**Evaluation of the potential risks of raw milk collected from different sources in El-Behaira Governorate**

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**Abstract**

Contamination of raw milk by different types of microorganisms from different sources would constitute a public health hazard. Therefore, this study aimed to assess chemical, sanitary and microbiological quality of raw cow bulk milk collected from different sources (dairy farms, collecting & distributing centers and dairy shops & supermarkets), 50 samples from each source. The obtained results revealed that gross chemical composition of raw bulk milk from dairy farms and collecting center nearly similar but low values was noticed in bulk milk collected from dairy shops and supermarket, bulk milk collected from collecting centers have higher values of pH and acidity than other source. All bulk milk samples from different source were contaminated with total bacterial count with different levels, while *Staph. aureus* and total Enterobacteriaceae were detected in (4,4, 8%)and (4, 8, 10%) of examined raw bulk milk collected from dairy farm, collecting & distributing centers, and dairy shops & supermarkets, respectively. The most prevalent enterobacteriaceae species isolated from raw bulk milk from different sources where was Citrobacter freundii 22% followed by Klebsiella pneumonia 18% while the most prevalent mould species isolated from raw bulk milk were penicillium spp. and Aspergillus niger (14% for both) while Rhodotorula species (14%) followed by *candida tropicalis* (10%)were the most prevalent yeast species isolated from bulk milk collected from different sources.In conclusion, higher incidence of indicator microorganisms was noticed in raw bulk milk collected from dairy shops and supermarkets would lead to a public health hazard and/or economic losses due to spoilage of raw milk, therefore, strict hygienic conditions are required during the production cycle from producer to consumers.

**Key words:** Bulk milk, *Staph. aureus*, enterobacteriaceae, yeast, mould.

**INTRODUCTION**

Fresh milk is considered a full meal since it contains numbers of the vital nutrients in a balanced ratio, unlike other foods, such as lactose, fat, protein, minerals, and vitamins (**Hossain and Dev, 2013**). Milk's solid components, primarily fat and protein, make it a valuable economic and nutritional resource (**Negash et al., 2012**).

The amount and types of spoilage bacteria present in milk are strongly related to the price at which the milk may be sold, hence the concept of milk quality is important. There are several levels at which spoilage is aimed to be reduced.One strategy is to try to reduce bacterial load by avoiding contamination across the dairy supply chain (**Boor et al., 2017**).

Milk can be contaminated from different factors like animal health, from milking environment, feed and milkers’ hand, dairy animal, and storage temperature **(Negash et al.,2012)**. Milking equipment are believed to be inconvenient for hygienic cleaning and cause quality deterioration of milk and impose health risks on the consumers (**Azeze and Tera, 2015)**.

The total bacterial count (TBC), which is used to determine the quality of raw milk, has become one of the most widely accepted grading criteria (**Mhone et al., 2011**). TBC is low in high-quality raw milk (**Oliver et al., 2009**).

The presence of *Staphylococcus aureus* in raw milk could be related to mastitis in the cows, inexperienced workers, or poor cleanliness. Their presence in foods poses a health concern to humans, resulting in a public health issue known as food borne intoxication (**Quintana and Carneiro 2006**). The presence of *Staph. aureus* in milk could be used as an indicator of its quality, as well as the hygienic conditions in which it was produced and the health of the herd (**Guerreiro et al., 2005**).

The Enterobacteriaceae family is a huge, diverse collection of Gram-negative rods that live in the intestines of both humans and animals. They are divided into several genera (such as Escherichia coli, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, and Proteus). These genera are common in the environment and can contaminate milk through excrement, bedding, inadequately cleaned teats, milk handling, and dirt or polluted water-contaminated equipment (**Cohen et al., 2017**). The presence of these organisms is usually used as a reliable indicator of faecal contamination. As a result, the presence of Enterobacteriaceae and Coliform bacteria is frequently tested in order to determine the sanitary quality of milk and dairy products(**Martin et. al., 2010**).

Molds in milk are undesirable because they can affect the organoleptic qualities of dairy products, create mycotoxins, and provide a potential health risk (**Wouters et al., 2002**). In addition, presence of yeasts themselves are not commonly the cause of defect in milk unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeast or fruity flavour and obvious gas (**Davis and Wilbey, 1990).**

In developing nations, the chain of persons involved in dairy production runs from milk production farms (farmers, farmworkers, and veterinarians) to milk transportation to markets or small vendors, and then to final consumers via milk vendors or stores. There are requirements for adequate hygiene handling of milk and pasteurization to safe guard public health from milk-borne diseases, however these regulations are rarely followed in underdeveloped nations, resulting in a higher risk of milk-borne illness (**Rahamtalla et al., 2016**).So, this study is conducted to assess chemical composition, Sanitary and microbiological quality of raw cow’s milk collected from different sources.

