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Assessment of The Microbiological Quality of Raw Cow's Milk Sold in The City of N'Djamena

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Abstract: Milk is a food with high nutritional value. Its importance stems from its high water, protein, fat, mineral, and vitamin content. The objective of this study is to assess the microbiological quality of raw cow's milk sold in the open air in N'Djamena (capital of Chad). A total of twenty (20) samples were analyzed. The results of microbiological analyses, when compared with microbiological criteria standards, reveal a total absence of Escherichia coli and Salmonella spp. However, toxin-producing bacteria were present : Total Aerobic Mesophilic Flora (TAMF) was present with an average microbial load of 4.83×10^5 CFU/g, for coliforms the microbial load was 2.64×10^4 CFU/g, Staphylococcus aureus at 2.38×10^3 CFU/g, Bacillus cereus with an average microbial load of 2.5×10^3 CFU/g, and yeasts and molds were present with an average of 1.8×10^4 CFU/g. In view of these results, raw milk is of good hygienic quality despite the unsatisfactory quality observed. Efforts to raise awareness of hygiene measures must be made in the dairy sector to restore the expected microbiological quality. Good hygiene practices can reduce the bacterial load in raw cow's milk.

Keywords: Microbiological quality, raw cow's milk, Escherichia coli, Salmonella spp. toxinogenic bacteria, hygienic quality.

INTRODUCTION

Monitoring the microbiological quality of our food remains essential. This is because it prevents dangerous or non-compliant products from being sold and consumed. This is done through regulated analyses governed by (national or international) standards, which often require food to be free of all pathogenic germs or microbial toxins and to have low levels of total flora (Guergouri, 2020).

According to Sikine *et al.,* (2013), raw milk is unprocessed milk that has not undergone pasteurization, sterilization, thermization, or microfiltration. It is milk that has never exceeded a temperature of 40ºC, which is close to the animal's body temperature.

In Chad, a developing country, milk is a widely consumed product and represents a significant food expense for households. Milk is an important foodstuff in the human diet. It is a biological fluid collected from mammals, particularly dairy cows. It is a complete food, containing the main nutrients essential for development.

In Chad, a developing country, milk is a widely consumed product and represents a significant food expense for households. Milk is an important foodstuff in the human diet; it is a biological fluid collected from mammals, particularly dairy cows. It is a complete food, containing the main nutrients essential for development. Each country must therefore ensure sufficient production and take all appropriate measures to feed and maintain its cattle herd. Assessing the microbiological quality of a foodstuff involves testing for germs of hygienic interest, fecal contamination, pathogens, and toxin-producing bacteria (Marnissi *et al.,* 2013).

Milk quality control is a fundamental necessity. Failure to comply with hygiene rules can seriously compromise milk quality and lead to a number of alterations and contaminations by microorganisms, some of which are pathogenic and can cause various diseases and human poisoning (Petranxiene and Lapied, 2002).

In addition, milk may contain germs that are pathogenic to humans. It can become coagulable when boiled if there is slight acidification with a pH drop from 6.7 to 6.3. This food product elicits reactions from consumers, health professionals, processors, and producers alike.

The overall objective of this study is to evaluate the microbiological quality of raw cow's milk sold in the open air in N'Djamena.

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MATERIALS AND METHODS

Type and periods of study

The work was carried out in the city of N'Djamena, specifically in three districts (6th, 7th, and 10th). This was a cross-sectional, prospective, and descriptive study conducted from October 12, 2022, to January 12, 2023. We obtained authorization from the dean of the Faculty of Exact and Applied Sciences and the director of the microbiology department at CECOQDA/DCQM.

MATERIAL

Biological material

The biological material sampled in the three districts of the city of N'Djamena consisted mainly of raw cow's milk, which was selected for microbiological quality assessment analyses. This is a food consumed daily by the population of the city of N'Djamena.

Sample collection

This is a cross-sectional, prospective, descriptive study conducted between October 12, 2022, and January 12, 2023. Sample collection is a crucial step and an important prerequisite for obtaining good results. To this end, the equipment used must be clean and sterilized.

