



RESEARCH PAPER

OPEN ACCESS

Microbiology of raw milk collected from different vending sources in Shendi area, River Nile State, Sudan

Abdulaziz Y. Al-Ghamdi¹, Ibtisam M. A. M. Ali², Laila E. O. AlSharqi³,
Mohamed O. M. Abdalla^{*3}

¹Department of Biology, Faculty of Science, Al-Baha University, Al-Baha, Saudi Arabia

²Department of Biotechnology, University of Shendi, River Nile State, Sudan

³Department of Biology, Faculty of Science, Al-Makhrwah, Al-Baha University, Saudi Arabia

Key words: Raw milk, Microbiology, Vending source, Bacterial isolation

<http://dx.doi.org/10.12692/ijb/16.5.336-344>

Article published on May 30, 2020

Abstract

This study was conducted to evaluate the microbiological quality of raw milk collected from different sources in Shendi area, River Nile State, Sudan. A total of 120 raw milk samples were collected from dairy farms (30 samples), milk vending shops (30 samples), pickup trucks (30 samples) and vendors on donkey cart (30 samples). The samples were transported to the microbiology laboratory at 4°C for microbiological examination (total viable bacteria, coliform bacteria and *Staphylococcus aureus*) in addition to isolation and identification of some bacteria. Results showed that total viable bacteria count (TVBC), total coliform bacteria count (TCC) and *Staphylococcus aureus* count were high in raw milk from vendors on donkey cart (log 8.09±0.270, log 6.01±0.189 cfu/ml and log 6.12±0.404 cfu/ml, respectively), and low in milk from pickup trucks (log 7.93±0.337 cfu/ml, log 5.89±0.110 cfu/ml and log 5.84±0.131 cfu/ml, respectively). During the study, several bacteria were isolated from raw milk of different sources and these included *Streptococcus epidermidis*, *Escherichia coli*, *Bacillus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Citrobacter diversus*. The study concluded that the raw milk of different vending sources was contaminated with microorganisms and it is recommended that this practice should be avoided as much as possible.

*Corresponding Author: Mohamed O. M. Abdalla ✉ abutahany@yahoo.com

Introduction

As a complex biological fluid, milk is a good medium for growth of many microorganisms, which may originate from different sources such as air, milking equipment, feed, soil, faeces and grass, and so it is an important vehicle for transmitting milk-borne pathogens to humans (Coorevits *et al.*, 2008; Senarath and Adikari, 2017). The bulky nature and nutritional characteristics of milk attract microbes that contribute to its perishability, and this creates a need to ensure that small-scale farmers produce clean milk, as this depends on milking environment, milkers' hygiene, teats, and containers used for milk storage (John, 2016). Milk sold in the market as raw is much below standards in hygiene, and its bacteriological quality needs to be carefully monitored regarding the number and types of microflora present (Singh and Shankar, 2017). Milk is sterile in healthy udder cells and does not contain microorganisms in the mammary gland at the site of its secretion unless there is an intramammary infection and/or the animal has a systemic disease, but as soon as the milk is excreted, it is immediately contaminated with microorganisms that naturally dwell in the teat skin and the epithelial lining of the teat canal (Tekilegiorgis, 2018).

Milk is a good medium for the growth of various microorganisms, especially bacterial pathogens such as species of *Bacillus*, *Listeria monocytogenes*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Campylobacter*, *Escherichia*, *Klebsiella*, which may cause milk-borne diseases such as tuberculosis, brucellosis and gastroenteritis (Ruangwittayanusorn *et al.*, 2016).

Due to its characteristics, milk deserves special attention during its production, processing, marketing, and consumption, and several factors may influence the microbiological quality of milk products such as the health of the herd, sterility of the cleaning equipment and utensils, the health conditions of the milking place, the excretion from the udder of an infected animal and quality of water used in the farm (Nwankwo *et al.*, 2015). However, despite the fact that modern technology is used for milk production, many milk producers still use non-specialized

methods resulting in poor-quality raw milk (Nwankwo *et al.*, 2015). The chain of people involved in dairy production in under-developed countries extends from milk production farms (farmers, farmworkers, and veterinarians) and transportation of milk to the market or small vendors to reach the final consumers either through milk vendors or from shops (Rahamtalla *et al.*, 2016). To protect public health against milk-borne infections, there are regulations for proper hygiene handling of milk and its pasteurization, but in under-developed countries, such regulations are not usually adhered to, hence milk-borne health risk is greater (Rahamtalla *et al.*, 2016).

