Short communication

Microbiological survey of milk and dairy products from a small scale dairy processing unit in Maroua (Cameroon)

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ABSTRACT

In the African context, the production of milk and dairy products is affected by the application of improper procedures during milking and processing, leading to microbial spoilage of milk, especially in local small scale processing plants.

The aim of this survey was to identify potential hazards and evaluate the microbial contamination of milk and dairy products in a small scale dairy processing unit located in Cameroon.

During a 6 week period, raw and pasteurized milk samples were evaluated for Total Aerobic Bacterial Count – TBC, Total Coliforms, *Escherichia coli* and Coagulase Positive Staphylococci – CPS; dairy products (yoghurt, soft cheese, mozzarella and ricotta) were also submitted to the count of Total Coliforms, *E. coli* and CPS.

Contamination levels of raw milk samples varied widely; about half of the samples complied with threshold values for TBC (<6.3 Log CFU/ml), while high coliforms counts (>5.0 Log CFU/ml) were detected in some samples.

In pasteurized milk, high residual counts were observed, indicating an insufficient efficacy of thermal treatment applied at the small scale unit; residual values >3 Log CFU/ml were detected for TBC, in some cases associated with high counts for the other parameters.

Among dairy products, mozzarella and soft cheese resulted the most contaminated by coliforms (mean value ≥ 2.7 Log CFU/g), while low contamination levels were detected for ricotta and yoghurt. Some samples of mozzarella also harboured high counts (>3 Log CFU/g) of *E. coli*.

Based on microbiological outcomes and milk production flow characterisation, Preventive and Corrective Measures were defined for milking and processing phases (thermal treatment, packaging and storage), focussing on training of farmers and dairy employees to improve the hygiene of the local milk and dairy production chain.

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1. Introduction

Unsafe food is still an important threat in most developing countries, especially in the African region (Sandrou & Arvanitoyannis, 2000; WHO, 2007). The main potential risk is represented by microbial contamination, and foodborne diseases caused by microorganisms are a large and growing public health issue. In fact, most countries with case-reporting systems have documented significant increases over the past few decades in the foodborne diseases incidence by pathogenic microorganisms (WHO, 2012).

The food safety challenges in the African region include unsafe water and poor environmental hygiene, weak foodborne disease surveillance, inability of small and medium scale producers to provide safe food, outdated food regulations and inadequate law enforcement, and insufficient cooperation among stakeholders.

Milk and dairy products are considered a high risk category for potential microbial contamination, due to insufficient animal health control, as well as inadequate training of farmers and dairy processing employees about milk hygiene, and weakness in the cold chain during production processes and storage (Aaku, Colison, Gashe, & Mpuchane, 2004; Chye, Abdullah, & Ayob, 2004; Chizari, Jannat, & Abbasi, 2008; Cunin et al., 1999; Tourette, Messad, & Faye, 2002).

Milk microbial contamination is also responsible for significant economic losses at different steps of milk production chain. Official data from World Bank indicate that 20% of milk production in developing countries is lost due to early spoilage, resulting in

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a 12 $/month damage for farmers (Bayemi et al., 2005; Bonfoh et al., 2003).

The World Health Organization requires that small scale dairy processing plants in the developing countries urgently comply with the *Codex Alimentarius* principles (WHO, 2007). Hazard Analysis of Critical Control Points (HACCP) and Good Manufacturing Practices (GMP) are also recommended for the small production units.

Few African countries have enacted foodborne disease surveillance systems; in Cameroon regulations concerning hygienic control of dairy products have been issued, but they are rarely enforced, and the hygienic condition of the milk chain is not sufficiently controlled (Njomaha, personal communication). A study carried out in the North West of Cameroon (Bayemi, Webb, Manjeli, & Naoussi, 2007) showed that a high level of milk microbial contamination causes the loss of 1 L of milk per day for each farmer.

Contamination of milk and dairy products can derive from the cow itself, from human procedures and from the environment (Alais, 2000). Udders can harbour different commensal and pathogenic microorganisms (e.g. streptococci, staphylococci, enteric bacteria), especially in case of clinical or subclinical mastitis. Traditional milking procedures can represent another risk source as they are performed manually in outdoor areas not specifically dedicated. Milking is also preceded by calf suckling to promote the release of oxytocin, without previous sanification of teats, milkers' hands and containers. In outdoor milking environment, several factors are involved in milk spoilage, such as faecal contamination of animal skin, the use of unsafe water for rinsing the udder, milkers' hands and equipment, and a high presence of dust and faeces (Chye et al., 2004; Gran, Mutukumira, Wetlesen, & Narvhus, 2002; Gran, Wetlesen, Mutukumira, & Narvhus, 2002).

