bacterial activity, uncleanliness, or long storage of milk samples during the production process (Hossain et al., 2011).

Total bacterial count is a microbiological test that could reflect the microbial contamination during milk collection, handling, and production (Hasan et al., 2016). As presented in Table 4 and Fig. 1, TBC was significantly elevated in investigated B ($P < 0.05$), C ($P < 0.01$), and D ($P < 0.001$) brands in comparison with the A brand. Hence, 100% of A, 90% of B, 80% of C, and 75% of D samples proved their excellent sanitary quality during processing, handling and production as they were compatible with the Egyptian Standard (2005) that stated that TBC of UHT milk should be not more than 10 CFU/mL.

Table 3. pH and titratable acidity % of examined UHT milk samples

Sample type	Sample No.	pH			Titratable acidity		
		Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
A brand	20	6.38	7.16	6.45 ± 0.27 NS	0.08%	0.16%	0.115% ± 0.004 NS
B brand	20	6.25	7.34	6.34 ± 0.32 NS	0.09%	0.18%	0.123% ± 0.0021 NS
C brand	20	6.24	7.05	6.37 ± 0.23 NS	0.07%	0.19%	0.125% ± 0.0026 NS
D brand	20	6.14	6.98	6.21 ± 0.15 NS	0.09%	0.18%	0.129% ± 0.002 NS

NS – non-significant differences ($P > 0.05$). Min – minimum; Max – maximum.

Table 4. Comparisons of bacterial load of UHT milk samples

Variables	A brand (n = 20)					B brand (n = 20)					C brand (n = 20)					D brand (n = 20)				
	Pos.	%	Min.	Max.	Mean ± SE	Pos.	%	Min.	Max.	Mean ± SE	Pos.	%	Min.	Max.	Mean ± SE	Pos.	%	Min.	Max.	Mean ± SE
TBC	0	0	0	0	0 ± 0	9	45	1	11	4.16 ± 1.35*	11	55	1	15	4.76 ±1.42**	15	75	0	18	5.16 ± 1.11***
TCC	0	0	0	0	0 ± 0	3	15	0	1	0.15 ± 0.018*	5	25	0	3	0.45 ± 0.043**	8	40	0	5	0.80 ± 0.066***

*Significance at $P < 0.05$. **Significance at $P < 0.01$. ***Significance at $P < 0.001$.
Min – minimum; Max – maximum.

Coliforms are mostly present in heat untreated milk but their presence in UHT milk reflects the inadequate sanitation of milk utensils and/or improper handling of milk during manufacturing (Salman and Hamad, 2011). As displayed in Table 4 and Fig. 2, TCC was significantly elevated in investigated B ($P < 0.05$), C ($P < 0.01$), and D ($P < 0.001$) brands compared with the A brand. Furthermore, the current study proved that 100% of A, 85% of B, 75% of C, and 60% of D brand samples had high hygienic quality as they were compatible with the current Egyptian Standard (2005) which states that UHT milk must be free from coliform organisms. Otherwise, 3 (15%) of B, 5 (25%) of C, and 8 (40%) of D brand were incompatible with the standard, and this a consequence of their low hygienic quality and/or fecal contamination during the manufacturing process that usually enhance rapid deterioration of the products and cause serious public health hazards (Saha et al., 2018).

Isolation and identification of coliform species (*E. coli*, *Salmonella*, and *Klebsiella*) can be simply applied to assess the hygienic and sanitary level during UHT milk production. Henceforth, we further cultured isolated coliforms from TCC positive samples on XLD and MacConkey medium to screen for the presence of gram-negative enteric bacteria especially *Salmonella* and *E.coli* which cause food-borne gastroenteritis. Fortunately, as shown in Table 5, all investigated samples were negative for *E.coli* and *Salmonella*. Based on typical colony characteristics onto specific and differential MacConkey media, our study suspected the presence of gram-negative psychrotrophic (GNP) bacteria (*Pseudomonas* and *Klebsiella*) in all coliform positive UHT samples as some of the isolates morphologically appeared as 2–3 mm, flat and smooth non-lactose fermenting colonies suspected to be *Pseudomonas*, also some of the isolates appeared as large, shiny, dark pink in color, mucoid in appearance and lactose fermenting colonies suspected to be *Klebsiella*. Further, by gram staining and microscopic examination, the isolated colonies presented as gram-negative rods with single, in pairs and irregular arrangement (Chen et al., 2011; Saha et al., 2018; Mwambete and Nakembetwa, 2015).

The applied biochemical test confirmed the presence of GNPs in 3 (15%), 5 (25%), and 8 (40%) of all examined B, C, and D samples that further classified into 2 (75%), 3 (60%) and 6 (75%) of *pseudomonas* and 1 (25%), 2 (40%) and 2 (25%) of *Klebsiella*, respectively (Table 6). The presence of these GNPs in some of the examined samples indicates the milk spoilage either by an inadequate sanitary condition or the adequate heat treatment process (Chen et al., 2011). GNPs could produce proteolytic and lipolytic enzymes as a consequence of their metabolic activities that can resist UHT processing resulting in unpleasant properties of milk (Tondo et al., 2004).