**2.MATERIAL AND METHODS**

**2.1. Collection of samples:**

One hundred and fifty samples of raw cow bulk tank milk from different sources including (dairy farm, dairy collecting &distributing centers and bulk dairy shops & supermarkets) 50 samples of each source, were collected randomly at El-Bahaira governorate. the samples were transferred as soon as possible in an insulated icebox at 4 ± 1oC to the laboratory(Animal Health Research Institute, Damanhour Branch) with a minimum of delay to be examined. The Examined samples were divided into two parts, 1st part for chemical and sanitary examination, 2nd part for microbiological evaluation.

**2.2. Chemical evaluation ofbulk tank milk samples:**Chemical properties (fat %, protein%, SNF %, TS %) were analyzed by Lactoscan Milk Analyzer which was manufactured by Milkotronic Ltd, Bulgaria (Lactoscan) according to **(FAO, 2009).**

**2.3. Sanitary and Keeping quality tests of bulk tank milk samples:**

**2.3.1.** Determination of pH value using pH meter according to (**APHA, 1992**).

**2.3.2.** Determination of titratable acidity percent according to (**AOAC, 1990**).

**2.4. Microbiological examination of bulk tank milk**

**2.4.1. Preparation of serial dilutions:** One ml of well mixed milk sample was added separately to 9 ml of peptone water 1% solution then thoroughly mixing for preparation of 10-fold serial dilutions, from which the decimal dilutions were prepared to detect according to (**APHA, 2004**).

**2.4.2.Total bacterial count** was evaluated on plate count agar (Merck, Germany), plates were incubated at 30°C for 72 hours for aerobic mesophilic counts (**ISO 4833/2003**).

**2.4.3. Total staphylococcal count** using Baird Parker agar with Rabbit plasma Fibrinogen (RPF) supplement agar (Biokar Diagnostics, France), plate incubate at 37±1 °C for 48 ±4hours according to (**ISO 6888-2/1999**).

**2.4.4. Total Enterobacteriaceae count** was carried out on Violet Red Bile Glucose (VRBG) agar (Merck, Germany) incubated at 37°C for 24 ±2 hours according to (**ISO 21528:2017).** Isolated colonies were purified on plate count agar at 37oC/24h and identified according to **Krieg and Holt, (1984).**

**2.4.5. Total yeast and mould count according to Koburger (1970)** was carried out on Sabouraud’s dextrose agar medium supplemented with chloramphenicol and chlortetracycline (100 mg of each) with final 6.6±0.2, plates incubated at 25±2 ºC for 3-5 days Mould as well as yeast were counted separately and recorded as total mould and yeast count/ml of milk, The isolated fungi were identified individually by macro- and microscopic characteristics according to **Samson (1979)**; **Pitt and Hoching (2009)**, by the lactophenol-cotton-blue stain which identifies mould by culture characteristics, morphology of hyphae cells, spores and fruiting bodies,while yeast isolates were identified according to **Kriger (1984)** and **Tibor and Larry (1996).** by gram stain, the yeast was identified on the basis colony morphological character (blastocondia and chlamydospores according to **Raper and Fennel (1965)**&**Frey et al., (1979).**Germ tube test was used for differentiation between Candida species in which a very light suspension of yeast like organisms in 0.5-1.0 ml of sterile rabbit serum can be used . Incubation was occurred at 37 o C for no longer than 3 hrs . then one drop of yeast- serum mixture was placed on a slide slip and was examined microscopically for germ tube production.

* 1. **2.5. Statistical analysis:** Data was expressed as mean ± SEM and statistically analyzed according to **SAS (2010)**.

**RESULTS**

**Table (1):Mean values ofchemical parameters for bulk milk collected from different sources.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Dairy farms** | **Collecting & distributing centers** | **Dairy shops & Supermarkets** |
| **Mean ±SEM** | **Mean ±SEM** | **Mean ±SEM** |
| **Fat** | **4.16±0.20** | **3.96±0.21** | **2.53±0.34** |
| **Protein** | **3.12 ±0.04** | **3.13±0.03** | **3.00±0.14** |
| **SNF** | **8.64±0.13** | **8.30±0.09** | **8.65±0.30** |
| **Total solid** | **12.83 ±0.24** | **12.50 ±0.31** | **11.38±0.60** |

**Table (2):Mean values of Sanitary and keeping quality testfor bulk milk collected from different sources.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Dairy farms** | **Collecting & distributing centers** | **Dairy shops & Supermarkets** |
| **Mean ±SEM** | **Mean ±SEM** | **Mean ±SEM** |
| **pH** | **6.84±0.09** | **7.04±0.05** | **6.77±0.15** |
| **Acidity degree** | **16.06±0.22** | **17.09±0.26** | **15.71±0.21** |

**Table (3):Statistical analytical results of total bacterial count for bulk milk collected from different sources (n=50 for each source)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sources** | **NO. of examined samples** | **Total bacterial count cfu /ml** | | |
| **Positive sample** | | **Mean ±SEM** |
| **No** | **%** |
| **Dairy farms** | **50** | **50** | **100** | **1.5×104 ±0.23×104** |
| **Collecting & distributing centers** | **50** | **50** | **100** | **2.28×105±0.35×105** |
| **Dairy shops & Supermarkets** | **50** | **50** | **100** | **8.93×105±1.85×105** |