In Sudan, raw milk distributed for consumption is not subjected to proper quality control measures (Mohamed and ElZubeir, 2007). In Khartoum State, 95% of milk is distributed as raw to the consumers (Salman and Hamad, 2011). Moreover, most of the milk producers in Khartoum State are unaware of the effect of animal health and environmental conditions on producing safe milk due to absence of full certification of employees, absence of staff and technicians, retardation of milk production and processing system and lack of training and extension programs (Abdalla and Elhagaz, 2011).

This study is conducted to evaluate the level of microbiological contamination of raw milk collected from different milk distributing channels around Shendi area, River Nile State, Sudan.

Materials and methods

Milk collection and sampling

One hundred and twenty (120) samples of raw milk were collected randomly from four different sources in Shendi area (30 samples from dairy farms, 30 samples from milk vending shops, 30 samples from vendors on donkey carts and 30 samples from pickup trucks).

The samples were collected in sterile containers and transported in an icebox at 4°C to the microbiology laboratory and analyzed for total viable bacteria (TVB), coliform bacteria and *S. aureus* counts, in addition to isolation and identification of some pathogenic bacteria.

Microbiological examination

Preparation of sample dilutions

Eleven milliliters (11mL) of raw milk were added to 99mL sterile distilled water and mixed to make 10^{-1} dilution, then 1mL from the above-mentioned dilution (10^{-1}) was added to 9mL sterile distilled water to make 10^{-2} dilution. This process was repeated to make serial dilutions of up to 10^{-8} .

Enumeration of bacteria

The bacterial count was determined according to Houghtby *et al.* (1992) using a standard plate count agar. The plates were incubated at 32°C for 48 hr and colonies were counted (Cheesbrough, 2006). *S. aureus* count was determined using mannitol salt agar medium. The plates were incubated at 37°C for 48 hr and colonies were counted (Cheesbrough, 2006). Total coliform bacteria count was determined using MacConkey agar medium, the plates were incubated at 37°C for 45 hr and the colonies were counted (Cheesbrough, 2006).

Purification of identification of organisms

Purification was carried out by sub-culturing of a well isolated typical colony on nutrient agar medium for 24 hr, and the plates were checked by Gram stain, then the colonies were transferred to a plate containing a fresh solidified corresponding medium (Barrow and Feltham, 1993). The purified isolates were identified by Gram staining, oxidase test, catalase test, motility test, Kligler's iron agar (KIA) test, DNase test (for Gram +ve cocci), indole production test, citrate utilization test, starch hydrolysis test, coagulase test, gelatin hydrolysis test and reaction on blood agar (Barrow and Feltham, 1993).

Statistical analysis

Data analysis was carried out by Statistical Analyses Systems (SAS, ver. 9). General linear models (GLM) were used to determine the effect of source and sample number on the microbiological characteristics of raw milk. Mean separation was done by Duncan's multiple ranges test ($P \leq 0.05$).

Results and 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 an effect on the quality of finished products microbially, organoleptically and chemically as well as its shelf life (De Silva *et al.*, 2016).

The mean TBVC of milk did not show a significant ($P > 0.05$) difference among the different sources, although the higher count was in milk from vendors on donkey cart ($\log 8.09 \pm 0.270$ cfu/ml), followed by milk from dairy farms ($\log 8.01 \pm 0.307$ cfu/ml), milk vending shops ($\log 7.99 \pm 0.275$ cfu/ml) and pickup trucks ($\log 7.93 \pm 0.337$ cfu/ml) (Table 1). The range of TVBC of samples from dairy farms, vending shops, pickup trucks and vendors on donkey cart were $\log 7.34 \pm 0.67 - 8.39 \pm 0.92$ cfu/ml, $\log 7.71 \pm 0.88 - 8.51 \pm 0.86$ cfu/ml, $\log 7.33 \pm 0.09 - 8.50 \pm 0.92$ cfu/ml and $\log 7.70 \pm 0.70 - 8.51 \pm 0.09$ cfu/ml, respectively (Tables 2 and 3). Similar results were reported by Kas *et al.* (2013) and Worku *et al.* (2012). Gemechu *et al.* (2014) reported that the total bacterial count was significantly ($P < 0.05$) higher in milk from dairy cooperative milk collection centers and hotels compared to small shops and small-scale producers, and they attributed this 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. These results are slightly lower than those of Rahamtalla *et al.* (2016) who reported a significant ($P < 0.001$) variation in total viable bacteria count from pickup trucks ($\log 9.22 \pm 0.84$ cfu/ml), farms ($\log 9.06 \pm 0.64$ cfu/ml) and vendors on donkey cart ($\log 8.82 \pm 0.84$ cfu/ml), and Edward and Inya (2013) who reported a total heterophilic count of $\log 8.99$ cfu/ml in milk from different locations in Nigeria. Karthikeyan and Pandiyan (2013) reported lower total viable count of

