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# Enumeration and characterization of aerobic bacillus species in cow's milk from farms in Khartoum North, Sudan

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## Abstract

This cross-sectional study was conducted with the objectives of determining the loads and types of spore-former bacteria from the genus Bacillus in cow's milk. One hundred samples of raw milk were randomly obtained from 20 dairy farms in Hillat Kuku and El seelait complexes in Khartoum North. Fifty samples from 10 dairy farms from each location were included (3 individual cows and 2 bulk milk). All collected milk samples were examined for enumeration of the spore forming bacteria and isolation and identification of *Bacillus* species. This study revealed that 45% of the examined milk samples showed prevalence of spore-forming bacteria. Moreover, the higher count was found in the bulk milk ($5.2\times10^3$ cfu /ml) compared to the individual milk samples ($3\times10^3$ cfu /ml). The 45 isolates found are belong to *Bacillus* spp. included *B. cereus* (15.5%), *B. pantothenticus* (8.8%), *B. licheniformis* (13.3%), *B. mycoides* (11.1%), *B. coagulans* (37.7%), *B. megaterium* (4.4%) and *B. subtilis* (8.8%). The rate of isolation for *B. cereus* were 24% and 10%, *B. pantothenticus* 8% and 8%, *B. licheniformis* 5% and 20%, *B. mycoides* 8% and 12%, *B. coagulans* 24% and 55%, *B. megaterium* 4% and 4%, and *B. subtilis* 4% and 13% from an individual and bulk samples, respectively. In conclusion, this study showed that almost half of milk samples produced in the dairy farms in the studied areas were harbouring a lot of *Bacillus* species with high load and occurrence in the bulk milk suggesting that extra sources of contamination might arise during storage. Hence the study suggested the need for improved hygienic practices at the farms level. Moreover, for quality assurance, regular control programs have to be applied for quality production and consumption of milk and its products in Sudan.

***Keywords:*** *Bacillus species; dairy farms; enumeration; identification; milk; Spore-forming bacteria.*

## 1. Introduction

The ability of the spore to resist the harsh environmental conditions is of particular concern for survivals of spore-forming bacteria (Logan and De Vos 2009; Postollec et al. 2012). On the dairy farm, spore-forming organisms have been isolated from various sources including soil and teats (Postollec et al. 2012), pasture (Christiansson et al. 1999; Slaghuis et al. 1997); bedding, silage, and feed (Crielly et al. 1994; Te Giffel et al. 2002; Magnusson et al. 2007), fecal material (Huck et al., 2008), and raw milk (Boor et al. 1998; Huck et al. 2007; Martin et al. 2011). The spores are commonly found and associated with the farm environment such as soil, bedding material, feces and feed (Eneroth et al., 2001; Vissers and Driehuis 2007; Simoes et al. 2010; Postollec et al. 2012). The spores found in the feces and bedding material are transmitted to milk from the teats surface during milking (Te Giffel et al. 2002). The exterior and interior of the cow's teats are the major routes for transmutation of spores into the milk, in addition to milking equipment contaminated surfaces (Vissers and Driehuis 2007). Moreover, the ability of survival of aerobic spore-forming bacteria during the industrial pasteurization and formation of biofilms within the pipelines and stainless-steel equipment were reported for the genus Bacillus (Gopal et al. 2015). Because the spore forming bacteria are heat resistance organisms that able to survive and germinate in the raw milk and heat-treated dairy products resulting in deterioration of the dairy products quality due to their proteolytic and lipolytic enzymes that are produced at low temperature in the fluid milk (Nassib et al. 2018).

Raw milk and the farm environment may contain spore counts up to $10^4$ cfu /ml and are regarded as primary sources of milk contamination (Coorevits et al. 2008). Members of *B. cereus*, *B. subtilis* and *B. licheniformis* are commonly present in milk (Bartoszewicz et al. 2008). Moreover, their species are most commonly isolated from raw milk (Crielly et al. 1994). The alimentary diseases caused by *B. cereus* could be due to the psychrophilic nature of the organism and its ability to grow in the dairy products during the cold storage (Christiansson 1992). However, *B. licheniformis*, *B. subtilis* and *B. pumilus* are mesophilic. The health hazards associated with most of *B. cereus* and *B. licheniformis* strains isolated from milk and other food is mainly because of their pathogenic properties (Mikkola et al. 2000; De Clerck and De Vos 2004; Nieminen et al. 2007).