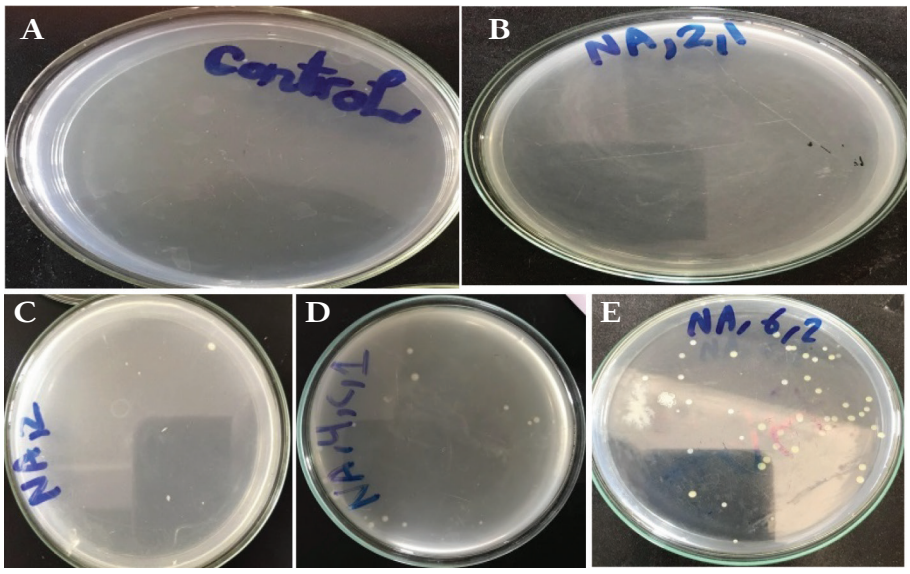

Fig. 1. TBC on nutrient agar.
A – control (-ve); B – absence of bacterial colonies in A brand;
C, D, E - presence of bacterial colonies in B, C and D UHT milk brands.

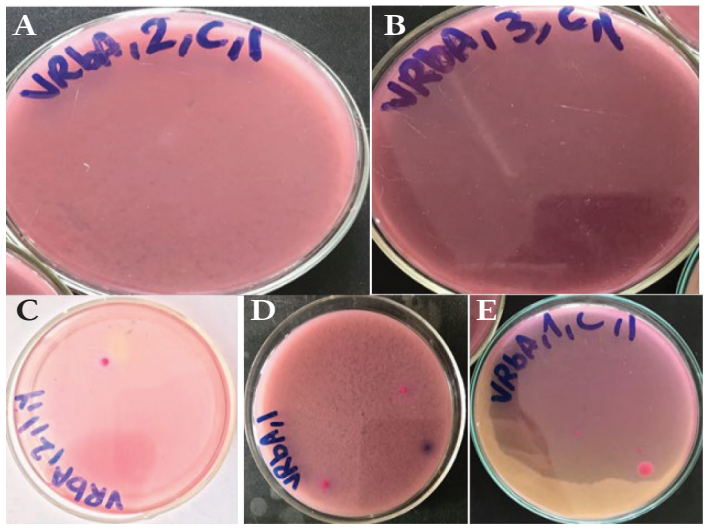

Fig. 2. TCC on violet red bile agar (VRBA).
A – control (-ve); B – absence of coliform colonies in A brand;
C, D, E – presence of coliform colonies in B, C, and D UHT milk brands

Table 5. Identified bacteria from UHT milk brands

Type of samples	Gram-negative isolate identified on McConkey agar	*E. coli*	*Salmonella*	*Pseudomonas*	*Klebsiella*
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
B brand	3 (15)	0 (0)	(0) 0	2 (75)	1 (25)
C brand	5 (25)	0 (0)	(0) 0	3 (60)	2 (40)
D brand	8 (40)	0 (0)	(0) 0	6 (75)	2 (25)

Table 6. Confirmative biochemical tests for the obtained gram-negative isolates

Isolates	C	O	NR	I	MR	VP	Citrate	Urease	TSI		
									Slant/Butt	Gas	H2s
Pseudomonas	+	+	+	–	–	–	+	+	R/R	–	–
Klebsiella	+	–	+	–	–	+	+	+	Y/Y	+	–
E. coli	+	–	+	+	–	–	–	–	Y/Y	+	–
Salmonella	+	–	+	–	–	+	+	+	Y/Y	+	+

C – catalase test; O – oxidase test; NR – nitrate reduction test; I – indole production; MR – methyl-red test; VP – Voges-Proskauer test; Citrate – citrate utilization test; Urease – urease activity; TSI – triple sugar iron fermentation; Y – yellow/acidic; R – red/alkaline; V – variable; '+' – positive; '–' – negative.

Conclusion

The current study concluded that there is somewhat inferior quality, adulteration and a potential risk hazard of consuming B, C, and D UHT milk brands regularly introduced to the resident patients in MUH as some pathogenic bacteria such as *Pseudomonas* and *Klebsiella* were isolated and identified from these brands. So, our study could play an important role in informing these dairy industries about the lack of heat treatment, sanitary processing, packaging, handling of milk during the production process which necessitates the extreme application of hygienic production practices and HACCP.

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