Microbial and Chemical Adulterants Assessment of Raw Cow Milk Collected from Dairy Farms of Hlabisa Villages, KwaZulu-Natal Province, South Africa

N.H. Xulu [1], S. Jamal-Ally [2], K.D. Naidoo [1*✉] iD

1. Discipline of Food Security, School of Agriculture, Earth and Environmental Sciences, Pietermaritzburg, 3209, South Africa
2. Discipline of Microbiology, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, 3209, South Africa

HIGHLIGHTS

- Total bacterial count of teats, milking buckets, and communal milk pooling buckets were 6.91, 6.06, and 6.06 log Colony Forming Unit/ml, respectively.
- The most found chemical adulterant was urea detected in 23 out of 68 (33.8%) samples.
- This study revealed the lack of standard operating sanitation in dairy farms of Hlabisa villages, South Africa.

ABSTRACT

Background: Milk is one of the most nutritious foods providing a variety of proteins, fats, minerals, and vitamins needed to maintain, grow, and develop the body. The aim of this study was to assess microbial and chemical adulterants of raw cow milk collected from dairy farms of Hlabisa villages, KwaZulu-Natal Province, South Africa.

Methods: A total of 68 raw cow milk samples were obtained from teats sampling points, milking buckets, and communal pooling buckets. The bacteriological analysis was conducted for the detection of various bacteria in milk samples. Biochemical tests were also done to detect some chemical adulterants in milk samples.

Results: Total bacterial count of teats, milking buckets, and communal milk pooling buckets were 6.91, 6.06, and 6.06 log Colony Forming Unit (CFU)/ml, respectively. The most found chemical adulterant was urea detected in 23 out of 68 (33.8%) samples, followed by hydrogen peroxide showed in 22 out of 68 (32.3%) samples. However, none of the samples were contaminated with formalin, starch, and neutralizer.

Conclusion: The present study revealed high microbial contamination of raw cow milk produced by rural small-scale dairy farmers of Hlabisa villages, KwaZulu-Natal Province, South Africa, indicating the lack of standard operating sanitation. It was also stated that raw milk samples contained various types of chemical adulterants that may lead to severe health problems. Good hygiene practices must be adopted by small-scale dairy farmers at every stage of their milk handling and processing.

Introduction

Milk is one of the most nutritious foods providing a variety of proteins, fats, minerals, and vitamins needed to maintain, grow, and develop the body. It is an important diet in all age groups, but mostly children under five

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* Corresponding author (K.D. Naidoo)
✉ E-mail: Naidook12@ukzn.ac.za
ORCID ID: https://orcid.org/0000-0002-5320-0538

years old (Mahmoudi et al., 2014). Consumption of milk is too vital for improving the nutritional status of people suffering from hidden hunger in many developing countries in Africa (Knight-Jones et al., 2016; Msalya, 2017).

The lack of standardized hygienic operating procedures results in early microbial spoilage of milk for rural small-scale dairy farmers at various stages of procurement, processing, and distribution (Hamid et al., 2013). Contamination with microorganisms could also result from various unhygienic environmental factors such as the udder, barn, milk collection materials, ingredients added to dairy products, etc. (Garedew et al., 2012; Mesfine et al., 2015). Milk from cows; affected with mastitis, poor sanitation of utensils, and unsanitized transport practices; maybe contaminated with pathogenic bacteria (Mesfine et al., 2015). Also, contaminated milk can result in spreading of some zoonotic diseases during milk processing (Abbas et al., 2013). In addition, milk adulteration has also been identified as one of the major challenges in diary industries affecting nutritional quality of the product (Azad and Ahmed, 2016; Karimuribo et al., 2015; Swai and Schoonman, 2011).

The aim of this investigation was to assess microbial and chemical adulterants of raw cow milk collected from dairy farms of Hlabisa villages, KwaZulu-Natal Province, South Africa.

Materials and methods

Sampling

A total of 68 raw cow milk samples were collected from 23 rural small-scale dairy farms located in Hlabisa villages, KwaZulu-Natal Province, South Africa during March 2018. Before sampling, information was collected through the questionnaire on environmental hygiene, personal hygiene, milk collection, storage utensils, storage condition, and water used in sanitation and milking procedures. The milk assessment for the smell, color, any deposits, and cleanliness of containers was done using standard methods.