Because of the survival of the Bacillus spores to the procedures of thermal inactivation, longer time and temperature are needed to achieve the required sterilization (Scheldeman et al. 2006; Schubert and Beaudet 2011). Moreover, the numbers of thermoduric bacterial on both teats and milk could be minimised via washing, drying with individual paper towels and disinfecting of the teat before milking (Gleeson et al. 2013).

In Sudan, the studies conducted on milk quality revealed low level of hygiene and the quality control measures were not satisfactory (Yagoub et al. 2005; El Zubeir and Ahmed 2007; Mohamed and El Zubeir 2007; Warsma et al. 2020). As some problems are arising from aerobic spore-forming bacilli in milk chain (Bellow et al. 2007). Therefore, the objectives of conducting this study are enumeration, isolation and phenotypic characterization of aerobic spores from the genus Bacillus from cow milk in Khartoum North.

## 2. Materials and methods

### Area of study

Hillat Kuku and Elseelait complexes comprising the largest dairy production complexes in Khartoum North, Sudan were chosen to conduct the present study.

### Study design

This study is cross-sectional study which contains two types of samples: individual cow and bulk tank milk. The study design was based on determining the bacteriological quality of milk and its safety in some dairy farms located in Khartoum North.

### Sources and sampling of the milk

One hundred samples of raw milk were randomly collected from 20 dairy farms located in Hillat Kuku and El seelait complexes, Khartoum North; 10 dairy farms in each area and 5 samples from each farm. All the 100 samples were divided into two types: individual cow samples and bulk tank samples (3 individual cows and 2 bulk tanks). Sixty individual cows' samples were collected under aseptic condition by disinfecting the teat area with a piece of cotton containing 70% alcohol, 40 bulk tank samples were collected from the last container which sold to the consumers. Both types of samples were collected in sterile container (about 40 ml) and kept in an ice box and were transported to the University of Khartoum, Faculty of Public and Environmental Health, Department of Food Hygiene and Safety laboratory, where the bacteriological examination was conducted.

Microbiological analysis was held in the Laboratory of Microbiology over a period of three months.

### Culture media

Nutrient agar (Micromaster, DM180), Standard plate count agar (Mast, DM195 D), Mannitol- egg yolk polymyxin agar (Scharlau, 01-262), Simmons citrate medium (Scharlau, 01-177), urea agar base medium (Scharlau, 01-261), nutrient broth medium (Scharlau, 02-144), starch medium (SDFCL, 40264 k05), ammonium salt sugars medium were prepared as described by the instructions of manufacturers'.

### Sterilization

Sterilization was done according to Harrigan and McCane (1976), Sterilization using dry heat was done for glass wares in the hot air by oven at 180° C for one hour. Moist heat was used for sterilization of all culture media, whereas autoclave (121° C for 15 minutes) was used to sterilize the solutions. Solution of 70% alcohol was used for sterilization of benches. Sterilization by flame was used for sterilization of loops and mouth of bottles. For sterilization of the udder and teats, 70% alcohol solution was used.

### Enumeration of total spore count

Under aseptic conditions, the samples of both raw bulk and individual cows' milk were taken from the selected dairy farms using sterile containers. The samples were labelled and brought to the laboratory using an ice box. All the milk samples were prepared for enumeration of total spore-forming bacteria as was outlined by Harrigan and McCane (1976). About 10 ml of each raw milk sample was aseptically taken into a sterile test tube. Then the tubes were placed in a water bath for boiling the milk (80° C for 10 minutes) and immediately cooled using cold water. After that the serial dilutions from each sample were performed by taken 1 ml from treated sample and placed in 9 ml normal saline solution and shacked well. This operation was repeated to make tenfold dilutions from $10^{-1}$ – $10^{-6}$ (Richardson, 1985). Then the total spore count was done by placing 0.2 ml of selected serial dilutions on the surface of the poured standard plate count agar medium after it was dried at 30°C in an oven (Miles et al. 1938). The plates were brought to an incubator (37° C) for 24 hours. In the next day, after the morphology of the developed colonies was observed, the plates were counted by taking the average numbers of the appropriate colonies. The obtained numbers were multiplied by the reciprocal number of the diluting factors and the results were presented as cfu/ ml.