Due to these criticisms, collection, transport and processing in compliance with good hygienic practices should be performed in order to avoid microbial spoilage of milk, especially in local small scale dairy processing plants.

The aim of this survey was to evaluate the microbial contamination of raw and pasteurized milk and dairy products in a small scale dairy processing unit located at Maroua, Extreme North Region of Cameroon. Based on microbiological outcomes and milk production flow characterisation, Preventive and Corrective Measures for farmers and dairy employees were also defined.

2. Materials and methods

2.1. The activity at Redou Gniwa dairy processing unit

The *Redou Gniwa* dairy processing unit was built in 2005 thanks to a cooperation and research project with the aim of collecting cow's milk from farmers located within a range of about 15 km around the town of Maroua (Extreme North Region of Cameroon). These farmers were gathered in a cooperative aiming to run jointly the dairy unit that processes about 400 L/week of milk. The dairy processing unit included a laboratory equipped for the execution of basic microbiological analysis. Milking was done twice a day, commonly in outdoor conditions, following traditional procedures: each farmer collected the milk in a gourd, which is an emptied pumpkin, and shortly afterwards all the milk was gathered into a single churn. The farmers themselves delivered the milk by bicycle to the *Redou Gniwa*; the transport from the village to the processing unit took from 45 min to 90 min. Milk quantity from each village ranged between 10 and 60 L/day.

At the arrival at the dairy unit, milk was evaluated for pH and density. If defined ranges were respected (pH $\geq$ 6.55, density $\geq$ 1.026 g/L), the milk was accepted, filtered and stocked into a cooler tank at 4 °C. Part of the milk was sold raw, while the remaining share was used for the production of yoghurt, soft cheese, mozzarella and ricotta. Milk was processed the day of reception or the day after.

Yoghurt was obtained by the overnight fermentation of pasteurized milk (72 °C, 15 s) by ferments (LYOFAST Y450B, Sacco, Cadorago − Como, Italy). For soft cheese production, milk was pasteurized and added with rennet (94% chymosin, 6% bovine pepsin, 1:50.000). After about 2 h, whey was poured off, while the curd was cut and added with salt, then put into batches, pressed and ripened for 15 days. Mozzarella production included: addition of rennet to raw milk, coagulation (2 h), double cut of the curd and stretching in hot water (85 °C). Then the curd was moulded by hand into a ball shape; the pieces obtained were placed in cold water (10 °C) and then put in brine. Ricotta was obtained from acidification and boiling of the residual whey from the production of soft cheese and mozzarella. All the products were sold on the local market.

Milking and processing procedures at *Redou Gniwa* unit were evaluated in order to identify potential hazards that could affect consumer's health or product quality. Considering flow process chart, Critical Points (CPs) were identified, taking into account microbiological and chemical hazards (Table 1).

2.2. Microbiological analyses

The analyses were performed in a 6 week period including the end of the rainy season and the early dry fresh season (September–October), characterized by high humidity and high environmental temperatures (min. about 25 °C and max. about 36 °C). Raw milk samples from 42 churns at their arrival at the *Redou Gniwa* and the same 42 samples after pasteurization were submitted to microbiological analyses in order to evaluate the milk contamination level and the efficiency of pasteurization process. The samples were evaluated for Total Bacterial Count (TBC), Total Coliforms, *Escherichia coli* and Coagulase-Positive Staphylococci (CPS).

The microbial analyses on yoghurt, soft cheese, mozzarella and ricotta were performed once a week for 6 weeks on a single sample of each product. Yoghurt and mozzarella were sampled at packaging; cheese and ricotta were tested at the end of production processes, just before entering the refrigerator for the storage. The samples were evaluated for Total Coliforms, *E. coli* and CPS. Samples dilutions were prepared and plated into Petrifilm® (3M Italia, Pioltello, Italy) containing specific culture media and incubated for 24 h at 30 °C (TBC), 37 °C (CPS, Total Coliforms) or 44 °C (*E. coli*). Analyses were performed in duplicate for each sample. Repeatability of microbiological count method was verified by index of dispersion (K_p); proportional dilution between two series was verified by the G^2 test (ISO 7218:2007).