milk samples obtained from local vendors (16×10^4 - 2.71×10^5 cfu/ml), private manufacturers (1.7×10^2 - 2.9×10^4 cfu/ml) and organized dairies (7×10^2 - 4×10^3 cfu/ml). Gwandu *et al.* (2018) outlined that the mean total viable count (TVC) of milk container surfaces was $\log 9.7 \pm 10.5$ cfu/ml, while total coliform count (TCC) was $\log 7.8 \pm 8.5$ cfu/ml. Up to 55.1% of milk had TVC beyond the recommended levels. The average TVC was $\log 11.02 \pm 11.6$ cfu/ml and TCC was 6.7 ± 7.3 log cfu/ml. Up to 26.5% of milk samples had the TCC beyond levels. 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 (Ali *et al.*, 2010; Farougou *et al.*, 2012; Shunda *et al.*, 2013).

The bacteria can enter the milk while it is still in the udder and most microorganisms found in raw milk are contaminants from the udder outer surface, milking utensils or vendors (Farougou *et al.*, 2012). Elzubeir and Ahmed (2007) reported that the higher total bacterial count of 2.6×10^{10} 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. Orregard (2013) reported a significant difference between farmers and agents, and between farmers and shop's milk, while no significant difference was observed between small-scale and large-scale agents' milk in terms of total bacterial counts. Previous results revealed that higher microbial counts for SPC and TCC were observed in chilling centers, transportation vessels, and farmers when compared to the standards.

Table 1. Microbiological quality (cfu/ml) of raw milk collected from different milk sources (means \pm SD).

Source of milk	Total viable bacteria	Total coliform bacteria	<i>S. aureus</i>
Dairy farms	8.01 \pm 0.307 ^a	5.96 \pm 0.238 ^a	5.99 \pm 0.190 ^a
Milk vending shops	7.99 \pm 0.275 ^a	5.95 \pm 0.165 ^a	5.98 \pm 0.477 ^a
Pickup trucks	7.93 \pm 0.337 ^a	5.89 \pm 0.110 ^a	5.84 \pm 0.131 ^a
Vendors on donkey cart	8.09 \pm 0.270 ^a	6.01 \pm 0.189 ^a	6.12 \pm 0.404 ^a
CV (%)	20.02	23.79	21.12
SL	NS	NS	NS

Means in each column bearing similar superscripts are not significantly different ($P > 0.05$)

NS = Not significant

SL = Significance level

SD = Standard deviation

CV = Coefficient of variation

Table 2. Microbiological quality (cfu/ml) of raw milk samples collected from dairy farms and vending shops (mean \pm SD).

Sample No.	Milk distribution channels					
	Dairy Farms			Vending Shops		
	Total viable bacteria	Total coliform bacteria	<i>S. aureus</i>	Total viable bacteria	Coliform bacteria	<i>S. aureus</i>
1	8.27 \pm 0.24 ^a	ND	6.11 \pm 0.69 ^a	8.17 \pm 0.97 ^{ab}	5.85 \pm 0.75 ^b	5.64 \pm 0.86 ^c
2	8.39 \pm 0.92 ^a	ND	6.13 \pm 0.77 ^a	7.71 \pm 0.88 ^b	6.09 \pm 0.71 ^{ab}	5.71 \pm 0.83 ^c
3	8.37 \pm 0.74 ^a	ND	6.13 \pm 0.68 ^a	8.40 \pm 0.13 ^a	5.95 \pm 0.81 ^b	5.85 \pm 0.74 ^c
4	7.85 \pm 0.04 ^b	ND	5.83 \pm 0.77 ^a	7.89 \pm 0.97 ^b	5.81 \pm 0.84 ^b	5.56 \pm 0.05 ^c
5	7.86 \pm 0.83 ^b	ND	5.83 \pm 0.87 ^a	7.88 \pm 0.65 ^b	5.91 \pm 0.87 ^b	5.82 \pm 0.04 ^c
6	7.34 \pm 0.67 ^c	5.61 \pm 0.90 ^a	5.96 \pm 0.90 ^a	7.76 \pm 0.86 ^b	6.34 \pm 0.81 ^b	6.11 \pm 0.72 ^b
7	8.04 \pm 0.10 ^a	6.08 \pm 0.73 ^a	5.95 \pm 0.88 ^a	8.51 \pm 0.86 ^a	5.89 \pm 0.97 ^b	5.85 \pm 0.74 ^c
8	7.91 \pm 1.60 ^b	6.12 \pm 0.73 ^a	5.95 \pm 0.87 ^a	7.79 \pm 0.82 ^b	5.79 \pm 0.77 ^b	6.14 \pm 0.82 ^b
9	8.00 \pm 1.02 ^a	6.17 \pm 0.70 ^a	6.36 \pm 0.16 ^a	7.96 \pm 0.80 ^b	5.84 \pm 0.80 ^b	7.23 \pm 0.09 ^a
10	8.04 \pm 1.94 ^a	5.82 \pm 0.73 ^a	5.71 \pm 0.89 ^b	7.86 \pm 0.83 ^b	5.99 \pm 0.86 ^b	5.85 \pm 0.75 ^c
SL	*	NS	NS	***	**	*