The primary identification was done by Gram stain to examine the cell morphology and the presence of spores and identification of their position and shape (Bartholomew 1962). All Gram-positive bacilli were selected for culturing on nutrient agar plates that incubated for 24 hours at 37° C. Then the cultures were kept in refrigerator for conducting the biochemical tests.

All isolates were cultured in Mannitol– egg yolk polymyxin (MEYP) agar that incubated at 37° C for 24 hours. The plates were checked for lecithinase production and if a zone of precipitation surrounding the growth, it indicates positive result. If the resulted growth and its surrounding medium are eosin pink, the mannitol is not fermented by isolate. However, when a yellow color was developed it indicates that acid is produced from mannitol.

Presumptive *B.* cereus was rough dry colonies with pink base surrounded by a zone of egg yolk precipitation, and usually lecithinase-positive and mannitol-negative. The presumptive *B.* cereus was then confirmed with appropriate biochemical tests.

All biochemical tests were done according to Barrow and Feltham (1993).

Catalase test, Oxidase production test, motility test, anaerobic growth, citrate utilization test, urease activity test, test for growth in 10% NaCl, Indole production, Voges –Proskauer test, growth at 50°C, starch hydrolysis test and casein hydrolysis test were done according to Barrow and Feltham (1993)

**Statistical analysis**

The Statistical Package for Social Science version 15 (SPSS 15.0) was used for the analysis of the present data. The variations in the bacterial spore counts in the milk samples as affected by locations and sources were calculated using oneway ANOVA test. Also, the least significant differences were done for means separation ($P<0.05$).

## 3. Results

### Enumeration of the total aerobic spore count

The total aerobic spore count was ranged between $5\times10^2$ cfu/ml to $1\times10^4$ cfu/ ml for the individual cow's milk samples collected from Hillat Kuku area, and the mean was $3\times10^3$ cfu/ml. Whereas aerobic spore count in El seelait area ranged between $5\times10^2$ cfu/ml to $15\times10^3$ cfu/ml, while the average mean was $3\times10^3$ cfu/ml. The total aerobic spore count revealed an overall average mean for two locations was $3\times10^3$ cfu/ml (Table 1).

**Table (1):** Comparison of total spore count of an individual and bulk milk samples collected from dairy farms in Hillat Kuku and El seelait complexes

| Locations | Source | | | | | | Level of significance |
|---|---|---|---|---|---|---|---|
| | Individual milk | | | Bulk milk | | | |
| | Total spore count (cfu/ml) | | | Total spore count (cfu/ml) | | | |
| | Mean± SD | Min. | Max. | Mean± SD | Min. | Max. | |
| Hillat Kuku | $3\times10^3 \pm 1.4\times10^3$ | $5\times10^2$ | $1\times10^4$ | $6.5\times10^3 \pm 3.8\times10^3$ | $5\times10^2$ | $45\times10^3$ | NS |
| El seelait | $3\times10^3 \pm 1.6\times10^3$ | $5\times10^2$ | $15\times10^3$ | $4\times10^3 \pm 4.2\times10^3$ | $5\times10^2$ | $5\times10^3$ | NS |
| Total | $3\times10^3 \pm 4\times10^3$ | $5\times10^2$ | $15\times10^3$ | $5.3\times10^3 \pm 11\times10^3$ | $5\times10^2$ | $45\times10^3$ | NS |
| Over all mean | $3\times10^3 \pm 1\times10^3$ | | | $5.2\times10^3 \pm 3\times10^3$ | | | NS |

NS= not significant ($P>0.05$)
cfu = colony forming unit
SD= Standard deviation
Min. = Minimum
Max. = Maximum

Aerobic spore count obtained from bulk tank samples was ranged between $5\times10^2$ cfu/ml to $45\times10^3$ cfu/ml with a mean of $6.5\times10^3$ cfu/ml in Hillat Kuku area. The result obtained from El seelait area ranged between $5\times10^2$ cfu/ml to $5.5\times10^3$ cfu/ml, the mean was $4\times10^3$ cfu/ml, whereas the overall mean was $5.2\times10^3$ cfu/ml (Table 1).

**Isolation and identification of *Bacillus* species**

A total of forty-five isolates of *Bacillus* species were isolated, they include *B. cereus* (15.5%), *B. pantothenticus* (8.8%), *B. licheniformis* (13.3%), *B. mycoides* (11.1%), *B. coagulans* (37.7%), *B. megaterium* (4.4%) and *B. subtilis* (8.8%) as shown in Table 2.