Sampling for microbiological assessment involved teats (n=25), milking buckets (n=25), and the communal pool-ing buckets (n=18). The milking and communal pooling buckets were swabbed at the bottom round corners using sterile dry swabs before the milking process. An area of 100 cm^2 was swabbed by rubbing firmly across the area several times in all directions. The swabs were immersed in 5 ml of tryptone soy broth (Merck, South Africa) in sterile 50 ml centrifuge tube and stored in cold storage before analysis (De Muynck et al., 2010). The swab and milk samples were transported to the University of KwaZulu-Natal Laboratory (South Africa) for the next microbiological analysis.

Serial dilution and isolation

The milk samples were immediately analyzed using the total plate count, biochemical identification tests, and milk adulteration tests. Each milk sample was diluted before plating and the dilutions were made in sterilized distilled saline water solution. One ml of milk from each sample was poured into 9 ml of sterilized distilled saline water in a test tube to get a dilution of 1:10. One μl of the inoculum was plated on the tryptone soy agar medium and spread using a hockey stick. The plates were then left for half an hour on the bench then incubated at 37 °C and examined after 24 h for bacterial growth (Eggermont et al., 2017). The colony count was carried out and the total viable bacterial count was calculated by multiplying the number of colonies with the reciprocal of the dilution used. The analyses were done in triplicate.

For isolation of bacteria from incubated milk plates and swabs, samples were streaked based on morphology on tryptone soy agar, which was incubated aerobically at 37 °C for 24 h. Plates that showed no growth were further incubated for 48 h before discarded as negative. The bacterial isolates were purified by repeated subculture.

Bacterial identification

Unique colonies were subcultured to obtain pure colonies of isolates. The pure isolates were maintained on agar plates and their probable identities were established using biochemical identification test kits (HiMedia Ltd, India) and carried out according to the manufacturer instructions (Hemraj et al., 2013). The KB003 and KB019 kit provided a comprehensive test system for the identification of *Enterobacteriaceae* and Gram-negative non-fermenters species.

Chemical adulterants analysis

According to Kandpal et al. (2012), all the milk samples were screened for the presence of commonly chemical adulterants using K088A milk adulteration kits (HiMedia Ltd, India) based on the manufacturer instructions. The kit contained biochemical tests for detection of alizarine, urea, detergents, salt, starch, sucrose, formalin, skim milk powder, glucose, and hydrogen peroxide.

Results

Total bacterial count of samples from teats, milking buckets, and communal milk pooling buckets examined in this investigation were 6.91, 6.06, and 6.06 log

Colony Forming Unit (CFU)/ml, respectively. Bacteria presented in the samples from teats, milking bucket, and communal milk pooling bucket are indicated in Table 1. Different pathogenic bacterial species were reported contaminating the raw cow milk, including *Enterobacter aerogenes, E. gergoviae, Klebsiella oxytoca, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Burkholderia mallei, Shigella dysentery, Sh. sonnei, Morganella morganii, Alkaligenes denitrificans,* and *Xanthomonas* spp. As the most abundant bacteria, *E. gergoviae* and *K. oxytoca* were detected in 100% of teats samples.

Chemical adulterants found in milk samples are presented in Table 2. The most found chemical adulterant was urea detected in 23 out of 68 (33.8%) samples, followed by hydrogen peroxide showed in 22 out of 68 (32.3%) samples. However, none of the samples were contaminated with formalin, starch, as well as neutralizer.