Our samples for microbiological analysis consisted of raw cow's milk collected in three (3) districts (6th, 7th, and 10th) of the city of N'Djamena. In total, we carried out two sampling trips on October 12, 2022, and January 12, 2023, which yielded a total of twenty (20) samples for microbiological analysis. These trips enabled us to collect 20 samples. Strict aseptic rules were followed during sampling to avoid any kind of contamination and prevent an increase in the initial microbial load, in order to obtain reliable results.
For the sample size in Excel, we used the SCHWARTZ formula:

$$n = z^2 \times p\,(1-p) / m^2$$

Z: confidence level; p: target population size; m: margin of error; and n represents the sample size.

Transport of samples

The volume of milk collected (500 ml) was immediately labeled and numbered, taking care not to contaminate the outside of the bottle or its cap. transported at +4°C without exceeding the time limit set by the microbiology laboratory of the Food Quality Control Study Center, Food, Water, and Beverage Microbiological Quality Control Department.

Preparation of the stock solution

Dilution is a process that reduces the concentration of a substance in a solution. To this end, decimal dilutions for the samples to be analyzed (previously prepared stock solutions) are carried out in cascades.

Cascade dilution

Cascade dilution was carried out 15 minutes after removal from Stomascher, in strict compliance with aseptic rules to avoid any kind of contamination.

Procedure

- The diluent tubes are labeled (10-1 ; 10-2 ; 10-3 ; 10-4) ;
- The stock suspension is aseptically withdrawn using a 1 mL sterile graduated pipette equipped with a suction bulb; The sample is homogenized using a homogenizer (standard NF ISO 7218).
- Transfer the 1 mL sample aseptically to the first tube (10-1), ensuring that the pipette does not penetrate the 9 mL of diluent.
- Discard the used pipette in an appropriate container. Using a second sterile 1 ml pipette, repeat the process from tube 10-1 to tube 10-2.
- Repeat for the last two tubes, using a new pipette for each sample.

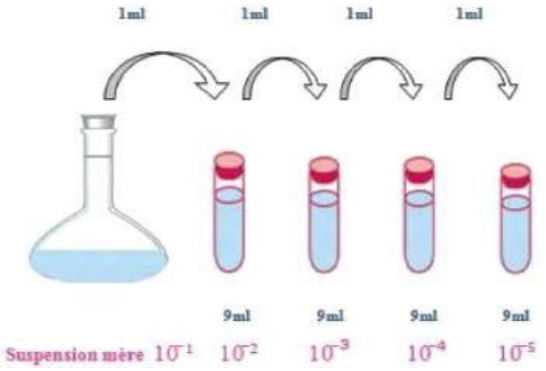

Figure 1: Cascade dilution (Sameh, 2017)

Preparation of culture media

Microorganisms require nutrients to grow ; these nutrients are provided in the laboratory by nutrient media or culture media that enable them to grow properly. The medium must contain the essential nutrients in sufficient quantities and in the right proportions, and must also be nutritious and balanced.

Microorganisms and the conditions for their development vary greatly, so there is obviously no universal medium on which all microorganisms are able to multiply. However, certain media are suitable for the development of a wide variety of microbial germs; the choice of such media is based on knowledge of the natural habitat and dietary physiology of the group of germs to be cultured. This is a fundamental step in microbiological analysis and must be carried out with the utmost rigor.

- The laboratory is cleaned and the equipment to be used is disinfected.
- The balance is turned on for 15 minutes and calibrated.
- Distilled water in the inert material container (polyethylene) is stored.

***Corresponding Author:** Renaud Nonmarmbaye

Before preparing a medium (when the box is first opened), it is recommended to perform a performance test that takes into account productivity, selectivity, and specificity. The mass of the complete dehydrated culture media is weighed according to the instructions on the box.

For media requiring heating and autoclaving, they were mixed with the recommended amount of distilled water, then heated and homogenized with a magnetic stirrer on a hot plate until completely dissolved to obtain a homogeneous suspension. The media were then autoclaved at 121°C for 15 minutes.

Media to be heated without autoclaving, such as Hektoën, VRBG, and VRBL agar, were heated using a hot plate. These media were removed from the hot plate, cooled to between 45°C and 47°C in a water bath, and then 15 ml of these media were poured into Petri dishes.