**Table (2):** Frequencies of *Bacillus* species in milk samples collected from different farms in El seelait and Hillat Kuku

| Areas / *Bacillus* spp. | El seelait | | Hillat Kuku | | Total |
|---|---|---|---|---|---|
| | N | Percent | N | Percent | |
| *B. cereus* | 1 | (4%) | 6 | (30%) | 7 (15.5%) |
| *B. pantothenticus* | 4 | (16%) | 0 | (0%) | 4 (8.8%) |
| *B. licheniformis* | 5 | (20%) | 1 | (5%) | 6 (13.3%) |
| *B. mycoides* | 5 | (20%) | 0 | (0%) | 5 (11.1%) |
| *B. coagulans* | 6 | (24%) | 11 | (55%) | 17 (37.7%) |
| *B. megaterium* | 1 | (4%) | 1 | (5%) | 2 (4.4%) |
| *B. subtilis* | 3 | (12%) | 1 | (5%) | 4 (8.8%) |

N= numbers of samples

*Bacillus* species from individual samples included *B. cereus;* 4 (20%) and 2 (8%), *B. pantothenticus;* 0 (0%) and 2 (8%), *B. licheniformis;* 1 (5%) and 0 (0%), *B. mycoides*; 0 (0%) and 2 (8%), *B. coagulans;* 4 (20%) and 1 (4%), *B. megaterium;* 0 (0%) and 1 (4%), and *B. subtilis;* 0 (0%) and 1 (4%). Whereas *Bacillus* species from bulk samples included *B. cereus;* 2 (10%) and 0 (0%), *B. pantothenticus;* 0 (0%) and 2 (8%), *B.*

*licheniformis;* 0 (0%) and 5 (20%), *B. mycoides;* 0 (0%) and 3 (12%), *B. coagulans;* 7 (35%) and 5 (20%), *B. megaterium;* 0 (0%) and 1 (4%), and *B. subtilis;* 1 (5%) and 2 (8%) as shown in Table 3.

**Table (3):** Frequencies of *Bacillus* species in milk samples collected from an individual and bulk milk samples

| Source / *Bacillus spp.* | Individual milk | | | | | Bulk milk | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hillat Kuku | | El seelait | | | Hillat Kuku | | El seelait | | |
| | N | % | N | % | Total | N | % | N | % | Total |
| *B. cereu* | 4 | 20 % | 1 | 4 % | 24% | 2 | 10 % | 0 | 0% | 10% |
| *B. pantothenticus* | 0 | 0% | 2 | 8 % | 8% | 0 | 0 % | 2 | 8% | 8% |
| *B. licheniformis* | 1 | 5% | 0 | 0 % | 5% | 0 | 0 % | 5 | 20 % | 20% |
| *B. mycoides* | 0 | 0% | 2 | 8 % | 8% | 0 | 0 % | 3 | 12 % | 12% |
| *B. coagulans* | 4 | 20 % | 1 | 4 % | 24% | 7 | 35 % | 5 | 20 % | 55% |
| *B. megaterium* | 0 | 0% | 1 | 4 % | 4% | 0 | 0 % | 1 | 4% | 4% |
| *B. subtilis* | 0 | 0% | 1 | 4 % | 4% | 1 | 5 % | 2 | 8% | 13% |

All those isolates were bacilli in shape, positive for Gram stain and catalase test, while the oxidase reaction and indole production were negative. Moreover, the majority of the isolates were citrate, urease, and Voges Proskauer (VP) positive and able to grow at 50°C and anaerobic growth. Also, variable results were obtained for tolerating 10% NaCl, casein hydrolysis and starch hydrolysis. The biochemical tests of the *Bacillus* spp. results were summarized in Table 4. Seven isolates showed the typical *B. cereus* colonial morphology on MEYP agar.