Means in each column bearing similar superscripts are not significantly different ($P > 0.05$)

The data are means of three replicates ($n = 3$)

*** = $P < 0.001$

** = $P < 0.01$

* = $P < 0.05$

NS = Not significant

SL = Significance level

SD = Standard deviation

Table 3. Microbiological quality (cfu/ml) of raw milk samples collected from pickup trucks and vendors on donkey cart (mean±SD).

Sample No.	Milk distribution channels					
	Pickup trucks			Vendors on donkey cart		
	Total viable bacteria	Total coliform bacteria	<i>S. aureus</i>	Total viable bacteria	Total coliform bacteria	<i>S. aureus</i>
1	7.66±0.72 ^b	5.90±0.81 ^a	5.72±0.64 ^a	8.30±0.92 ^a	5.85±0.02 ^a	5.67±0.82 ^b
2	8.31±0.10 ^a	5.76±0.96 ^a	6.19±1.00 ^a	7.70±0.70 ^b	5.97±0.79 ^a	5.73±0.86 ^b
3	8.19±1.00 ^a	5.77±0.80 ^a	5.83±0.72 ^a	7.82±0.73 ^{ab}	6.22±0.74 ^a	6.43±0.21 ^{ab}
4	8.50±0.92 ^a	5.90±0.82 ^a	5.86±0.68 ^a	7.95±0.88 ^{ab}	6.13±0.65 ^a	6.21±0.91 ^{ab}
5	7.93±0.77 ^{ab}	5.95±0.81 ^a	5.84±0.80 ^a	8.51±0.09 ^a	6.11±0.87 ^a	5.98±0.93 ^b
6	7.87±0.64 ^{ab}	5.96±0.02 ^a	5.75±0.77 ^a	8.33±1.00 ^a	6.00±0.99 ^a	7.04±0.59 ^a
7	7.94±0.62 ^{ab}	6.12±0.67 ^a	5.82±0.04 ^a	8.18±0.07 ^a	5.59±1.00 ^a	5.99±0.84 ^b
8	7.33±0.09 ^b	5.82±0.78 ^a	5.76±0.10 ^a	7.86±0.90 ^{ab}	5.93±0.77 ^a	5.78±0.74 ^b
9	7.74±0.71 ^b	5.91±0.75 ^a	5.82±0.10 ^a	8.33±0.16 ^a	6.11±0.77 ^a	6.22±0.75 ^{ab}
10	7.81±0.75 ^{ab}	5.79±0.83 ^a	5.79±0.77 ^a	7.96±0.87 ^{ab}	6.20±0.77 ^a	6.18±0.75 ^{ab}
SL	***	NS	NS	**	NS	*

Means in each column bearing similar superscripts are not significantly different ($P > 0.05$).

The data are means of three replicates ($n = 3$)

*** = $P < 0.001$

** = $P < 0.01$

* = $P < 0.05$

NS = Not significant

SL = Significance level

SD = Standard deviation

The SPC and TCC showed a significant difference ($P < 0.05$) between low and high-risk chilling centers, low and high-risk farmers, chilling centers and transportation vessels and chilling centers and farmers (Senarath and Adikari, 2017). The mean total bacterial count of raw milk samples collected from the local producers, collectors, and dairy markets was $5 \times 10^3 - 3.18 \times 10^8$ cfu/ml (Tekilegiorgis, 2018).

The TCC of milk from different sources indicated a non-significant variation ($P > 0.05$), although the higher count was in milk from vendors on donkey cart ($\log 6.01 \pm 0.189$ cfu/ml), and the lower count was in milk from pickup trucks ($\log 5.89 \pm 0.110$ cfu/ml) (Table 1). Coliform bacteria were not detected in some samples (samples 1-5), and in other samples, the count ranged between $\log 5.61 \pm 1.90$ and $\log 6.17 \pm 0.70$ cfu/ml in milk from dairy farms, while in milk from vending shops the count was $\log 5.81 \pm 0.84 - 6.34 \pm 0.81$ cfu/ml, and in samples from pickup trucks and vendors on the donkey cart, the count was $\log 5.76 \pm 0.96 - 6.12 \pm 0.67$ cfu/ml and $\log 5.59 \pm 1.00 - 6.22 \pm 0.74$ cfu/ml respectively (Tables 2 and 3). Mohamed *et al.* (2017) found a lower mean value of coliform counts ($\log 3.91$ cfu/ml) in raw milk collected from farmers and dairy producers in Djibouti, which was higher than the maximum

