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RESEARCH ARTICLE

MICROBIOLOGICAL QUALITY OF VARIOUS MILK MILES PRODUCED IN URBAN ENVIRONMENT IN NIGER

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ABSTRACT

Milk, a complete, easily perishable food is processed to increase its shelf life, often even at the household level. One of its by-products, the curd of large consumption in Niger, could be at the origin of certain infections. The objective of this study is to contribute to the improvement of the hygienic quality of curd produced in households and small milk production units of Niamey 60 milk samples were collected including 30 raw and 30 curds. Thus, total aerobic mesophilic flora, total coliforms, Staphylococcus aureus, Escherichia coli, and Salmonella were determined. The results, presence of the total mesophilic aerobic flora in all samples ranging from 2.4.10 8,2.10 4 to 6 in the two types of milk;raw milk and curd were respectively 86.66% and 70% contaminated with total coliforms;33.33% of raw milk samples are contaminated with salmonella and 16.66% contaminated with curdled milk.Staphylococcus aureus is present(80%) in raw milk and 13.33% in curd, E-coli (50%) in sour milk and (53.33%) in raw milk.Lactobacilli oscillate 2,36.10 3,33.10 4 to 5. Yeasts were enumerated in 53.33% of the raw milk and in 70% of the curd, the molds were present in 3.33% of the samples of the two types of milk. High levels of coliforms, E. coli, Staphylococcus aureus and Salmonella show that milk (curds and raw) productsand sold in Niamey and its peripheries represent health risks for consumers and consequently for public health.Permanent support of producers to good production practices is essential to protect consumers from contamination.

INTRODUCTION

Even today, milk is an exclusive source of food for certain populations of all ages. In the same way, the milk of the animals replaces in many foci, the breast milk for the infant. Milk is used in a variety of other ways and it is when it combines with other foods in a combination diet that milk becomes very valuable. Thus, the complementarity relations that exist between milk and cereals are a well-established phenomenon: lactic proteins provide lysine, tryptophan and other amino acids thus improve the biological value of the proteins of the mixture (MADOUGOU, 2010). In the class of mammals, the first days of life are ensured exclusively by milk, which is the only food of the newborn. To play this role, milk should be a complete feed that contains most of the nutrients needed for the growing needs of the young. Milk is pretty much the only food that can balance most of a man's nutritional needs. We understand why at the dawn of civilization, the man used for his food the milk of large domestic animals (Madougou, 2010).

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In Niger, traditional peri-urban dairy farming systems are the work of ethnic groups (Fulani or Tuareg) who have ancestral farming practice. These populations, transhumant, have gradually settled around cities to meet everincreasing urban milk demand (Viasetal, 2003). These producers have settled around the city with 72% within 15 km and 28% beyond 15 km (SIM B, 2014). Milk is a valuable nutritious food that has a short shelf life and must be handled with care. Milk is very perishable because it is an excellent growth medium for microorganisms - particularly pathogenic bacteria - that can alter the product and cause disease in consumers.http://www.fao.org/agriculture/dairygateway / milk processing / en / #. WJtw 6L-nIUviewed on 19/02/2017. In general, milk includes four types of important constituents, namely: lipids, consisting essentially of ordinary fats (triglycerides), proteins (casein, albumin and globulin), carbohydrates, essentially lactose, salts.But many other constituents are present in minimal amounts such as vitamins, enzymes, nucleotides, dissolved gases;don t have some great importance due to their biological activity. This composition varies according to different factors generally related to animals and the environment.(VIGNOLA, 2002) cited by CONTE, 2008.

Milk contains few microorganisms when taken under good conditions from a healthy animal (less than 103 germs / ml).It is essentially saprophytic germs of udders and ducts galactophores: micrococci but also lactic streptococci (Lactococcusand Lactobacillus). Raw milk is protected against bacteria by inhibiting substances called " Lactenins " but their action is of very short duration (about 1 hour) (GUIRAUD, 1998).Other microorganisms can be found in milk when it comes from a sick animal. They are generally pathogenic and dangerous from a health point of view (CONTE, 2008). The lactic microflora of milk is part of the normal flora of milk and is characterized by its ability to ferment lactose with the production of lactic acid and thus a lowering of pH. (ALAIS, 1984, CLAUDE and CHAMPAGNE, 1998). Products derived from milk processing are often prepared under questionable hygienic conditions. Few studies have been conducted on curdled milk in Niger.Indeed, this work puts a particular emphasis on the production of curd in some households and small dairy units in the city of Niamey (Niger). As curd is an important part of people's eating habits in Niamey, it is therefore necessary to evaluate the nutritional microbiological quality of this foodstuff. The overall objective of this study is to contribute to improving the hygienic quality of curd produced in Niamey's households and small milk production units.