**Table (4):** Biochemical tests used for the identification of *Bacillus* species isolated from raw milk

| Tests | *Bacillus* spp. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Gram stain | + Rod | + Rod | + Ro d | + Rod | + Rod | + Rod | + Rod |
| Chain of cells | + | +/- | +/- | + | +/- | +/- | + |
| Motility | + | + | + | - | + | +/- | + |
| Catalase | + | + | + | + | + | + | + |
| Oxidase | +/- | - | - | +/- | +/- | - | - |
| Growth at 50°C | - | + | + | - | + | + | - |
| Growth in 10% NaCl | +/- | - | - | +/- | + | +/- | - |
| Growth in 10% NaCl | +/- | - | - | +/- | + | +/- | - |
| Anaerobic growth | + | + | + | + | + | - | - |
| Utilization of citrate | +/- | +/- | + | +/- | - | + | + |
| Urease | - | - | +/- | +/- | - | - | +/- |
| Indole | - | - | - | - | - | - | - |
| VP | + | + | +/- | + | +/- | + | - |
| Casein hydrolysis | + | + | + | + | + | + | + |
| Starch hydrolysis | + | + | + | + | - | - | + |
| Glucose | + | + | + | + | + | + | + |
| Xylose | - | - | + | - | - | +/- | + |
| Mannitol | - | +/- | + | - | - | + | +/- |

1. *Bacillus cereus*
2. *Bacillus coagulans*
3. *Bacillus lincheniformis*
4. *Bacillus mycoides*
5. *Bacillus pantothenticus*
6. *Bacillus subtilis*
7. *Bacillus megaterium*

## 4. Discussion

In the present study, the microbiological safety of raw milk that produced in Hillat Kuku and El seelait complexes was determined by enumeration of aerobic spore-forming bacilli that might represent public health concern in the dairy industry. The aerobic spore count for milk samples collected from individual cows in Hillat Kuku and El seelait complexes revealed $5\times10^2$-$1\times10^4$ cfu/ml and $5\times10^2$-$15\times10^3$ cfu/ml, respectively. The data in Table 1 showed that the average mean for total spore count was high in bulk milk samples ($5.2\times10^3\pm3\times10^3$ cfu/ml) compared to the individual milk samples ($3\times10^3\pm1\times10^3$ cfu/ml). The higher maximum level of spore forming bacteria in the individual samples might be the reason for elevating the bulk milk samples. The high counts of aerobic spore-forming bacteria associated with raw milk increased the possibility of spoiling the final product and hence reducing its shelf life (Yacoub et al. 2017). The results revealed the same mean value ($3\times10^3$ cfu/ml) for the total aerobic spore count of milk samples collected from two study areas (Table 1). This might indicate the similarity of farming systems, environmental conditions (less hygienic conditions in the environment and during milking procedures). Vissers et al. (2007) estimated and compared the contaminated teats with soil versus feed as sources of contamination of tank milk; they found that 33% vs. 2% of the samples contain more than 1000 spores/L, respectively.

The values for aerobic spore– forming bacilli found in the bulk milk samples ranged from $5\times10^2$ to $45\times10^3$ cfu /ml in the milk samples collected from Hillat Kuku dairy complex. Similarly, the samples obtained from Elseelait dairy complex were found to range from $5\times10^2$ to $5.5\times10^3$ cfu /ml. The overall mean revealed $5.2\times10^3$ cfu /ml (Table 1). Similarly, Khater and Abdella (2017) reported that the minimum, maximum and the means of total aerobic spore formers counts in raw milk samples were $3.2\times10^3$, $2.7\times10^4$ and $1.5\times10^4$ cfu/ml, respectively Egypt. These results indicate less hygienic quality during milk procedure, since neither washing of udder or teats prior to milking was not a common practice as was reported previously (Ahmed and El Zubeir 2013a; Mohamed and El Zubeir 2015). Poor environmental sanitation and milking was usually done under poor hygienic condition (Mohamed et al. 2014; Ahmed and El Zubeir 2013b). They added that the general hygiene in most of the dairy farms in Khartoum State was poor due to the absence of farms cleaning and sanitation programs and practices. This situation will enable the spores to transfer from the soil, feed and feces of the cows and its bedding material to contaminate the milk (Crielly et al. 1994; Te Giffel et al. 2002; Magnusson et al. 2007; Postollec et al. 2012). Moreover, Vissers and Driehuis (2007) reported that when feces or bedding materials contaminate the cow's teats, the cleaning of the teat prior to milking can result in partial reduction of the attached dirt and spores. The present findings showed that the mean was relatively higher than the mean obtained from Hillat Kuku than those obtain from El seelait area (Table 1). Holm et al. (2004) reported similar results when examining the microflora of downgraded Danish bulk tank raw milk to identify the main causes of increased microbial counts. Their results were $5.2\times10^3$ cfu /ml for *Bacillus* spp. Also, in Estonia, Stulova et al. (2010) investigated the farm raw milk from bulk tank for the microbial quality during 2004-2007, they reported that the aerobic spore forming bacterial count were $5.2\times10^3$ cfu /ml during the period from April to May 2004. Scheldeman et al. (2005) also reported $5.46\times10^3$ cfu $ml^{-1}$ for average spore count in the raw milk which was tested from Belgian dairy farms. Sutherland and Murdoch (1994) stated that the occurrence of highly contaminated raw milk samples with *Bacillus* spores might reach up to $10^3$ cfu $ml^{-1}$. However, El Zbueir and Ahmed (2007) obtained higher mean count of milk samples from some selected dairy farms in Khartoum State ($6.0\times10^3$ -$3\times10^9$ cfu ml $^{-1}$) during summer. Also Mohamed and El Zubeir (2007) reported that $7.15\times10^6 \pm 1.79\times10^7$ cfu $ml^{-1}$ for spore-forming bacterial count during summer.