recommended value. The results in this study are in disagreement with these of Rahamtalla *et al.* (2016) who reported that coliform bacteria count was significantly higher ($P < 0.001$) in milk from pickup trucks followed by farms and vendors on the donkey cart. Fresh milk collected from different sources is heavily contaminated and possible reasons for this could be due to infected udders, unhygienic procedures or equipment and/or inferior microbiological quantity of water used for cleaning utensils and animals as well as milk storage conditions (Ali *et al.*, 2010).

The presence of high numbers of coliforms in milk provides an index of the hygienic standard used in the production of milk or unclean udder and teats can contribute to the presence of coliforms from a variety of sources such as manure, soil, feed, personnel and even water (Farougou *et al.*, 2012; Hadrya *et al.*, 2012). Orregard (2013) stated that farmers' milk had significantly ($P < 0.001$) lower coliform counts than agents' milk and milk from shops implying that farmers' milk is of better quality. Karthikeyan and Pandiyan (2013) reported that coliform bacteria count was higher in milk from local vendors ($3 \times 10^2 - 2.9 \times 10^3$ cfu/ml) compared to private manufactures ($1 \times 10^2 - 6.0 \times 10^2$ cfu/ml) and organized dairies

(1.0×10^2 - 3.0×10^2 cfu/ml). Gemechu *et al.* (2014) reported that coliform bacteria count was significantly higher in milk from dairy cooperatives compared to hotels, small shops and small-scale milk producers. Singh and Shankar (2017) reported that the coliform count of samples from milk vendors was $\log 3.33$ - 4.65 cfu/ml, with a mean of $\log 4.01 \pm 0.1413$ cfu/ml, while the count of milk samples from vending shops was $\log 3.46$ - 5.39 cfu/ml, with a mean of $\log 4.36 \pm 0.180$ cfu/ml. The total coliform count of raw milk samples collected from the local producers, collectors, and dairy markets were 1.81×10^2 - 3.08×10^6 cfu/ml (Tekilegiorgis, 2018).

Staphylococcus aureus count was high ($\log 6.12 \pm 0.404$ cfu/ml) in milk from vendors on donkey cart, followed by dairy farms ($\log 5.99 \pm 0.190$ cfu/ml), milk vending shops ($\log 5.98 \pm 0.477$ cfu/ml) and pickup trucks ($\log 5.84 \pm 0.131$ cfu/ml) although the difference was not significant (Table 1). *S. aureus* count ranged between $\log 5.71 \pm 0.89$ cfu/ml and $\log 6.36 \pm 0.16$ cfu/ml in dairy farms, while in milk from vending shops, pickup trucks and vendors on donkey cart the count was $\log 5.56 \pm 0.05$ - 7.23 ± 0.09 cfu/ml, $\log 5.72 \pm 0.64$ - 6.19 ± 1.00 cfu/ml and $\log 5.67 \pm 0.82$ - 7.04 ± 0.59 cfu/ml, respectively (Tables 2 and 3).

Table 4. Number of isolates and prevalence (%) of different bacterial species in raw milk collected from dairy farms and vending shops.

Bacterial species	Milk distribution channels			
	Dairy Farms		Vending Shops	
	Number of isolates	Prevalence (%)	Number of isolates	Prevalence (%)
<i>Staphylococcus aureus</i>	36	60	17	26.2
<i>Bacillus</i> spp.	0	0	17	26.2
<i>Escherichia coli</i>	9	15	15	23.0
<i>Klebsiella</i> spp.	0	0	14	21.5
<i>Streptococcus epidermidis</i>	15	25	2	3.1
Total	60	100	65	100