Table 1
Critical Points and associated hazards for processing procedures at *Redou Gniwa* unit.

Critical point	Hazard
CP 1 − Milk delivery	Microbiological: contamination by pathogenic/spoilage bacteria
	Chemical: unsuitable pH or density for dairy transformation
CP 2 − Pasteurization	Microbiological: pathogenic/spoilage bacteria overcoming thermal treatment
	Chemical: protein thermal damage − enzymes inactivation
CP 3 − Yoghurt bottling	Microbiological: recontamination by pathogenic/spoilage bacteria
CP 4 − Mozzarella packaging	
CP 5 − Products refrigerated storage	Microbiological: bacterial contamination by environment − microbial growth during storage

2.3. Statistical analysis

Mean, standard deviation, median, minimum and maximum values, 25th and 75th percentiles were calculated for each microbiological parameter (SPSS, 2007). For the calculation of mean values, a standard value of 0.48 Log CFU/ml or g was assigned to samples with microbial counts <1 Log CFU/ml or g, based on the count limit indicated by McCrady table for Most Probable Number calculation (3 CFU/ml or g).

3. Results and discussion

3.1. Microbiological analyses

The results of the microbiological analyses of raw and pasteurized milk and dairy products are outlined in Tables 2 and 3; threshold values for each parameter are also reported.

In order to make an evaluation of microbiological data considering the dairy production context of this area, as Cameroon legislation does not indicate specific microbiological requirements, threshold values defined for raw milk by *Kenyan Bureau of Standards* (KEBS, 1996) for Total Aerobic Count and Total Coliforms were considered. Microbiological threshold values from *Kenyan Bureau of Standards* are more suitable for tropical and small scale processing units than internationally recommended levels indicated by Vairamuthu, Sinniah, and Nagalingam (2010) (5 and 3 Log CFU/ml, respectively), as already suggested by several authors (Duteurtre, 2004; Hempen, Unger, Münstermann, Seck, & Niamy, 2004, p. 54; Omore, Arimi, Kangethe, & McDermott, 2004; Omore et al., 2001). For pasteurized milk, threshold values reported by Mhone, Matope, and Saidi (2011) were adopted.

For *E. coli* and CPS in raw milk, and for microbiological parameters considered in the analysis of dairy products, as threshold values fitted for African productions were missing, other sources were used (Commission Regulation 1441/2007; CSR, 2007; Gilbert et al., 2000).

Contamination levels of raw milk samples widely varied. The mean and median values of Total Bacterial Count slightly exceeded 6 Log CFU/ml, and were near the threshold settled by KEBS; approximately half of the samples analyzed (55.6%) had a contamination level lower than or equal to the threshold value. A high percentage (87.1%) of samples showed coliforms levels below the threshold, but some samples showed a high contamination level (>5 Log CFU/ml), indicating an improper hygienic status of milk. A low mean contamination level by *E. coli* was found, and in 20.5% of samples the number of *E. coli* was not detectable (<10 CFU/ml). Contamination level by CPS was very low (mean and median values fell into the range 2.5–3 CFU/ml), and 82.5% had a concentration less than or equal to the limit settled for raw milk marketing in Europe.

Table 3
Microbiological results and threshold values for dairy products.

		Mean ± S.D.	Median	Min.	Max.	Threshold values
Total coliforms Log$_{10}$ CFU/ml	Yoghurt	1.62 ± 1.33	1.30	<1	3.39	4[b]
	Mozzarella	2.79 ± 1.52	2.97	<1	4.16	4[b]
	Soft cheese	2.70 ± 1.89	3.55	<1	4.27	4[b]
	Ricotta	1.52 ± 1.77	1.41	<1	3.27	4[b]
E. coli Log$_{10}$ CFU/ml	Yoghurt	0.80 ± 0.76	<1	<1	1.70	2[b]
	Mozzarella	1.89 ± 1.52	2.40	<1	3.49	2[b]
	Soft cheese	0.96 ± 1.50	<1	<1	3.21	3[a]
	Ricotta	0.72 ± 1.02	<1	<1	2.16	2[b]
CPS Log$_{10}$ CFU/ml	Yoghurt	0.89 ± 0.72	1.09	<1	1.60	2[b]
	Mozzarella	0.30 ± 0.73	<1	<1	1.78	2[b]
	Soft cheese	0.60 ± 0.96	<1	<1	2.18	2[a]
	Ricotta	1.02 ± 1.17	<1	<1	2.11	2[b]

[a] Commission Regulation (EC) 1441/2007.
[b] Gilbert et al., 2000.