Discussion

We reported the presence of coliform bacteria such as *E. aerogenes and E. gergoviae* in milk samples which are indicators of poor hygiene conditions. Our results were comparable with the studies carried out on microbial contamination of milk samples in Tanzania (Gwandu et al., 2018) and Eastern Ethiopia (Mesfine et al., 2015). Enterobacteriaceae family are prevalent residents of the intestinal tract of multiple domestic animals such as cow and might be a possible indication of contamination from the udder, milking utensils, water, or milk handler (Akabanda et al., 2010; Wanjala et al., 2017). *K. oxytoca,* the main pathogenic *Klebsiella* spp. causes pneumonia while *M. morganii* is mainly an opportunistic pathogen associated with soft tissue infection, respiratory tract infection, and urinary tract infections (Liu et al., 2016; Singh et al., 2016). Also, the species of *Shigella* identified from the raw milk of Hlabisa (South Africa) were *Sh. sonnei* and *Sh. dysenteriae* (Table 1).

Table 1: Microbial contamination rate (%) of teats, milking buckets, and communal pooling buckets from dairy farms of Hlabisa Villages, KwaZulu-Natal Province, South Africa

Microorganism	Contamination rate (%)		
	Teats	Milking buckets	Communal pooling buckets
Enterobacter aerogenes	0	52	39
Enterobacter gergoviae	100	38	14
Klebsiella oxytoca	100	72	17
Morganella morganii	0	36	27
Shigella dysenteriae	2	6	0
Shigella sonnei	1	5	1
Alkaligenes denitrificans	0	52	2
Pseudomonas aeruginosa	3	4	2
Burkholderia mallei	36	68	22
Stenotrophomonas maltophilia	16	96	33
Xanthomonas spp.	10	16	6

Table 2: Chemical adulterants found in milk samples (n=68) from dairy farms of Hlabisa Villages, KwaZulu-Natal Province, South Africa

Chemical adulterants	No. of contaminated samples	Percentage
Alizarine	20	29.4
Formalin	0	0
Urea	23	33.8
Starch	0	0
Neutralizer	0	0
Detergent	20	29.4
Sodium chloride	8	11.9
Skim milk powder	10	14.7
Sucrose	4	5.8
Glucose	4	5.8
Hydrogen peroxide	22	32.3

This study indicated the presence of *P. aeruginosa, Stenorophomonas maltophilia*, and *B. mallei* in milk samples. These findings are consistent with the research carried out by Garedew et al. (2012) who stated that 18.52% of milk samples from Ethiopia were contaminated with *P. aeruginosa*. Pseudomonadaceae family are distributed ubiquitously in diverse environmental sources such as tap water or contaminated solution. Thus, *P. aeruginosa* found in this survey may be entered into bucket by contaminated water or imperfect udder sanitizing before milking. Similar presence of *Pseudomonas* spp. has been previously showed in bulk milk from 131 dairy herds in Eastern South Dakota and Western Minnesota, USA (Jayarao and Wang, 1999).

Among the microorganism detected in the present survey, *S. maltophilia* was identified as more predominant isolate in clinical mastitis milk samples (Zhang et al., 2015). Clinical mastitis is the main cause of permanent teat blockage, which was observed most frequently in the dairy cattle of Kwa-Hlabisa rural small-scale dairy farmers and led to less milk being produced. Therefore, farmers must be equipped with adequate skills on dairy cow management, through visiting successful farmers or working with livestock extension personnel. Previous studies indicated that *S. maltophilia* isolates were involved in a herd outbreak of mild mastitis in cattle in Japan (Ohnishi et al., 2012). *S. maltophilia* has also been found to be an environmental global emerging Gram-negative bacterial pathogen that can cause various infections in humans (Brooke, 2012; Looney et al., 2009). Furthermore, earlier studies conducted in China have shown that highly concentrated feed causes a significantly high percentage of environmental pathogen like *Stenotrophomonas* in cow dung (Zhang et al., 2015). Cow dung contamination may be the main source of *Stenotrophomonas* infection among dairy cows of Kwa-Hlabisa, and bacteria may be transferred between the lying surface and the teats. As a result, cow dung management needs to be practiced among the small-scale dairy farmers to ensure and limit the presence of bacterial cross-contamination.