MATERIAL AND METHODS

Geographical location of the study area: The communes I, II, III, IV are all on the left bank of the Niger River while the commune V is on the right bank. Figure 1 illustrates the map of the five municipalities in the Niamey region, of which four municipalities are concerned by this study (I, II, IV and V).

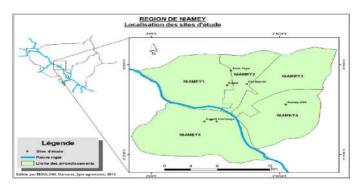


Figure 1. Map of the urban community of Niamey

Biological material

It consists of 60 milk samples, including 30 raw milk and 30 sour milk from the three communes.

Collection equipment

- a cooler + ice, for transporting samples of curds and raw milk under cold conditions;
- Sterile bags to collect the samples to be analyzed; a marker for the numbering of the samples;

MICROBIOLOGICAL ANALYSIS METHODS

Preparation of the stock solution and decimal dilutions: In this work, the following stages of analysis will be ideal for all microorganisms and for all products. In a flask containing 225

ml / 90 ml of buffered peptone water (EPT) or distilled water, 25 ml / 10 ml of the analyte is added. The whole is mixed using a sterile pipette .The mother solution thus obtained corresponds to a 1/10 dilution, ie D1 (10⁻¹). From D1, successive dilutions were carried out in test tubes each containing 9 ml of peptone water (PE). From D1 to 1/10 (225 ml of EPT + 25 ml of the product), 1 ml of the same pipette is withdrawn and transferred to a test tube containing 9 ml of EP.This gives a dilution to 1/100 ie D2 (10^{-2}) and homogenized with another sterile pipette. From D2, the same operations are carried out in order to successively obtain dilutions at 10 $^{\text{-3}}$, 10 $^{\text{-4}}$, 10 $^{\text{-5}}$, 10 $^{\text{-6}}$, 10 $^{\text{-7}}$;10 $^{\text{-8}}$ is D3, D4, D5, D6, D7, D8. The product or its decimal dilutions were used for the seeding of specific culture media for the purpose of isolation and numeration of the desired microorganisms. The incubation is done in boxes in the inverted position (lids at the bottom) to avoid the confluence of the superficial colonies because of the condensation water on the lid.On reading, the number found is multiplied by the dilution factor.

Enumeration of total aerobic mesophilic flora at 30 ° C

It is performed by mass counting on PCA (Plat Count Agar) media. The total flora count (mesophilic aerobic flora) was carried out according to the international standard ISO 4833, May 2003. The seeding is done in double test on the medium "Agar Plate Count Agar (PCA)" with 0.1ml sample. The dishes are incubated in an oven set at 30 $^{\circ}$ C for 72 h \pm 3h.

Research and enumeration oftotal formsof coli

Total coliforms were counted according to the international standard ISO 4832 (2006). Seeding is done on EMB agar (Eosin Blue Methylene). The dishes are incubated at 37 $^{\circ}$ C for total coliforms and at 44 $^{\circ}$ C for thermotolerant c oliforms , in an oven for 24h \pm 2h. Typical colonies are bright red to pinkish or purple because fermenting lactose and have a diameter of more than 0.5 mm.

Enumeration of fungal flora (Yeasts and molds)

The international standard ISO 7954 (1988) was used for the enumeration of yeasts and molds. Seeding was carried out with 0.1 ml of the sample on SABOURAUD agar with chloramphenicol in Petri dishes. The dishes are incubated at 25 $^{\circ}$ C./30 $^{\circ}$ C. in an oven for 3, 4 or 5 days.

Research and enumeration of Staphylococcus aureus

This research is carried out according to standard NF-V08-057-1 (November 1994).Baird Parker (BP) is used as the culture medium, to which egg yolk with tellurite or Chapman medium is added.The dilutions used are $^{\rm 1}$ and 10 $^{\rm 2}$.Seeding is done on the surface with 0.1 ml by dilution on BP previously cast in Petri dishes and incubated in an oven + 37 $^{\circ}$ C for 24 hours. Colonies of Staphylococcus aureus appear shiny black, curved and surrounded by an opaque white border and a lightened halo.