**Table (4):Statistical analytical results of *Staphylococcus aureus* count for bulk milk collected from different sources.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sources** | **NO. of examined samples** | ***Staphylococcus aureus* Cfu /ml** | | |
| **Positive sample** | | **Mean ±SEM** |
| **No** | **%** |
| **Dairy farms** | **50** | **2** | **4** | **2.5×102±0.50×102** |
| **Collecting & distributing centers** | **50** | **2** | **4** | **2.5×102±0.50×102** |
| **Dairy shops & Supermarkets** | **50** | **4** | **8** | **4.2×102±1.93×102** |

**Table (5):Statistical analytical results of total Enterobacteriaceae count for bulk milk collected from different sources.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sources** | **NO. of examined samples** | **Total Enterobacteriaceae count Cfu /ml** | | |
| **Positive sample** | | **Mean ±SEM** |
| **NO** | **%** |
| **Dairy farms** | **50** | **2** | **4** | **2.5×103±0.50×103** |
| **Collecting centers** | **50** | **4** | **8** | **2.46×103±0.57×103** |
| **Dairy shops & Supermarkets** | **50** | **5** | **10** | **8.5×102±4.3×102** |

**Table (6): Incidence of Enterobacteriaceae species of bulk milk collected from different sources**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species**  **Sources** | **Klebsiella pneumonia** | | **Citrobacter freundii** | | **E.coli** | | **Salmonella typhi** | | **Shigella boydii** | |
| **No** | **%** | **No** | **%** | **No** | **%** | **No** | **%** | **No** | **%** |
| **Dairy farms** | **2** | **4** | **3** | **6** | **-** | **-** | **-** | **-** | **-** | **-** |
| **Collecting center** | **3** | **6** | **3** | **6** | **-** | **-** | **-** | **-** | **-** | **-** |
| **Shops and supermarkets** | **4** | **8** | **5** | **10** | **3** | **6** | **2** | **4** | **2** | **4** |
| **Total** | **9** | **18** | **11** | **22** | **3** | **6** | **2** | **4** | **2** | **4** |

**Table (7):Statistical analytical results of total yeast and mould count for bulk milk collected from different sources (n=50 for each source)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sources** | **Yeast count Cfu /ml** | | | **Mould count Cfu/ml** | | |
| **Positive sample** | | **Mean ±SEM** | **Positive sample** | | **Mean ±SEM** |
| **NO** | **%** | **NO** | **%** |
| **Dairy farms** | **2** | **4** | **2.0×10±0.02×10** | **2** | **4** | **2.0×102± 0.06×102** |
| **Collecting centers** | **2** | **4** | **1.0×103± 0.04×103** | **2** | **4** | **1.05×103±0.1×103** |
| **Shops & Supermarkets** | **3** | **6** | **1.1×103±0.9×103** | **3** | **6** | **2.03×103±0.1×103** |

**Table (8):**I**ncidence of fungal isolates in bulk milk collected from different sources (N=50)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Milk samples** | **Yeast species** | | | | | | **Mould species** | | | | | |
| ***C.albicans*** | | ***C.tropical*** | | ***Rhodotorula*** | | ***A.flavus*** | | ***A.niger*** | | ***Penicillium*** | |
| **No** | **%** | **NO** | **%** | **No** | **%** | **No** | **%** | **No** | **%** | **No** | **%** |
| **Dairy farms** | **1** | **2** | **1** | **2** | **2** | **4** | **2** | **4** | **2** | **4** | **2** | **4** |
| **Collecting centers** | **1** | **2** | **2** | **4** | **2** | **4** | **2** | **4** | **2** | **4** | **2** | **4** |
| **Shops&Supermarkets** | **2** | **4** | **2** | **4** | **3** | **6** | **2** | **4** | **3** | **6** | **3** | **6** |
| **TOTAL** | **4** | **8** | **5** | **10** | **7** | **14** | **6** | **12** | **7** | **14** | **7** | **14** |

**DISCUSSION**

The use of plastic containers for milk storage by farmers and vendors can compromise milk quality since plastic can easily crack and these cracks harbour spoilage bacteria and are difficult to clean, thus the stainless steel and aluminum cans are advised in milk storage as they are easily cleaned **(John, 2016)**. It is very important to wash the udder correctly by using unperfumed soap and Luke warm water followed by drying with a clean cloth (**De-Silva et al., 2016)**. The use of unclean milking and transport equipment also contributes to the poor hygienic quality of the milk. The quality deterioration of raw milk had influenced effect on the quality of finished products microbially, organoleptically and chemically as well as its shelf life (**De-Silva et al., 2016**).