Forty-five *Bacillus* spp. isolates were identified (Table 2), they include *B. cereus* (15.5%), *B. pantothenticus* (8.8%), *B. licheniformis* (13.3%), *B. mycoides* (11.1%), *B. coagulans* (37.7%), *B. megaterium* (4.4%), *B. subtilis* (8.8%). Similarly, Crielly et al. (1994); Sutherland and Murdoch (1994) identified *Bacillus* spp. from the milk of the dairy farms. This result showed broad diversity of *Bacillus spp*. in the examined milk samples, the predominant organism in the raw milk in the two studied areas was *B. coagulans*. The bacteriological quality and safety of milk is not only affected by the bacterial count, but also by type and strain of the bacteria. The presence of these organisms might result from excretion from infected udder of an animal (Oliver et al. 2005). These results also supported Aouadhi et al. (2014) who observed that *Bacillus* species occurred in 47.5% of the raw milk in the dairy farms in Tunisia included *B. cereus* (22.5%), *B. licheniformis* (12.5%) and *B. subtilis* (12.5%). However, Warsma et al. (2020) assessed the milk quality status in Khartoum State and found 4.4% of *B. cereus*, 6.6% of *B. licheniformis*, 0.9% of *B. pumilus*, 0.9% of *B. sphaericus*, 0.7% of *B. amyloiquefaciewns* and 0.7% of *Bacillus coaguln*s. The occurrence of food borne causative agents in milk is mainly because of the direct contact between the contaminated sources in the environment of dairy farm as well as the udder excretion of diseased animal (Oliver et al. 2005; El Zubeir et al. 2006). Usually the spore forming bacteria are found in water, silage, bedding and manure as well as paper towels (Khater and Abdella 2017). They exist in a dormant state as spores, which can survive many of the unfavorable conditions such as high heat (e. g., pasteurization), drying, acidity and radiation.

*B. coagulans* was the most frequently isolates (37.7%) followed by *B. cereus* (15.5%), in particular *Bacillus cereus* is of major concern due to its risk as hazard for public health. The important point is that *Bacillus coagulans* is a known thermo-tolerant organism in addition to its high endospores resistance. *Bacillus coagulans* is also of great economic importance due to its food spoilage capacity (De Clerck et al. 2004). Moreover both safety and quality of milk are found to be affected by the presence of aerobic spore-formers as the spores generated by these bacteria are resistant to the applied heat treatments and subsequently germinate into vegetative cell form, spoiling milk via the production of deteriorating enzymes, toxins, nitrates reduction or gas production (Coorevits et al. 2010).

## 5. Conclusion

From this study, it was shown that the raw cow's milk is of low microbial quality in the selected areas as it was produced under unhygienic condition. The isolates of *Bacillus cereus* and *B. coagulans* are considered of being one of the most important microbial contaminations, and might represent public health concerns. Therefore; measures to improve the hygiene of milk production should be instituted at the farm level. Lowering of spore load by good hygienic measures should be done as its further lead to reduction of the level of contamination in raw milk and minimizing the occurrence of aerobic spore-forming bacteria in milk and dairy products. Moreover, awareness programs should be organized targeting dairy cows, owners to raise their skills and practices on adequate farm hygiene (housing, feeding, milking and storage and marketing of milk) and personal hygiene in order to improve the milk hygienic status.

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