Search for Escherichiacoli

This study is based on four properties of E.coli: it is able to develop in a bile medium, it supports a temperature of 44 ° C, it ferments lactose by producing gas, it is indologen.

Two tubes of bright green birch lactose broth (BLBVB) containing Durham bells were seeded with 1 ml of the product and incubated 48 hours at 37 ° C. The bright bile-green combination inhibits most enterobacteria. The fermentation of lactose which results in the appearance of gas in the bells of Durham signs the presence of coliforms. This gas release must be at least equal to one tenth of the volume of the bell so that the test can be considered as positive. At this stage, E. coli is identified by the Mackenzie test. The positive cultures are parallel transplanted on BLBVB and on indo-free EP, then incubated 48 hours at 44 ° C. The presence of E. coliis confirmed in the only case where there is:

- Gaseous release in BLBVB's Durham bells,
- Indole production, revealed by the addition of Kovacs reagent.

The indole produced by E.coli gives, with the acyl alcohol contained in the Kovacs reagent, a coloration ranging from pink to red.

Salmonella search

For the detection of salmonella in milk, the following scheme was defined by EDEL and KAMPELMACHER (1969) and retained by National and International Standards:

Pre-enrichment (6-18 hours)

D1 suspension was incubated at 37 °C for 24 hours. This phase aims to allow injured (stressed) bacteria to recover their stability. It is therefore necessary to push the selection even further.

Enrichment (in selective liquid media 24-48 hours)

At the end of these 24 hours, 10 ml of the pre - enrichment medium are removed and added to 100 ml of Na tetrathionate broth and brilliant green (MULLER- Kauffmann). Bright green inhibits Gram + hulls.Incubation is at 37 or 44 ° C for 24-48 hours.

This medium promotes salmonella growth, even in the presence of a competing polymicrobial population .The following broths can also be used:

- Selenite broth of Na (with or without Cystine and Novobiocine).
- RAPPAPORT-VASSILIADIS broth (rv10) with magnesium chloride or Malachite Green.

Isolation on solid selective media

It is carried out on solid selective media from selective liquid enrichment media. Incubation of the selective media is at 37 °C for 24 to 48 hours. These media mainly contain selective agents (bile salts and colorants for example), sugars including lactose, salts to reveal the production of H $_2$ S and pH indicators. Bile salts also inhibit flora and accompany salmonella. The main media that can be used are: bright green agar, Hektoen agar, DCLS agar, XLD agar. Also suitable are SS agar, Mc CONKEY agar, Bismuth sulfite agar.

Identification

It is carried out from the enrichment medium in parallel on the bright green and phenol red agar (VBRP) and on the

deoxycholate citrate lactose sucrose (DCLS) medium. The culture is streaked on previously solidified media. The incubation is conducted at 37 °C for 24 to 48 hours. On reading, the germs fermenting lactose turn the medium to yellow. Salmonella does not ferment lactose, their colonies will be smooth and red on VBRP and red or colorless (lactose - and saccharos e -) black center (H2S + colonies) or not on DCLS. These indications of culture and color are in fact only a presumption. Thus a thorough search on Kligler's medium is carried out. The pellet is seeded by puncture, the slope streaks . Salmonella are - Glucose +, yellow pellet,

- Gas +: air bubbles in the pellet,
- -H₂ S + black ring between base and slope, black net along seeding line in base, lactose - and gives a red color on the seed slope.

For further clarification, confirmation can be continued by testing urea-indole, orthonitrophenyl, galactopyranoside (ONPG), lysine decarboxylase (LDC). The French Api 20 E system facilitates this identification. Salmonella are: indole -, urea - ONPG -, LDC +.

Lactobacillus Research

The enumeration of the lactic acid bacteria was carried out according to the ISO standard 21414 (1998). Seeding is carried out with 0.1 ml of the sample in Petri dishes containing agar ManRogosa and Sharp or MRS agar. The incubation of the dishes is carried out at 37 °C in hermetically sealed anaerobic jars (Biolab) containing CO2 generators (anaerocults) and placed in an oven for 72 to 96 hours.

Expression of Results

The formula for the expression of the results is the following:

$$N = \frac{\sum C}{V \times 1.1 \times d}$$

N = Number of microorganisms per gram or per milliliter of product, expressed as a number between 1.0 and 9.9 multiplied by 10^{x} (where x is the appropriate power of 10).

- ΣC is the sum of the colonies counted on the two boxes kept after two successive decimal dilutions, at least one of which contains at least 10 colonies.
- V is the volume of inoculum placed in each box, in milliliters.
- d is the dilut ion corresponding to the first dilution retained (d = 1 when the undiluted liquid sample is retained).