The results in this study are higher than those of Khan *et al.* (2008) who reported staphylococcal count of $\log 2.812$ - 2.988 cfu/ml in raw farm milk, Hadrya *et al.* (2012) who reported an average *S. aureus* count of 1.4×10^5 cfu/ml in informally marketed raw milk, and slightly lower than those of Ali *et al.* (2010) who

reported *S. aureus* count of $\log 7.08$ cfu/ml in raw milk collected from Khartoum North city, Sudan. Rahamtalla *et al.* (2016) reported that *S. aureus* count was significantly ($P < 0.001$) higher in milk from pickup trucks, followed by milk from vendors on the donkey cart and milk from farms. Kas *et al.* (2013) reported a count of 3.0×10^2 - 6.5×10^3 cfu/ml in raw cow's milk. Orregard (2013) reported that *S. aureus* count in the shops' milk was significantly ($P < 0.001$) higher than milk from farmers, and there was no significant difference in the count between the milk of small-scale and large-scale agents, neither was there any significant difference between agents' and shops' milk. *S. aureus* is recognized as a causative agent of clinical and subclinical mastitis (Farougou *et al.*, 2012), and the presence of *S. aureus* in milk is related to environmental conditions and the tropical climate seriously disposes animals to infection by the pathogen *E. coli* (Afif *et al.*, 2008; Fook *et al.*, 2004). Out of 160 raw milk samples collected from 80 dairy herds in Northern China, 52.50% were *S. aureus* positive, and that the prevalence of *S. aureus* was influenced by season, herd size, milking frequency and disinfection frequency (Lan *et al.*, 2017). 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 1.6×10^3 to 5.1×10^4 cfu/mL (Pyz-Lukasik *et al.*, 2015).

During the study, several bacteria were isolated from milk of different sources. In addition to *S. aureus*, *Streptococcus epidermidis* (25%) and *E. coli* (15%) were isolated from milk from farms, while *Klebsiella* spp. (22%), *S. epidermidis* (16%), *Salmonella paratyphi* (4%) and *Citrobacter diversus* (2%) were isolated from milk of vending shops, *Bacillus* spp. (26.2%), *Klebsiella* spp. (21.5%) and *S. epidermidis* (3.1%) were isolated from milk of pickup trucks, and *S. epidermidis* (6.2%), *Klebsiella* spp. (6.2%), *Pseudomonas aeruginosa* (13.8%) and *Bacillus* spp. (43.0%) were isolated from milk of vendors on donkey cart (Tables 4 and 5). Shunda *et al.* (2013) isolated *S. aureus* from dairy farms (13.3%), milk vending shops (4.4%) and houses and cafeterias (8.9%), while *Streptococcus* spp. were isolated in

percentages of 8.9%, 11.1%, and 6.7% respectively, and *E. coli* was isolated in percentages of 11.1%, 11.1%, and 22.2%, respectively. Elzubeir and Ahmed (2007) isolated *S. aureus*, *E. coli* and *Salmonella* spp from bulk raw milk of some dairy farms in Khartoum State.

Table 5. Number of isolates and prevalence (%) of different bacteria species in raw milk collected from pickup trucks and vendors donkey cart.

Bacterial species	Milk distribution channels			
	Pickup trucks		Vendors on donkey cart	
	Number of isolates	Prevalence (%)	Number of isolates	Prevalence (%)
<i>S. aureus</i>	10	15.4	14	28
<i>E. coli</i>	10	15.4	14	28
<i>Streptococcus epidermidis</i>	4	6.2	8	16
<i>Klebsiella</i> spp.	4	6.2	11	22
<i>P. aeruginosa</i>	9	13.8	0	0
<i>Salmonella paratyphi</i>	0	0	2	4
<i>Citrobacter diversus</i>	0	0	1	2
<i>Bacillus</i> spp.	28	43.0	0	0
Total	65	100	50	100

Mubarak *et al.* (2010) reported that the dominant flora associated with raw milk in Coimbatore district of India was in the order of *Lactobacillus* spp. > *S. aureus* > *E. coli* > *Bacillus* spp. > *Pseudomonas fluorescens* > *Salmonella* spp. They added that the presence of these bacteria in milk is suggestive of contamination from various sources such as animal, human, environment, utensils, and others. Adjlane-Kaoucke *et al.* (2014) stated that *S. aureus* was detected in 33% of the samples at the end of the collection period. Salman and Hamad (2011) isolated and identified different species of coliforms from vendor and market milk of Khartoum State, Sudan which included *E. coli* (32%), *Enterobacter cloacae* (13.6%) and *Enterobacter aerogenes* (11.3%). Edward and Inya (2013) reported that among the organisms isolated from raw milk *E. coli*, *S. aureus*, and *Streptococcus* ssp. had a 100% occurrence being present in all samples tested. Worku *et al.* (2012) isolated *E. coli* (12.89%), *Klebsiella pneumoniae* (6.67%), *Klebsiella oxytoca* (5.33%), *S. aureus* (6.78%), *Pseudomonas aeruginosa* (2.67%), *Salmonella typhi* (6%), *Salmonella typhimurium* (5%) and *Citrobacter diversus* (2.78%) from milk drawn directly from cow udder. Msalya (2017) isolated ten groups or species of bacteria from raw milk, and these were *E. coli*, *Salmonella* spp.,

Klebsiella spp. and *Proteus* spp. (four groups of bacteria not identified to species level) in addition to six species including *P. aeruginosa*, *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *S. aureus*, and *B. abortus*. Hassan *et al.* (2015) isolated *E. coli*, *S. aureus*, *Bacillus cereus*, *E. coli* O157:H7 and *Salmonella* spp. from raw market milk samples collected randomly from different supermarkets and retailer shops in Cairo and Giza governorates.