It is evident from Table (1) that the average fat percentage of raw milk from different sources varied from 2.35 ±0.34 to 4.14 ±0.20; average of protein content varied from 3.00 ±0.14 to 3.13 ±0.03; average SNF ranged from 8.30 ±0.09 to 8.65 ±0.30; in addition, average total solid in raw milk collected from different source varied from 11.38 ±0.60 to 12.83 ±0.24.It is notice that the mean values of fat percentages and total solid content of raw milk collected from supermarkets are slightly lower than the raw milk collected from other source, it may occur due to skimming of milk, this result was supported by **Towhida et al., (2021) .** According to **Egyptian standard (2005)** established that fat percent of raw cow milk should be less than 3% and SNF not less than 8.25 %; the lowest mean values were noticed in bulk milk collected from dairy shops and supermarkets.

Our findings of fat percent were supported by **Debnath et al. (2009)** who demonstrated that the fat of milk from different sources from Chittagong metropolitan area, Bangladesh varied from 3.52 to 4.01%. In addition, nearly similar means of protein content in raw milk collected from different sources were supported by **Towhida et al., (2021)** who reported that protein content of raw milk collected from different five milk marketing agencies in Chattogram metropolitan area, Bangladesh was 3.11±0.08.

Data reported in Table (2) showed that the pH of raw milk collected from different sources nearly similar and varied from 6.77 ±0.15 to 7.04 ±0.05. In addition, titratable acidity content of bulk milk samples collected from different source varied from 15.71 ± 0.21 to17.09 ± 0.26.

Titratable acidity is a common mean to judge the sanitary quality of milk. It has a strong economic impact, because it is one of the criteria used to determine whether or not raw milk enters the food chain as a premium-priced fluid product **(Schmidt et al., 2014)**. Acidity degree of bulk milk collected from different sources within normal range according to **(Ebner et al., 2017)** recorded that the acidity degree of normal milk is about 16 to 18.

The pH of milk indicates its real acidity. It is a better method for recording the acidity for milk and milk products than the titratable acidity because it is less variable, although it is influenced by temperature (**Walstra et al., 2006**).

Mentioned results in Table (3) revealed that higher TBC was in bulk milk collected dairy shops and supermarkets (8.93×105 ±1.85×105 Cfu/ml), followed by bulk milk from collecting and distributing centers (2.28×105 ±0.35×105 Cfu/ml) and milk from dairy farms (1.5×104 ±0.23×104 Cfu/ml). These findings might attribute to further contamination of milk from dairy cooperatives during transportation, use of poorly cleaned milk containers and absence of cooling systems in milk selling points (**Al-Ghamdi et al., 2020).**

The higher bacterial count which exceeded the microbiological criteria applicable to raw milk indicates substandard hygienic conditions practiced during production and subsequent handling which include poor hygiene during milking or equipment used for milking and udder infection of the cow (**Shunda et al., 2013**) and this findings were supported by **El-Zubeir and Ahmed (2007)** who reported that the higher total bacterial count of 2.6x1010 cfu/ml, which exceeded the international standards of raw milk, could be due to unsatisfactory hygiene and control measures and the health supervision applied to the farms.

The recorded data in Table (4) illustrated that 4, 4 and 8% of examined bulk milk collected from dairy farm, collecting & distributing centers and Dairy shops & Supermarkets were contaminated with Staph. aureus with mean values of 2.5×102 ±0.50×102, 2.5×102 ±0.50×102 and 4.2×102 ±1.93×102  Cfu/g, respectively. Higher incidence of staph. aureus in bulk milk collected from dairy shops &supermarkets might attribute to neglected hygienic measure and lack of personal hygiene in milk selling points. According to **Egyptian standards (2005)** stated that Staph. aureus should excess 100 cells/ml, all mean values of raw bulk milk were exceed the normal range.

Our findings were agreed with **Orregard (2013)** who reported that Staph. aureus count in the shops' milk was significantly higher than milk from farmers. In addition, **Shunda et al. (2013)** isolated S. aureus from milk collected from dairy farms, milk vending, and houses &cafeterias at incidence rate 13.3, 4.4 and 8.9 %, respectively. The microbiological analysis of samples from all direct sale points revealed that Staphylococcus spp. were found in all milk samples with a count ranging from 1.6×103 to 5.1×104 cfu/mL (**Pyz-Lukasik et al., 2015).**

The results shown in Table (5) reported that that 4, 8 and 10% of examined bulk milk collected from dairy farm, collecting & distributing centers and dairy shops & supermarkets were contaminated with enterobacteriaceae with mean values of 2.5×103±0.50×103, 2.46×103±0.57×103 and 8.2×102±4.3×102 cfu/g, respectively.Higher prevalence of enterobacteriaceae were detected in 84% of the examined raw milk samples collected from small dairies, groceries, and supermarkets from different localities at Kafr El-Sheikh Governorate, Egypt with a mean count of 1.02x 06±1.98 x105 Cfu/ml (**Sobeihet al., 2020)**. In addition,**El-Mokadem et al., (2020)** reported that incidence of enterobacteriaceae in bulk cow milk collected from governmental farms at El-Gharbia Governorate, Egypt was 68%.Enterobacteriaceae counts in milk are useful indicators of hygiene conditions during production cycle from producers to consumers (**Pyz-Lukasik et al., 2015).**