For pasteurized milk samples, considering the efficiency of thermal treatment, high residual counts detected in a high percentage of samples indicate an insufficient fall in bacterial populations. Residual TBC ranged between 3 and 4.5 Log CFU/ml, with almost half of the samples with counts higher than the threshold value, and some samples showed high counts (>3 Log CFU/ml) for the other microbiological parameters, exceeding suggested threshold values for Total Coliforms and *E. coli*.

Among dairy products, considering coliforms, mozzarella and soft cheese resulted the most contaminated (2.7–2.8 Log CFU/g), with some samples exceeding the threshold value of 4 Log CFU/g. For *E. coli*, mozzarella showed a high contamination level, as more than 50% of the samples had values >2 Log CFU/g; only one sample of ricotta and one sample of soft cheese presented values above the threshold. For CPS, all the products presented a high percentage of samples with a low contamination level (mean and median values were near or below 1 Log CFU/g) and the maximum contamination level observed in soft cheese and ricotta was only slightly above the limit of 2 Log CFU/g. All the samples of yoghurt showed values below the threshold for all the considered microbiological parameters.

The results of the study indicate that contamination level of raw milk delivered to *Redou Gniwa* unit agrees with the results obtained in other studies performed in the subsaharian region (Bonfoh et al., 2003, 2006; Kameni, Imele, & Mbanya, 2002; Mhone et al., 2011). *Kenyan Bureau of Standards* set higher acceptance levels than international ones, nevertheless a relevant percentage of samples exceeded threshold values. High presence of coliforms and *E. coli* might be related to the application of traditional milking procedures, which are the most important source of faecal contamination, leading to milk deterioration and consequently to a significant economic loss for local farmers (Bayemi et al., 2007; Bonfoh et al., 2003; Gran, Wetlesen et al., 2002). Jayarao, Pillai,

Table 2
Microbiological results and threshold values for raw and pasteurized milk.

		Mean ± S.D.	Median	Min	Max	25thPerc.	75th Perc.	Threshold values
TBC Log$_{10}$ CFU/ml	Raw	6.23 ± 0.63	6.19	4.93	7.46	5.93	6.58	6.30[a]
	Pasteurized	3.79 ± 0.62	3.54	3.17	4.74	3.30	4.40	3.70[b]
Total coliforms Log$_{10}$ CFU/ml	Raw	3.83 ± 0.86	3.88	2.30	5.41	2.95	4.38	4.70[a]
	Pasteurized	1.92 ± 1.86	2.57	<1	4.18	<1	3.52	2[b]
E. coli Log$_{10}$ CFU/ml	Raw	2.25 ± 1.44	2.48	<1	5.18	1.70	3.28	2[c]
	Pasteurized	1.08 ± 1.54	0.70	<1	3.72	<1	2.36	0[b]
CPS Log$_{10}$ CFU/ml	Raw	2.65 ± 0.89	2.92	<1	3.71	2.33	3.17	3.3[d]
	Pasteurized	1.48 ± 1.21	1.00	<1	3.01	<1	2.71	3[b]

[a] KEBS, 1996.
[b] Mhone et al., 2011.
[c] Gilbert et al., 2000.
[d] CSR, 2007.

Sawant, Wolfgang, and Hedge (2004) evidenced that bacterial population of milk from udders contaminated by faeces can be 5 times higher than that of milk form clean udders.

Milking procedures are also critical for milk contamination by Staphylococci which are linked to subclinical mastitis but are also usually present on animal and human skin (Bertocchi, Varisco, Bolzoni, Bravo, & Bonometti, 2005; Jayarao et al., 2004; Wilson, Helena, Gonzalez, & Sears, 1997). Nevertheless, the level of contamination observed in milk samples was not high, and bacterial numbers potentially associated to the production of thermo-resistant enterotoxins (4 Log CFU/ml) were not detected (Alais, 2000; Jørgensen, Mørk, & Rørvik, 2005). The results of microbial parameters were likely influenced by milk collection and transport procedures: raw milk was exposed for prolonged periods to the high environmental temperatures.