Conclusion

Milk from pickup trucks was the best having low total bacteria, total coliform bacteria and *S. aureus* counts. The high count of *S. aureus* from all sources which is higher than the standards indicates that the milk is of low quality and may be hazardous to the consumers. In addition, the isolation of pathogenic bacteria such as *S. aureus*, *E. coli*, *Klebsiella* spp., *Salmonella typhimurium*, *Bacillus* spp. also indicates that the milk is produced and distributed under unhygienic conditions, 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

- Abdalla MOM, Elhagaz FMM.** 2011. The Impact of applying some hygienic practices on raw milk quality in Khartoum State, Sudan. *Research Journal of Agriculture and Biological Sciences* 7, 169-173.
- Adjlane-Kaouche S, Benhacine R, Ghozlane F, Mati A.** 2014. Nutritional and hygienic quality of raw milk in the mid-Northern region of Algeria: Correlations and risk factors. *The Scientific World Journal*, Volume 2014, Article ID 131593, 7 pages.
- Afif A, Farid M, Chigr F, Najimi M.** 2008. Survey of the microbiological quality of the raw cow milk in the Tadla area of Morocco. *International Journal of Dairy Technology* 61(4), 340-346.
- Ali AA, Irshad NB, Razaz SA Manahil AA.** 2010. Microbiology safety of raw milk in Khartoum State, Sudan: 1-Khartoum and Omdurman Cities. *Pakistan Journal of Nutrition* 9(5), 426-429.

- Barrow GL, Feltham KA.** 1993. Cowan and Steel's Manual for the identification of Medical Bacteria, 3rd Edn, Cambridge, UK: Cambridge University Press, 21-48.
- Cheesbrough M.** 2006. District Laboratory Practice in Tropical Countries, Second Edition, Part 2. Cambridge, UK: Cambridge University Press, 45-70.
- Coorevits A, De Jonghe V, Vandroemme J, Reekmans R, Heyrman J, Messens W, De Vos P, Heyndrickx M.** 2008. Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms. *Systematic and Applied Microbiology* 31(2), 126-140.
- De Silvaa SASD, Kanugalab KANP, Weerakkody NS.** 2016. Microbiological quality of raw milk and effect on quality by implementing good management practices. *Procedia Food Science* 6, 92-96.
- Edward KC, Inya IM.** 2013. The microbial quality of raw milk from four locations in Abia State, Nigeria. *Journal of pharmacy and Biological Sciences* 5(3), 30-33.
- Elzubeir IEM, Ahmed MIA.** 2007. The hygienic quality of raw milk produced by some dairy farms in Khartoum State, Sudan. *Research Journal of Microbiology* 2(12), 988-991.
- Farougou S, Sessou P, Yehouenou B, Dossa F.** 2012. Microbiological quality of raw milk in processed from cows raised under extensive system in the Republic of Benin. *Research Journal of Microbiology* 7(7), 337-343.
- Fook Y, Chye A, Ayob A, Khan M.** 2004. Bacteriological quality and safety of raw milk in Malaysia. *Food Microbiology* 21(5), 535-541.
- Gemechu T, Beyere F, Eschetu M.** 2014. Handling practices and microbial quality of raw cow's milk produced and marketed in Shashemeno Town, Southern Ethiopia. *International Journal of Agricultural and soil Sciences* 2(9), 153-162.
- Gwandu SH, Nonga HE, Mdegela RH, Katakweba AS, Suleiman TS, Ryoba R.** 2018. Assessment of Raw Cow Milk Quality in Smallholder Dairy Farms in Pemba Island Zanzibar, Tanzania. *Veterinary Medicine International*, Volume 2018, **Article ID 1031726**, 9 pages.
- Hadrya F, Elouardi A, Benali D, Hami H, Soulaymani A, Senouci S.** 2012. Bacterial quality of informally marketed raw milk in Kenitra City, Morocco. *Pakistan Journal of Nutrition* 11(8), 662-669.
- Hassan GM, Meshref AMS, Gomaa SM.** 2015. Microbiological Quality and Safety of Fluid Milk Marketed in Cairo and Giza Governorates. *Current Research in Dairy Sciences* 7(1), 18-25, 2015.
- Houghtby AG, Maturin LJ, Koenig KE.** 1992. Microbiological count methods. In: Marshal RT, Ed. *Standard Methods for the Examination of Dairy Products*, 16th edition. Washington, DC: American Public Health Association, 213-246.
- John MN.** 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.
- Karthikeyan N, Pandiyan C.** 2013. Microbial quality of Khoa and Khoa based milk streets from different sources. *International Food Research Journal* 20(3), 1443-1447.
- Kas K, Megnanou RM, Akpa EE, Assidjo NE, Niamke LS.** 2013. Evaluation of physico-chemical, Nutritional and Microbiological Quality of Raw Cow's milk Usually Consumed in the Central Part of Cote D'Ivoire. *African journal of food, Agriculture, Nutrition and Development* 13(3), 7888-7904.
- Khan MTG, Zinnah MA, Siddique MP, RashidmmA, Islam MA, Choudhury KA.** 2008. Physical and Microbial Qualities of raw milk collected from Bangladesh Agricultural University Dairy farm and the surrounding villages. *Bangladesh Journal of Veterinary Medicine* 6(2), 217-221.