Higher prevalence of enterobacteriaceae in bulk milk collected from dairy shops & supermarkets followed by collected from collecting & distributing centers could be due to lack of sanitary practice at the point of sale. this suggest were supported by **El-Zubeirand Ahmed, (2007)** who reported that milk samples contaminated with enterobacteriaceae indicated lack of sanitary measures during production and handling of milk **Pyz-Lukasik et al., (2015)** reported that milk samples collected from 5 direct sale points in eastern Poland were contaminated with enterobacteriaceae ranged from 6.4×101 to 1.7×106 cfu/ml.

The obtained results in Table (6) revealed the incidence of enterobacteriaceae species in bulk milk collected from different sources where Citrobacter freundii was the most prevalent species 11 (22%) followed by Klebsiella pneumonia9 (18%). On the other hand, E.coli, salmonella typhi and shigella boydii were isolated only from raw cow bulk milk collected from supermarkets only by percent 6, 4 and 4%, respectively for each isolate.

**El-Mokadem et al., (2020)** reported that most prevalent enterobacteriaceae species isolated from raw cow milk samples of both governmental and private farms was Serratia marcsence with incidence rate of 19.23 and 18.75 %. In addition, **Sobeih et al., (2020)** found that the most frequent enterobacteriaceae members isolated from the examined raw milk samples were Hafnia alive (30.95%), Serratia liquefaciens (25.0%), and Klebsiella pneumonia (15.48%).On the other hand, **Oladipo et al., 2016**) could isolate Salmonella typhi and E.coli from raw cow milk samples were collected from the five different locations in Ogbomosho, Nigeria.

Klebsiella pneumoniae found in soil, water, sewage and constitute a part of the flora of the mouth and intestinal tract of human and animals. It responsible for pneumonia and upper respiratory tract infection and may be responsible for meningitis, pyaemia, and cystitis (**Martin and Bachman, 2018**).The presence of a large number of Enterobacteriaceae in raw milk indicate unsafe raw milk for human consumption (**Nyein et. al. 2002**) unless it is pasteurized as Enterobacteriaceae in raw milk were completely removed by pasteurization and not appear during the manufacturing process of dairy product (**Branciari et al., 2004**) so milk for human consumption must be pasteurized.

The results tabulated in Table (7) reported that raw cow bulk milk collected from dairy farm, collecting & distributing centers and dairy shops & supermarkets was contaminated with yeast at level 4, 4 and 6% with mean values 2.0×10± 0.02, 1.0×103 ± 0.04 and 1.1×103 ±0.9×103 Cfu/ml, respectively. while mould was detected at incidence rate 4, 4 and 6 % with mean values of 2.0×102±0.06, 1.05×103±0.1×103 and 2.03×103 ±0.1×103 Cfu/ml, respectively. Contamination of raw milk with mould and yeast is to be a reflection of hygienic conditions during the processes of milking and milk storage **Chipilev et al., (2015).**

Our results indicating that bulk milk collected from dairy shops & supermarkets most contaminated with yeast and mould than bulk milk from other sources. Higher incidence of yeast and mould in raw milk was obtained by **Chipilev et al., (2015)** who reported that 93.3 and 76.6% of raw milk collected from Bulgaria was contaminated with yeast and mould with log mean 3.5±0.42 and 3.4±0.23 Cfu/ml, respectively.

The most predominant mould species isolated from bulk milk collected from different source were Asp. niger and penicillium at incidence level (14 and 14%). while the most prevalent yeast species from the same sources was Rhodotorula with and incidence 14% followed by candida tropicalis with incidence level 10%(Table, 8). In addition, the most contaminated source with fungal isolates was dairy shops and supermarkets might be due to inappropriate hygienic conditions and unclean equipment during storage.

Our results about predominant yeast and mould species in raw milk similar to (**Gulbe and Valdovska, 2014**) who reported that mould strains identified from raw milk samples collected from dairy herd during the whole year belonged to 15 genera, of which Aspergillus (44.2%) and Penicillium (28.9%). in addition, **El-Kest et al.(2015)** revealed that the most predominant mould spp. in raw milk samples were Aspergillus spp. 33.3%. in contrary, **Spanamberg et al. (2014)** examined 588 raw milk samples and revealed that the most common isolated yeast species were Candida spp. (70.0%) followed by Rhodotorula species (11.70%) also **Chipilev et al., (2015)** found that the most common isolated yeast spp. from raw milk were Candida spp. 70 %.

Raw milk is generally considered an ideal growth medium for many fungal species, because of providing all important nutrients and conditions for fungal growth (**Gulbe and Valdovska 2014**).The occurrence of microscopic fungi in raw milk can take place during milking, handling, storage and other pre-processing activities; besides, it is influenced by the animal’s physiological state and the weather and breeding conditions (**Vacheyrou et al., 2011**).