Table 1: Targeted microorganisms, corresponding culture media, temperatures, and incubation times

Gems	Culture media	Temperature	Incubation time
FAMT	PCA	30°C	72H
Coliform bacteria	VRBG	37°C	24H
Staphylococcus aureus	Baird-Parker	37°C	24 à 48H
Escherichia coli	TBX	44°C	24H
Bacillus cereus	MYP	30°C	24H
Yeasts-Molds	OGA	25°C	24H
Salmonella spp	RVS, MKTTn, XLD, Hektoën et TSA	37°C	24H

In this section, we focus on testing the microbiological quality of raw cow's milk based on the parameters to be sought for their enumeration. The standard methods chosen by reference technicians (AFNOR and ATNOR methods) for official testing are used in this work.

Detection and enumeration of TAMF: (ISO 4833-1: 2013)

TAMF (Total Aerobic Mesophilic Flora) is a health indicator used to assess the number of CFU (Colony Forming Units) present in a product. Counting is carried out at 30°C and this procedure is governed by: (ISO 4833-1:2013). Dilutions for TMAF counting range from 10-1 to 10-4. One milliliter is aseptically sampled from each tube and placed in single-use Petri dishes. Fifteen milliliters of PCA (Plate Count Agar) medium, melted and then cooled in a water bath to 47°C, is added. The mixture is homogenized by swirling the Petri dishes. 5 ml of PCA medium is added after solidification. This prevents the dish from being invaded by germs that could make reading difficult. The dishes are incubated at 30°C for 72 hours, after which characteristic colonies will appear. Only dishes containing between 30 and 300 colonies are taken into account.

Detection and enumeration of coliforms at 37°C

According to Rachedi (2020), coliforms are rod-shaped, Gram-negative, oxidase-negative, aerobic or facultative anaerobic bacteria.

Coliforms are detected by deep inoculation on Lactose-Bile-Neutral Red-Violet Crystal (VRBL) agar (Petranxiene and Lapied, 2002).

The double layer technique was used to count coliforms. VRBL agar is a selective medium for the isolation and enumeration of coliforms in milk and other dairy products, dairy equipment, and other foodstuffs. We used dilutions of 10-1 and 10-2 to detect total coliforms.

For each sample in which coliforms were counted, 1 mL of each of the two successive decimal dilutions was introduced into two sterile Petri dishes using a sterile graduated pipette. We then poured approximately 15 mL of VRBL agar onto the inoculum. We homogenized the mixture and allowed it to solidify. We poured a second layer of approximately 5 mL of VRBL into each dish.

We incubated the dishes at 37°C for total coliforms. Suspicious colonies appear red.

Detection and enumeration of *Escherichia coli* (E. coli)

Escherichia coli-β-glucuronidase positive is a bacterium that, at 44°C, forms characteristic blue colonies on tryptone bile glucuronidase (TBX) medium, under the conditions specified in this part of ISO 16649 (2001).

The procedure for counting E. coli (ISO 16649-2001) was carried out in TBX (tryptone bile glucuronidase) medium, in 1 ml of dilutions (10-1, 10-2). This sample was taken and then placed in Petri dishes; the mixture was homogenized by circular movement of the dishes, resulting in the flow of 10 to 15 ml of TBX medium. After solidification, 5 ml of this medium was poured again to avoid contamination. Incubation took place at a temperature of 44°C for 24 to 48 hours.

Detection and enumeration of Staphylococcus aureus: (ISO 6888-2: 2018)

Staphylococci were counted using the following procedure : (ISO 6888-2 : 2018) : Chapman Mannitol medium was used for this test. One (1) ml of each dilution was placed in Petri dishes, then 10 to 15 ml of Chapman medium was added. The mixture was

***Corresponding Author:** Renaud Nonmarmbaye

homogenized by swirling the dish. After solidification, a second layer of 5 ml was poured onto the surface. Incubation was carried out at 37°C for 24 to 48 hours. Microorganisms that ferment mannitol produce yellow colonies. This characteristic is a guiding criterion for the identification of Staphylococcus aureus. *Staphylococcus aureus* is counted by surface plating on a selective solid medium (Baird Parker). Incubation is carried out at 37°C for 24 to 48 hours. (Joffin and Joffin, 2000).

Detection and enumeration of Bacillus cereus : (NF ISO 7932: 2005)

Bacillus cereus is a bacterium that can be found in many environments, particularly at all stages of the food chain. *Bacillus cereus* is a recurring problem in equipment used in the dairy industry, and this bacterium is very common in all dairy products.