- Lan XY, Zhao SG, Zheng N, Li SL, Zhang YD, Liu HM, McKillip J, Wang JQ.** 2017. *Short communication: Microbiological quality of raw cow milk and its association with herd management practices in Northern China Journal of Dairy Science* **100**, 4294-4299.
- Mohamed AF, Fourreh AE, Okieh AA, Said CN, Mérito A, Yagi S.** 2017. Evaluation of Microbiological Quality of Raw Milk from Farmers and Dairy Producers in Six Districts of Djibouti. *Journal of Food: Microbiology, Safety and Hygiene* **2(3)**, 1-7. 124.
- Mohamed NI, Elzubier IEM.** 2007. Evaluation of the hygienic quality of market milk of Khartoum State (Sudan). *International Journal of Dairy Sciences* **2(1)**, 33-41.
- Msalya G.** 2017. Contamination Levels and Identification of Bacteria in Milk Sampled from Three Regions of Tanzania: Evidence from Literature and Laboratory Analyses. *Veterinary Medicine International*. Volume 2017, **Article ID 9096149**, 10 pages.
- Mubarak HM, Doss A, Dhanabalah R, Balachander S.** 2010. Microbial Quality of raw milk samples collected from different villages of Coimbatore District, Tamilnadu, South India. *Indian Journal of Science and Technology* **3(1)**, 61-63.
- Nwankwo IU, Amaechi N, Adiele WA.** 2015. Microbial Evaluation of Raw Milk from Dairy Farms in Udi L.G.A. Enugu State, Nigeria. *IOSR Journal of Agriculture and Veterinary Science* **8(3)**, 60-65.
- 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.
- Pyz-Lukasik R, Paszkewik W, Tatara MR, Brodzki P, Belkot Z.** 2015. Microbiological quality of milk sold directly from producers to consumers. *Journal of Dairy Science* **98**, 4294-4301.
- Rahamtalla SA, Elsheikh NAH, Abdalla MOM.** 2016. Microbiological Quality of Raw milk produced and distributed in Khartoum State, Sudan. *ARPN Journal of Agricultural and Biological Science* **11(1)**, 24-29.
- Ruangwittayanusorna K, Promketa D, Chantiratikul A.** 2016. Monitoring the Hygiene of Raw Milk from Farms to Milk Retailers. *Agriculture and Agricultural Science Procedia* **11**, 95-99.
- Salman AMA, Hamad MI.** 2011. Enumeration and identification of Coliform bacteria from raw milk in Khartoum State, Sudan. *Journal of Cell and Animal Biology* **5(7)**, 121-128.
- Senarath HMIGAMK, Adikari AMJB.** 2017. Microbiological Quality of Raw Milk at Selected Chilling Centers in Anuradhapura District of Sri Lanka. *International Journal of Scientific and Research Publications* **7(11)**, 328-333.
- 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.
- Singh AK, Shankar U.** 2017. Microbiological Study of Raw Milk Collected from Local Milk Vendors of Lucknow District, UP, India. *International Journal of current Microbiology and Applied Science* **6(5)**, 2866-2873.
- Tekilegiorgis T.** 2018. Microbiological Quality Analysis of Raw and Pasteurized Milk Samples Collected from Addis Ababa and Its Surrounding in Ethiopia. *Approaches in Poultry, Dairy and Veterinary Sciences* **4(5)**, 374-381.
- Worku T, Negere E, Nurfeta A, Wellearegay H.** 2012. Microbiology Quality and Safety of Raw Milk Collected from Borana pastoral community, Oremia Regional State. *African Journal of food Science and Technology* **3(9)**, 213-222.