**CONCLUSION AND RECOMMENDATION**

In conclusion, bulk milk collected from dairy shops and supermarkets was the worst having high total bacteria, staph. aureus, enterobacteriaceae, and total yeast &mould count, indicating that the milk is distributed under unhygienic conditions, in addition, lack of sanitary measure at the point of sales. Therefore, it is the responsibility of the local authorities to monitor the production and distribution of the milk to reach the consumer with acceptable levels of microorganisms.

**REFERENCES**

**Al-Ghamdi, A.Y., Ibtisam, M.A.M. Ali, Laila E.O.,AlSharqi, Mohamed O. M. Abdalla, 2020**. Microbiology of raw milk collected from different vending sources in Shendi area, River Nile State, Sudan. International Journal of Biosciences, 16 (5): 336-344

**Ali, A.A., Irshad, N.B., Razaz, S.A.,Manahil, A.A. 2010.** Microbiology safety of raw milk in Khartoum State, Sudan: 1-Khartoum and Omdurman Cities. Pakistan Journal of Nutrition 9(5): 426-429

**AOAC, 1990.** Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington, D.C. USA.

**APHA, (American Public Health Association) 1992.** Standard Methods for Examination of Dairy Products. 16th Ed. American Public Health Association, Washington, DC.

**APHA, (American Public Health Association) 2004.** Standard methods for the examination of dairy products. 17th Ed., New York. USA.

**Azeze, T.,Tera, A. 2015.** Safety and quality of raw cow milk collected from producers and consumers in Hawassa and Yirgalem areas, Southern Ethiopia. Food Science and Quality Management, 44:63-72.

**Boor, K.J., Wiedmann, M., Murphy, S.,Alcaine, S. 2017.** A 100-year review: Microbiology and safety of milk handling. Journal of Dairy Science 100(12):9933-9951.

**Branciari, R., Goga B.T.C., Rea S., Avellini P. 2004.** Evaluation of hygienic characteristics of Italian “fossa” cheese, food-safetyassurance and veterinary-public-health volume 2: Safety assurance during Food Processing, 333.

**Chipilev, N., Daskalov H., Stoyanchev T. 2015.** Study on the prevalence of lipolytic yeasts and moulds in raw cow milk and white brined cheese. Bulg. J. Vet. Med., pp:1-10.

**Cohen, I., Powderly, W.G.,Opal, S.M. 2017.** Infectious Diseases, 4th Ed. El Sevier Ltd.

**Davis, J.G.,Wilbey, R.A. 1990.**Microbiology of Cream and Dairy Desserts. In: Dairy Microbiology (Ed.: Robinson, R.K.). London and New Yersey, Applied Science Publishers, 41–108.

**Debnath, G.K., Kober, A.K.M.H., Chanda, G.C. 2009.** Quality of fluid milk available in Chittagong Metropolitan Area, Proceedings of the Sixth Annual Scientific Conference of CVASU, Bangladesh.

**De-Silvaa, S.A.S.D., Kanugalab, K.A.N.P.,Weerakkody, N.S. 2016.** Microbiological quality of raw milk and effect on quality by implementing good management practices. Procedia Food Science 6: 92-96.

**Ebner, P., Ghoryar, M.A., Shakhes, S.A., Ghanizadah, A.Z. 2017**. Milk Quality and Safety Testing Manual. Afghanistan, 1(1):12.

**Egyptian standards 154-1/2005.** milk and milk products part 1: raw milk

**El-Kest, M., El- Hariri, M., Khafaga, E., Nagwa, I.M., Refai, M.K., 2015.** Studies on contamination of dairy products by aflatoxin M1 and its control by probiotics. Journal of Global Biosciences 4(1):1294- 1312.

**El-Mokadem E.A., El-Leboudy A.A., Amer A.A. 2020.** Occurrence of Enterobacteriaceae in dairy farm milk. Alexandria Journal of Veterinary Sciences, 64 (2): 66-71.

**El-Zubeir, I.E.M., Ahmed M.I.A. 2007.** The hygienic quality of raw milk produced by some dairy farms in Khartoum State, Sudan. Research Journal of Microbiology 2(12): 988-991.

**FAO, 2009.** Milk Testing and Payment Systems Resource Book a practical guide to assist milk producer groups. Rom, Italy: Food and Agricultural Organization United Nation.

**Frey, D., Old-Field, R.J., Bridjer, R.C. 1979.** A colour atlas of pathogenic fungi. Wolf Medical Public. Ltd. London.

**Guerreiro, P.K., Machado, M.R.F., Braga, G.C., Gasparino, E., Franzener, A.S.M. 2005.**Qualidademicrobiológica de leiteemfunção de técnicasprofiláticas no manejo de produção. Revista de CiênciaAgrotécnica 29: 216-222.