Nb: For technical reasons, the determination of lactobacilli was made in Ouaga (Burkina Faso), only twenty (20) samples of laurel were analyzed.

Data entry and analysis: The data collected were analyzed using one-way analysis of variance (ANOVA) at p = 0.05.

RESULTS AND DISCUSSION

Results and comments

Results of microbiological analyzes of raw milk: It is apparent from Table I that more than 50% of contaminated

samples by Staphylococcus aureus are off-specification, 33.33%, 46.66 and 30% are respectively contaminated by aerobic mesophilic floras total, Escherichia coli, and Salmonella are out of the ordinary.

associated with contamination of fecal origin and significance of testifying hygienic conditions deteriorated during milking, teat skin of improperly cleaned or during transport (Bachtarziet al., 2015).

Table 1. Microbiological analysis results of raw milk by grouping

GR (cfu/ ml)	BORN	NEC	% EC	MGT / ml	MxGT/ ml	NEHN	% HN
FAMT	30	30	100	2,410 4	8.2.10 ⁶	10	33.33
CT	30	26	86.66	<10	2,110 4	0	0
E-coli	30	16	53.33	<10	1.40 ²	14	46.66
stp aureus	30	24	80	<10	1,610 4	16	53.33
S	30	10	33.33	<10	10 ¹	9	30
yeasts	30	16	53.33	<10	1,410 4		
molds	30	1	3.33	<10	4		

GR: Wanted Sprouts; BORN: Number of samples; NEC: Number of samples; MGT: Minimum of found germs / ml; MxGT: maximumfound germs / ml; NEHN: number of exceptional samples FAMT: Aerobic total mesophilic flora, CT: total coliform; E-coli: Escherichia coliStp: Staphylococcus, S: Salmonella, E: E-coli: Escherichia coli; Stp:Staphylococcus, S: Salmonella, E: sample, FAMT: aerobic flora, total mesophilic, CT: total coliform, S: salmonella, EchC: Echerichiacoli, Ms: mold, Lb: Lactobacillus.

Table 2. Results of microbiological analyzes of curds by grouping

GR	BORN	NEC	% EC	MGT / ml	MXGT / ml	NEHN	% HN
FAMT	30	30	100	2,4,10 4	8.2.10 ⁶	30	100
CT	30	21	70	<10	3.6.10 4	21	70
E-coli	30	15	50	<10	4.10^{2}	15	50
stp aureus	30	21	70	<10	$1.4.10^{2}$	4	13.33
S	30	5	16.33	<10	3.10 1	5	16.66
yeasts	30	21	70	<10	$1.6.10^{4}$	21	70
molds	30	1	3.33	<10	$1.3.10^{-1}$	1	3.33
lactobacilli	20	20	100	2,36.10 4	3.33.10 5	0	100

GR: Wanted Sprouts; BORN: Number of samples; NEC: Number of samples; MGT: Minimum of found germs /ml; MxGT: maximum germs found / ml; NEHN: number of exceptional samples; FAMT: Aerobic total mesophilic flora, CT: total coliform; E-coli: Escherichia coli Stp: Staphylococcus, S: Salmonella

The microbiological analysis of curd (Tables II) shows that all the samples analyzed have a microbiological load above the standard for FAMT. 70% of samples contaminated with total coliforms and yeasts have a higher than normal load; 50%; 13.33%; 16.66% and 3.33% contaminated respectively by E-coli, Staphylococcus salmonella and mold are out of the ordinary.

DISCUSSION

Aerobic total mesophilic flora: Aerobic total mesophilic flora is considered as a general indicator of the overall quality of the dairy product.It reveals the conditions of production, more particularly the hygienic practices during milking. The enumeration of this total mesophilic aerobic flora for the 60 milk samples analyzed (raw and curd) showed that there is a significant contamination. The mean value of the raw milk MILT analyzed (1.33 10 5 CFU / ml) is lower than that found in South-Togo by Seme.KPitala.W, Osseyi, G. E (2015) on Nutritional and Hygienic Quality of suckling cow milks (5.9 10 ⁵ CFU / ml), also inferior to the results obtained by Taybiet al.(2.15 10^{-7} CFU / ml) (2014).For curdled milk the average value of FAMT is $2.17.10^{6}$ CFU / mlgreater than normal (≤ 10 ⁴ CFU / ml), The search for indicator microorganisms of faecal contamination makes it possible to judge the hygienic state of a product such as milk. Even at low levels, they would testify to degraded hygienic conditions during milking, transport, or processing.