The pasteurization process is widely considered a critical phase in small processing units, as showed by different authors (Breurec et al., 2010; Mhone et al., 2011). Our data on pasteurized milk indicate an inadequate microbial quality. The factors, which could contribute to the presence of high levels of residual contamination in pasteurized milk, include initial contamination level, treatment parameters and inadequate refrigeration of treated milk. The presence of high bacterial numbers in raw milk (>6 Log CFU/ml) can make a standard pasteurization treatment insufficient to assure milk safety and durability. In a study conducted in Cameroon dairies, Kameni et al. (2002) suggested to pasteurize milk at 74 °C for 10 min, showing the efficacy of such treatment, as residual counts were below 4 Log CFU/ml. The inadequate milk pasteurization at *Redou Gniwa* might also be related to excessively slow refrigeration of pasteurized milk. As known, some thermoresistant microorganisms, such as *Streptococcus* spp., spore-forming *Bacillus* spp. and *Clostridium* spp., can survive pasteurization and start a rapid growth phase when the temperature reaches critical values (25–30 °C) (Alais, 2000).

The analyses of dairy products outlined the presence of detectable numbers of Total Coliforms and *E. coli* in soft cheese and mozzarella. For cheese, it is likely caused by contamination during processing and short ripening. The ripening phase is very important for assuring safety and durability of cheeses; high environmental temperatures typical of this area do not allow a sufficiently long ripening period in order to obtain a significant decrease of bacterial populations. The critical factor in mozzarella production is the use of unpasteurized milk; the immersion of the curd in hot water (85 °C) for stretching seems to be insufficient to reach an efficient reduction of microbial populations. The lack of efficacy could be due to the high fat content of this product, which can protect microorganisms from the action of the heat (Salvadori del Prato, 1998). Microbial contamination of other dairy products (yoghurt and ricotta) was low. Regarding ricotta, the second heat treatment carried out during the production (typical of "re-cooked" product) should significantly lower bacterial populations.

The good microbial quality of yoghurt produced in *Redou Gniwa* unit was assured by the strong acidification (reaching pH values of 5.0–5.2) that is able to inhibit the growth of different microorganisms, in particular of coliforms (Robinson & Tamime, 1983).

3.2. Preventive and corrective measures

Based on identification of CPs and microbiological results, Corrective and Preventive Measures were defined and explained to farmers and dairy employees; such actions must be applied in case of failure to respect the microbiological limits set for each product.

Considering that traditional milking (CP 1) led to a high microbial contamination of milk delivered at *Redou Gniwa* unit, preventive and continuous training of farmers on milking hygiene was suggested.

Considering milk pasteurization (CP 2), the efficacy of thermal treatment depends on several factors, including initial microbial contamination level of milk, treatment parameters (time/temperature), refrigeration and storage of pasteurized milk. A possible intervention could be an adjustment of pasteurization parameters (e.g. an increased duration of thermal treatment) and an improvement of efficacy and rapidity of post-pasteurization phases, by the use of a water/ice bath.

Considering yoghurt production, as the natural acidification represents a protective factor for the hygiene of the product, preventive measures were pointed to the maintenance of the microbial quality during bottling (CP 3), ensuring the use of sanitized bottles.

During packaging of mozzarella (CP 4), a training of dairy workers on personal and environmental hygiene must be considered crucial to avoid recontamination and improve safety and durability of the product.

Taking into account the criticisms outlined in the production flow, the refrigerated storage (CP 5) should be controlled to minimize the microbial growth, mainly in subsaharian context characterized by high environmental temperature and high contamination level of the final products.

4. Conclusions

In subsaharian context, some constrains are experienced in developing and applying plans to improve milk and dairy quality; in particular, the strong linkage to traditional farming system represents an obstacle to the adoption of proper procedures. Training of farmers becomes essential to improve hygienic level of traditional milking. A specific effort should be made to increase the consciousness of farmers about the significant influence of microbial contamination of milk on dairy yield and on safety and durability of dairy products. Identifying a clean milking area, avoiding calf suckling during milking operations, tying the tail, cleaning the udder (washing and drying) and washing milkers' hands, eliminating the first milk outflows, cleaning the containers and reducing the time between milk collection and delivery must be considered as fundamental items to be included in training programmes for farmers.

The milk processing is deeply affected by environmental and working conditions, influencing milk and dairy quality and increasing the risk of microbial contamination and growth. Preventive measures should be established and applied to assure the safety of milk and dairy products. Training programs for dairy workers should be focused on the need for fast refrigeration to avoid bacterial spread and for minimizing milk recontamination by the use of sanitized containers. Packaging and storage of milk and dairy products are critical, mainly in extreme environment, and inadequate practices can negatively affect all the processing chain.

Acknowledgements

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