**Gulbe G., and Valdovska , A. 2014.**Diversity of Microscopic Fungi in the Raw Milk from Latvian Organic Farms. Proc. Latv. Univ. Agr., 31(326): 46-53.

**Hossain, M.B.,Dev, S.R. 2013.** Physiochemical characteristics of various raw milk samples in a selected dairy plant of Bangladesh, International Journal of Engineering and Applied Sciences, 1 (3): 91-96.

**ISO 21528-2:2017.** Microbiology of the food chain- Horizontal method for the detection and enumeration of Enterobacteriaceae- Part 2: Colony-count technique.

**ISO 4833/2003.** Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of microorganisms-Colony-count technique at 30°C. International Organization for Standardization, Genova, Switzerland, 1–9.

**ISO 6888-2/1999**. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase positive staphylococci (Staphylococcus aureus and other species) Part 2: Technique using rabbit plasma fibrinogen agar medium. International Organization for Standardization, Genova, Switzerland, 1–7.

**John, M.N. 2016.** Microbiological and compositional quality of raw milk delivered by small scale dairy farmers to the milk collection centers in Hai District, Tanzania. MSc thesis, University of Zimbabwe, Zimbabwe, 38-39.

**Koburger, J.A. 1970.** Fungi in Foods.1-Effect of inhibitor and incubation  temperature  on enumeration. J. Milk and Food Technol., 33(10):433-434.

**Krieg N.R., Holt J.G. 1984.** Bergey’s manual of systematic bacteriology. Vol. 1. Williams and Wilkins. Baltimore, London.

**Kriger Van-Rij, N.J.W.1984.** The yeasts:A taxonomic study. 3rd Ed. Amsterdam, Elsevier

**Martin R.M., Bachman, M.A. 2018.** Colonization, infection, and the Accessory Genome of Klebsiella pneumoniae. J. Front. cell. Infec. Microbial., 8(4).

**Martin, M.C., Martinez, N., del Rio, B., Ladero, V., Fernández, M., Alvarez, M.A. 2010.** A novel real-time polymerase chain reaction-based method for the detection and quantification of lactose-fermenting Enterobacteriaceae in the dairy and other food industries. J. Dairy Sci., 93(3): 860-867.

**Mhone, T.A.,  Matope, G., Saidi, P.T. 2011.**Aerobic bacterial, coliform, Escherichia coli and Staphylococcus aureus counts of raw and processed milk from selected smallholder dairy farms of Zimbabwe. Int. J. Food Microbiol., 151:223-228

**Negash, F., Tadesse, E., Woldu, T. 2012.** Microbial quality and chemical composition of raw milk in the Mid-Rift Valley of Ethiopia, African Journal of Agricultural Research, 7 (29):4167-4170.

**Nyein, M-M., Khine, T.T., Thin, K.K. 2002.** Bacteriological aspects of milk and milk products in Yangon during 2000, Myanmar. Health. Sci. Res. J., 14 (1/3): 35-41.

**Oladipo, I.C., Tona, G.O., Akinlabi, E.E., Bosede, O.E. 2016.** Bacteriological quality of raw cow’s milk from different dairy farms in Ogbomoso, Nigeria. Int. J. Adv. Res. Biol. Sci., 3(8): 1-6.

**Oliver, S.P., Boor, K.J. , Murphy, S.C.,Murinda, S.E. 2009.** Food safety hazards associated with consumption of raw milk Foodborne Pathog. Dis., 6: 793-806

**Orregard, M. 2013.** Quality analysis of raw milk along the value chain of the informal milk market in Kiambu Country, Kenya. MS thesis, Swedish University of Agricultural Sciences, Sweden, 24-27.

**Pitt,J.I., Hocking,A.D.2009.** Fungi and Food spoilage. 3rd Ed. Published by Springer Dordrecht Heidelberg, London,New York.

**Pyz-Lukasik, R, Paszkiewik, W, Tatara, M.R., Brodzki, P.,Belkot, Z. 2015.** Microbiological quality of milk sold directly from producers to consumers. Journal of Dairy Science 98:4294-4301.

**Quintana, R.C.,Carneiro, L.C. 2006.**Avaliação do leite in natura comercializadoclandestinamente no município de Morrinhos, GO. Rev. Inst. Adolfo. Lutz. 65: 194-198.

**Rahamtalla, S.A., Elsheikh N.A.H.,Abdalla M.O.M. 2016.** Microbiological quality of raw milk produced and distributed in Khartoum State, Sudan. ARPN Journal of Agricultural and Biological Science 11(1):24-29.

**Raper, K.B., Fennel, D.T. 1965.** The genus Aspergillus. The Williams and Wilkins co., Baltimore.

**Samson, R.A.1979.** Aspergilli described since 1965.Studies in Mycology No.18.

**SAS, 2010.** SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.

**Schmidt, K.A., Stupar, J.,Shirley, J.E. 2014.** Factors affecting titratable acidity in raw milk. America. Kansas Agricultural Experiment Station Research Reports.0(2): 60.