The culture medium, Mannitol Egg Yolk Polymyxin (MYP) agar, a selective and differential medium for Bacillus cereus, was chosen for the culture. Inoculation consisted of spreading 1 ml of dilution in each Petri dish. The dishes were read after incubation at 30°C for 24 hours ± 3 hours. The characteristic colonies are pink on the inside and dome-shaped.

Searching for and counting yeast and mold : ISO 21527-1 and 2: 2008.

We poured chloramphenicol Sabouraud agar into Petri dishes, seeded them with 1 ml of decimal dilutions, and incubated the dishes at 25°C for 5 days. Yeasts were identified by the development of creamy to white colonies, and molds were counted based on the development of filamentous colonies of different colors.

7. Detection and enumeration of Salmonella spp : NF ISO 6579: 2017.

Salmonella was detected in 25 g of sample diluted in 225 ml of EPT according to the following 4 steps:

- Pre-enrichment in a non-selective medium: this consists of incubating at 37°C for 24 hours in the stock solution;
- Selective enrichment: We inoculated 0.1 ml of the pre-enriched broth into 10 ml of Vassiliadis broth and incubated at 41.5°C for 21 hours ± 3 hours. At the same time, we seeded 1 ml of the pre-enriched broth into 20 ml of Müller Kaüffmann broth and incubated at 37°C for 21 hours ± 3 hours.
- Selective isolation: A streak from each of the enriched broths was seeded onto XLD and Hektoën agar plates, respectively. Incubation was carried out at 37°C for 24 hours. Colonies suspected of containing salmonella on XLD are red in color with or without a black center, and on Hektoën they are green in color with or without a black center.
- Purification: Suspected colonies are transferred to ordinary agar and incubated at 37°C ± 1°C for 24 hours ± 3 hours to obtain young, pure colonies .

Expression of results

Results are expressed in accordance with ISO standard 7218 of May 1996.

As a general rule: two successive dilutions that have produced at least one plate containing more than colonies are considered, and the weighted average is obtained using the following equation:

$$N = \frac{\sum C}{V \times (n1 + 0{,}1n2)\, d}$$

N = number of colonies; ∑C: total sum of colonies counted on the selected plates; V = dilution volume; 1/d = F = highest dilution factor counted ; n1: number of plates at the lowest dilution counted ; n2: number of plates at the highest dilution counted for boxes containing at least 10 colonies and where the number is between 10 and 150, the expression is:

$$N = \frac{\sum C}{V} \times \frac{1}{d}$$

Where N is the number of colonies, V is the dilution volume, and 1/d is the dilution factor.

In this study, we used a two-class sampling plan, with the samples analyzed divided into two categories: satisfactory and unsatisfactory or non-compliant, based on a limit value of "m=M."

- Quality is satisfactory when all observed values, N ≤ m,
- Quality is unsatisfactory or non-compliant when all observed values N > m.

Table 2: Criteria for interpreting results, (DSAa) : Luxembourg Food Safety Division (2016)

Result of the analysis	Conclusion
"Presence" of germs in a defined quantity of foodstuffs. • 1 result > m	"Unsatisfactory" quality
"Absence" of germs in a defined quantity of foodstuffs. • All results < or = m	"Satisfactory" quality

Data Analysis

For statistical analysis, our data was subjected to descriptive statistics and the various distributions were organized in tables, graphs, etc. using Microsoft Excel 2016.

***Corresponding Author:** Renaud Nonmarmbaye

RESULTS AND DISCUSSIONS

Results

During this study, several samples (20) were used to test for bacteria in raw cow's milk.

- Observations of personal hygiene rules Milking clothing
- Observations regarding personal hygiene and milking clothing revealed that five (5) out of 15 peoples complied with the rules, giving a percentage of 33.33%, while ten did not comply, giving a percentage of 66.66%.
- Compliance with hygiene rules for milking equipment
- Regarding the hygiene of milking equipment, we recorded a rate of 40%, representing six people who comply, compared to 80% who do not.
- Compliance with hygiene rules concerning udder cleaning
- When it comes to cleaning the udder before or after milking, we recorded a rate of 80%, representing twelve people who clearly comply, compared to 20%, corresponding to three people who do not comply.