The detection of coliform and pathogenic bacteria from our milk samples indicated that there might be poor hygiene either from the udder of cattle or utensils used for getting milk. It further indicated that there were poor milking management, ineffective milking practices, and deficient cattle care. Hence, lack of domestic infrastructures such as running water, electricity, and refrigerators might have contributed considerably to the predicament of the rural small-scale dairy farmers. Simple and appropriate solutions and or technologies for small-scale dairy farmers are one way to address poor hygiene; however, these solutions are often over-looked. Lues et al. (2012) reported that appropriate managerial practices could

improve and control clinical and sub-clinical udder infections, a practice which can affect most of the cattle positively in the current study if rural small-scale dairy farmers are aware of it. The contamination from external sources is considerably reduced when the cows and floor are cleaned, the manure are removed daily, utensils are sterilized, and the udders and teats of the cow are washed (Hagevoort et al., 2013). Most of the rural small-scale dairy farmer in this study used plastic buckets as milking utensils, which are difficult to clean and can be a potential source of bacterial contamination and invariably adulteration of milk. Similarly, two previous studies reported microbial contamination from the wide use of plastic buckets as milking utensils in rural dairy units and rural dairy producers in Ethiopia (Bereda et al., 2012) and South Africa (Lues et al., 2012). The presence of coliform isolates can also be ascribed to the neglect of post-milking teat dipping and absence of herd health management.

According to the findings of the current research, the total bacterial count of cow milk from teats, milking bucket, and communal pooling bucket ranged from 6.06 log to 6.91 log CFU/ml. The levels of bacterial contamination of raw milk from the rural small-scale dairy farmers in this study were higher than the recommended limit approved by milk and dairy product organization (Department of Health of Republic of South Africa, 2002) which is 2×10^5 CFU/ml. Similar with our findings, Ngasala et al. (2015) reported that the total bacterial count of raw milk from Arusha City and Meru District of Tanzania was 6.73 log CFU/ml.

Adulteration of food products especially milk is a serious problem in rural areas and may lead to severe health problems to milk consumers (Handford et al., 2016). In this study, formalin, starch, and neutralizer detection tests were negative. It can be assumed that the water used for hygiene practices met suitable standards for use. In contrary with our results, 20% of milk samples in Pakistan (Barham et al., 2014) and 32% of milk samples in India (Singuluri and Sukumaran, 2014) were contaminated with formalin which can cause potentially toxic effects on the consumers.

The extent of glucose adulteration in this study was somewhat similar to the findings of Barham et al. (2014) who reported 10% of glucose in market milk at Mirpurkhas, Pakistan. Surprisingly, Nirwal et al. (2013) reported a very high level of adulteration of milk with glucose (80%) in India. Additionally, the present study results revealed that 15% of milk samples were adulterated with skim milk powder, which was almost the same to the results of Barham et al. (2014), whereas higher percentage (80%) of skim milk adulteration was reported by Singuluri and Sukumaran (2014) in milk samples from India. These results correspond with the findings of

Lateef et al. (2009) in Pakistan who concluded that small-scale rural dairy farmers use skim milk powder to adulterate milk by adding inexpensive substances such as glucose or skim milk powder to maximize their profit, in order to improve total milk solids. Likewise, sugar was detected as an adulterant by 6% of the milk samples collected from Kwa-Hlabisa, South Africa. The main reason for the presence of cane sugar in raw milk is unknown. However, sugar is a cheap source of sweetener, and probably, it could be assumed that cane sugar is added to the diluted raw milk to improve its taste. Also, it can also be assumed that the presence of sucrose in milk was due to sugar cane fed as fodder to the cattle.

Conclusion

The present study revealed high microbial contamination of raw cow milk produced by rural small-scale dairy farmers of Hlabisa villages, KwaZulu-Natal Province, South Africa, indicating the lack of standard operating sanitation. It was also stated that raw milk samples contained various types of chemical adulterants that may lead to severe health problems. Appropriate hygiene practices must be adopted by small-scale dairy farmers at every stage of milk handling and processing.

Author contributions

N.H.X and K.D.N. designed the study; N.H.X. conducted the experimental work; N.H.X. and S.J-A. analyzed the data; N.H.X., K.D.N., and S.J-A. wrote the manuscript. All authors revised and approved the final manuscript

Conflicts of interest

There is no conflict of interest in the study.

Acknowledgements

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