**Shunda, D., Mabtamu, T.,Endale, B. 2013.** Assessment of Bacteriological Quality of Raw Cow Milk at Different Critical Points in Mekelle, Ethiopia. International Journal of Livestock Research 3(3b): 42-48.

**Sobeih, A.M.K., Al-Hawary, I.I., Elsayed, M.K., Nahla, A.E. 2020.**Prevalence of Enterobacteriaceae in raw milk and some dairy products. Kafrelsheikh Veterinary Medical Journal, 18 (2): 9-13.

**Spanamberg, A., Fraga, C.F., Ferreiro, L., Aguinsky, M.S., Sanches, E.M.C., Roehe, C., Lautert, C., Santurio, J. M., 2014.** Yeasts in the raw ewe's milk. Acta Scientiae Veterinariae 42:1236-1240

**Tibor, D., Larry R.B. 1996.**  Handbook of food  spoilage yeasts. 1st Edition (Contemporary Food Science) by CRC Press, Boca Raton, New York, London and Tokyo.

**Towhida, K., Eaftekhar, A.R., Md. Shafayat, J., Md. Fahad, B.Q., Mishuk, S., Omar, F.M. 2021.** Assessment of biochemical and microbial quality of different market and raw milk available in Chattogram metropolitan area, Bangladesh. Int. J. Adv. Res. Biol. Sci. 8(2): 80-85.

**Vacheyrou, M., Normand, A.C., Guyot, P., Cassagne, C., Piarroux, R., Bouton, Y. 2011.** Cultivable microbial communities in raw cow milk and potential transfers from stables of sixteen French farms. International Journal of Food Microbiology, 146:253–262.

**Walstra, P., Wouters, J.T.M.,Geurts, T.J., 2006.** Dairy Science and Technology, CRC Press, Boca Raton, FL, USA, pp. 497–512

**Washington, W.J., Stephan, A., Willium, J., Elmer, K.,Gail, W. 2006.**Koneman’s color Atlas and Textbook of diagnostic Microbiology,6th edd:1152-1232.

**Wouters, J.T.M., Ayad, E.H.E., Hugenholtz, J.,Smit, G. 2002.** Microbes from raw milk for fermented dairy products. Int. Dairy J. 12 (2-3): 91 - 109.

**تقييم المخاطر المحتملة للحليب الخام المجمع من مصادر مختلفة بمحافظة البحيرة**

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قد يشكل تلوث الحليب بأنواع مختلفة من الميكروبات من مصادر مختلفة خطرًا على الصحة العامة. لذلك هدفت هذه الدراسة إلى تقييم التركيب الكيميائي والجودة الصحية والميكروبيولوجية للحليب الخام المجمع من مصادر مختلفة مثل (المزرعة الألبان ومراكز التجميع والتوزيع ومحلات الألبان ومحلات السوبر ماركت)، 50 عينة من كل مصدر. أوضحت النتائج أن التركيب الكيميائى الإجمالي للحليب من مصادر مختلفة متشابهة تقريبًا ولكن قيم منخفضة لوحظت في الحليب الذي تم جمعه من محلات الألبان والسوبر ماركت. فيما يتعلق بالرقم الهيدروجيني والحموضة ، فإن الحليب الذي تم جمعه من مراكز التجميع له قيم عالية بالإضافة إلى ذلك . فيما يتعلق بالتقييم الميكروبيولوجي، فإن جميع عينات الحليب كانت ملوثة بالعدد البكتيري الكلي بمستويات مختلفة. ووجد أن نسبة تواجد الميكروب العنقودى الذهبى وإجمالي البكتيريا المعوية في الحليب المجمع من المزرعة ، مراكز التجميع والتوزيع ومحلات الألبان ومحلات السوبر ماركت كانت (4 ، 4 ، 8٪) ، (4 ، 8 ، 10٪) على التوالي.وكان أكثر أنواع البكتريا المعوية إنتشار كان السيتروبكتر فرونيدى بنسبة 22% و الكليبسيلا نيمونيى بنسبة 18% وكان أكثر أنواع الفطريات المعزولة من الحليب المجمع من مصادر مختلفة هىالبنسليم بنسبة (14%) و أسبريجليس نيجر (14%). بينما كانت الرودوتورولا(14%) والمبيضات الاستوائية (10%)المعزولة من الحليب الذي تم جمعه من مصادر مختلفة هي أكثر أنواع الخميرة انتشارًا. في الختام ، لوحظ ارتفاع التلوث الإصابة بالميكروبات في الحليب الذي تم جمعه من محلات الألبان والسوبرماركت مما قد يؤدي إلى مخاطر على الصحة العامة و / أو خسائر اقتصادية بسبب تلف الحليب الخام ، وبالتالي ، فإن الشروط الصحية الصارمة مطلوبة أثناء دورة الإنتاج من المنتج للمستهلكين.