Total coliforms

Raw milk

The average value of total coliforms is $2.14110~^3 CFU$ / ml.These levels of contamination exceed the current standards of $10~^3$ CFU / ml.The presence of Cototal liformes is often

Our results are superior to those reported by Labiouiet alin 2009 who achieved an average of $2.0\ 10^4$ CFU / ml, however they are lower than the counts ($3.02\ 10^5$ CFU / ml) found by Taybiet al, 2014 in Morocco.

Rotten milk: The average total coliform content in curd is 2.46×10^3 corresponding to a contamination rate of 70%. Our results are similar to those of NAMEGNI (2006) who counted 84% of the total coliform-contaminated curd samples. On the other hand, our results are superior to those obtained by NJASSAP (2001) in Cameroon, which counted 57% of the samples which are contaminated.

Staphylococcus aureus: Our analysis revealed that 53.3% of raw milk and 13.33% curds contaminated parStaphylococcus aureus. The presence of staphylococci in milk may have two main origins, either as a result of primary contamination, due to the presence in a herd ofStaphylococcus aureusmastitis, or it is ahumancontamination. Our results are similar to those obtained by HAMZA (1996) on the artisanal curd "TarmamounAdar" in Niger of which 18.18% of the samples were contaminated. NJASSAP (2007), found for the same type of study in Cameroon, a contamination rate of 37% by Staphylococcus aureus in raw milk, lower than our results.

Escherichia coli: It was found in 50% in curd and 53.33% in raw milk, which is well above the standards. The lower rate incurd versus raw milk is thought to be due to the effect of acidity which significantly reduces the percentage of E. Coli (NDIAYE A., 1994). Contamination can be of faecal origin, cleaning with contaminated water or from a mastitis Ecoli (BACHTARZI et al, 2015). Its presence in a water or food suggests that there wasduring their preparation a lack of hygiene that led to defilement byfeces. For NJASSAP (2007), the simple fact of finding E. coliin food, even in large numbers, is not enough to say that it is pathogenic. Some only

can be pathogenic for man andcause gastroenteritis.It is therefore important to make a typing for determining souch are enteric,

Salmonella

Salmonellae were found in 10 samples of raw milk and 5 samples of curdled milk which corresponds respectively to 33.33% and 16.66%. This low level of salmonella in curdled milk maybe justified not only by the pasteurization used by some households before milk curdling but also by the high sensitivity of salmonella to acidic pH. Indeed, POUEME NRS, (2006) found that salmonella do not resist pHs between 4.6 and 4.8.

Lactobacillus

The analyzes gave important values of lactobacilli in curd ranging from $2.36 \times {}^{10}$ to 3.33×10^{-5} (Table II). The number of lactobacilli of all samples is in accordance with the required standard (> 10^{-8}). Nevertheless, our results are inferior to those found by BIATCHO (2006) with lactobacilli in the order of 10^{-8} seeds / g in artisanal curd.

Fungal flora: Yeast and mold

Yeasts are found in 53.33% of raw milk and in 70% of curd, mold is present in 3.33% of samples of raw milk and curd. According to DIENG (2001) molds are not impeded by acidity, sucrose and lactose residue el, which is a source of energy for them. The fungal flora comes from poor hygiene conditions during handling, sale and ambient air. Ineffective or incomplete pasteurization as well as a defection of the cold chain, as well as a bad closing of the boxes are all factors favorable to their development. Our results are similar to those obtained by Diallo (1995) who found for the same type of product, a contamination of 75% by the lev ures and 26% by the molds. The presence of certain microorganisms could be justified by the certain hygiene practices observed from milking to manufacturing. To wash the containers 51% of the respondents use simple water (tap or borehole) against 49% who use soap.

Conclusion

This study focused on the microbiological analysis of curd milk products in households and small dairy units in Niamey. The evaluation of the microbiological quality of the raw milk and the curd showed a presence of total mesophilic aerobic flora in all the samples of the two types of milk of total analyzed.High levels coliforms, E. Staphylococcus aureus and Salmonella indicate somewhere that hygienic conditions are not or poorly met. As a result, the consumption of these products could pose a significant public health risk.It would therefore be important to continue studies in the same areas and to determine fecal coliforms and lactobacilli, and to broaden the scope of studies throughout the national territory to obtain a general overview of the quality of milk sold in Canada. Niger.

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