Table 3: Results of the breeder technical data sheet

Hygiene	Personal hygiene Milking clothing		Milking equipment hygiene		Cleaning the udder before and after milking	
Terms	Yes	No	Yes	No	Yes	No
6th, 7th, and 10th	5	10	6	9	12	3
Percentage	33, 33 %	66,66 %	40 %	60 %	80 %	20

Average pathogen loads

The results of the strain count (Table IV) showed an average load of 4.83 × 10^5 CFU/g of aerobic microorganisms at 30°C in raw milk, with extreme values below 40 and above 3 × 10^6 CFU/g. For coliforms, the extreme values obtained were below 40 and above 1.5 × 10^5 CFU/g, with an average load of 2.64 × 10^4 CFU/g, considering the tolerance threshold set at 10^2 CFU/g. The samples analyzed were not contaminated with salmonella. Yeasts and molds are present with an average value of 1.8 × 10^4 CFU/g. For Bacillus cereus, the extreme values obtained range from 0 to 2.3 × 10^4 CFU/g, with an average load of 2.5 × 10^3 CFU/g. Staphylococci (Staphylococcus aureus) are present with an average load of 2.38 103 CFU/g, corresponding to extreme values below 40 and above 1.5 104 CFU/g.

Table 4: Bacterial counts isolated from raw milk (CFU/g)

Germ	Average	Minimum	Maximum
Microorganisms at 30°C	4,83 10^5	< 40	> 3.10^6
Coliform bacteria	2,64 10^4	< 10	> 1,5 10^5
Escherichia coli	0,13 10^2	< 10	40
Staphylococcus aureus	2,38 10^3	< 40	>1,5 10^4
Bacillus cereus	2, 5 10^3	0	2,3 10^4
Yeasts – Molds	1,8 10^4	< 40	> 1,5 10^5
Salmonella spp.	Absence in 25g		

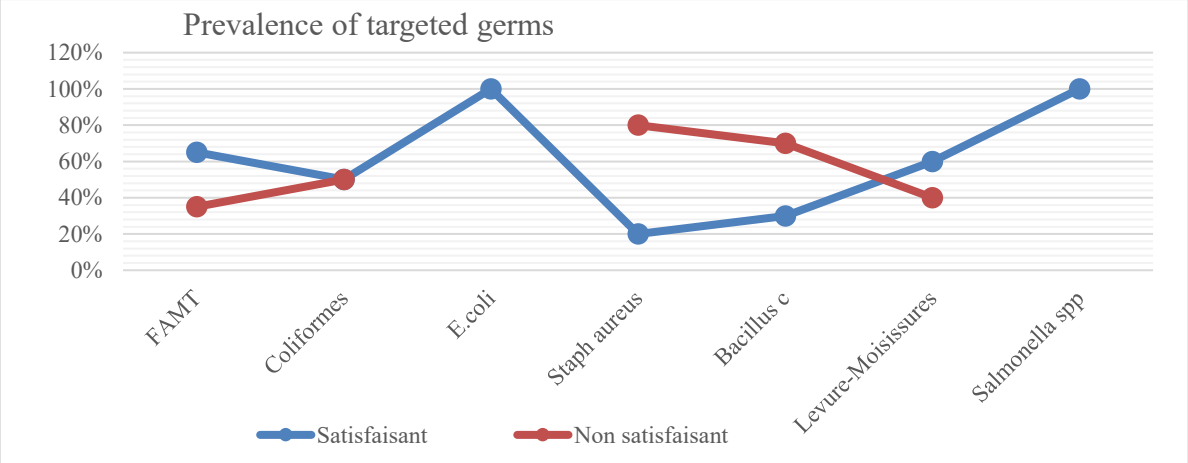

Figure 2: Prevalence of the germs tested for

DISCUSSIONS

This study, which took place in the city of N'Djamena, aimed to assess the microbiological quality of raw cow's milk sold in the open air in N'Djamena. It has been noted that raw milk is a perishable product, and bacteria that can cause contamination can multiply more

***Corresponding Author:** Renaud Nonmarmbaye

quickly, rendering it unfit for processing and human consumption.

We are interested in evaluating the microbiological quality of raw cow's milk, taking into account the results of analyses and discussing them in light of scientific knowledge and the results of other studies.

Based on the results of microbiological analyses of raw milk, we noted that out of 20 samples analyzed during the experiment, FAMT had an average load of 4.83 105 CFU/g, higher than the average of 1.08 108 CFU/ml in Benin according to the work of Farougou *et al.* 2011; These results are lower than those reported by Anago (2017). In northern Benin, with an average value of 6.38×10^6 CFU/g, the results vary between 1.6×10^6 and 2.5×10^6 CFU/ml, compared to those reported by Srairi *et al.* (2006).

In Algeria, research by Baazize (2006) shows that 81% are contaminated with FAMT.

According to the FAO (1998), milk from a perfectly healthy animal that has been milked under aseptic conditions is normally free of microorganisms. The number of germs leaving the udder is very low, generally less than 5,000 CFU/ml if standards are met.

Counting FMCA is a useful indicator for monitoring the sanitary conditions of raw milk production, but its presence may not indicate the direct source of contamination (Robinson, 2002). These germs may come from the water used during milking, either to wash the container used to collect the milk.

Our microbiological analysis results reveal an average coliform load of 2.64 104 CFU/g. However, our results contradict those of (Belarbi, 2015), which found an average load of 1.5 103 CFU/ml. In northern Benin (Anago, 2017), the average microbial load is 1.5×10^2 CFU/ml for total coliforms and 4.55×10^1 CFU/ml for fecal coliforms for the entire municipality.

In addition, certain strains can lead to intestinal infections, which can be explained by exogenous contamination. They could also originate mainly from the hands and hygiene of vendors, as well as from the sales environment (Kouamesina *et al.,* 2010).

The results of the research analyses showed no contamination of *Escherichia coli* in 25 g of all samples. This indicates no health risk for human consumption. In comparison, in Algeria (Gharbi *et al.,* 2019), the microbial load is ID : 2.6 ± 0.9 × 102 CFU/ml-1 ; D : 3.3 ± 1.0 × 102 CFU/ml ; these results are not similar to our results.

Microbiological analysis of the samples shows that staphylococci (Staphylococcus aureus) were recorded with an average load of 2.38×10^3 CFU/g. Meanwhile, Ghazi and Niar (2011) found an average load of 2.102 CFU/g. Although the microbial load in our S. aureus samples is below the accepted microbiological criteria, appropriate measures must be taken to counteract this contamination, as the presence of *Staphylococcus aureus* in food poses a potential risk to consumer health due to the production of enterotoxin (Godfay and Molla, 2000; Cenci-Goga *et al.,* 2003).

In Algeria (Belarbi, 2015 ; Feknous *et al.,* 2018), the samples analyzed showed a total absence of the bacterium.

Milk contamination is becoming a major public health issue, especially with the presence of Staphylococcus aureus, which is responsible for food poisoning.

The presence of germs considered pathogenic is probably due to poor hygiene in the containers used or dirty hands of the staff who handle the zebu cattle in the dairy industry.

The *Bacillus cereus* count gave a result with an average microbial load of 2.5 103 CFU/g.

Yeasts and molds are present with an average value of 1.8 104 CFU/g. In comparison, in northern Benin (Anago, 2017), the average yeast-mold load is 3.65×10^2 CFU/ml, with values of 1.2×10^2 CFU/ml in Kantchagou-tamou and 6.1×10^2 CFU/ml in Pouya. The contamination of milk by yeast and mold, as we have observed, is mainly the result of high external contamination and poor hygiene of the utensils used for milking and milk storage (Bonfoh, 2002).

The results of microbiological analyses for salmonella showed a total absence in 25g of all samples analyzed and are similar to those of (Anago, 2017) in northern Benin. This absence in 25g is consistent with those of (Maïworé *et al.,* 2018 ; Marnissi et al., 2013), who found no contamination in samples similar to our results.

CONCLUSION

Milk is a widely consumed foodstuff in the city of N'Djamena, as in all other cities in Chad and in other countries. The issue of quality is very important within a sector, as it largely determines its economic development. The major challenges are not only to guarantee food safety but also to ensure the sound economic development of the dairy sector.

The objective of our study is to evaluate the microbiological quality of raw cow's milk sold in open-air markets in N'Djamena. The study found that none of the samples analyzed met the Microbiological Criteria of the Food Safety Division (DSAa), with *Staphylococcus aureus* being the most prevalent contaminant. Furthermore, the total absence of *Salmonella spp.* and β-

***Corresponding Author:** Renaud Nonmarmbaye

glucuronidase-positive *Escherichia coli* bacteria in all raw milk samples indicates that the cows are in good health and that the milk remains of satisfactory microbiological quality despite the unsatisfactory results. The presence of bacteria is due to poor hygiene practices during milking, transport, and handling.

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