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Research Article

Staphylococcus in Raw Cow's Milk from Maroua (Cameroon), Determination of Their Resistance Level to Antibiotics

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Abstract

Raw cow's milk produced locally is a popular commodity but, its quality is a hindrance for the consumers. The objective of this study was firstly to identify the *Staphylococcus* present in raw cow's milk collected in Maroua (Cameroon) and, secondly to assess their level of resistance to antibiotics. Physico-chemical analyses based on the temperature, pH and titrable acidity were carried out. For the microbial analysis, the plating method was used to isolate and, the count of the total mesophilic aerobic flora (TMAF) and *Staphylococcus* was done. After identification, the resistance of *Staphylococcus* to antibiotics was assessed. The results obtained revealed that the temperature value was between 34.3 ± 0.282 and 36 ± 0.212°C; the pH between 5.18 ± 0.32 and 5.80± 0.2 and the density between 1.025 ± 0.0007 and 1.030 ± 0.0021. Microbiological analyses revealed that TMAF ranged from (9± 0.9)10^3 to (7.2± 0.9)10^9 CFU/mL while the abundance of *Staphylococcus* ranged from (1.2 ± 0.3)10^3 to (1.03± 0.08)10^4 CFU/mL. Nineteen (19) species of *Staphylococcus* were identified with a predominance of the *aureus* specie (68.62%). The antibiogram revealed 95% resistance to β-lactam, 78% to Macrolides, 42% to Glycopeptides, 16% to Quinolones, 5% to Aminosides and Cotrimoxazole and 0% to Chloramphenicol. Raw milk from Maroua has a high rate of *Staphylococcus* antibiotic-resistant.

Keywords: Raw Milk; *Staphylococcus*; Antibiotics; Resistance; Maroua

Abbreviations

FAO: Food And Agriculture Organization; N: North; E: East; ALT: Altitude; Ph: Potential Of Hydrogene; TMAF: Total Mesophilic Aerobic Flora; CFU: Colony Forming Unit; S1: Site1; T: Temperature; AFNOR: Association Française De Normalisation; S: Sensitive; R: Resistant; I: Intermédiaire; B-Lac: B-Lactam; GLYC: Glycopeptides; Amin: Aminosides; MACR: Macrolides; Quin: Quinolones; CHLO: Chloramphenicol; COTR: Cotrimoxazole; NITR: Nitrofurantoine; Fus A: Fusidic A; DAPT: Daptomycin; RIFA: Rifampicin; ANOVA: Analysis Of Variance

Introduction

Milk is a basic food in the human diet with great value thanks to its composition in nutrients like proteins, lipids, carbohydrates and calcium. It also plays an important role in food security for many people [1-3]. Nowadays, the needs for milk are more and more important since this product can be consumed in the fresh state, but also in pasteurized form, sterilized or transformed into derived products such as: fresh cheese, butter and milk fermented [4]. However, milk constitutes an excellent substrate for the proliferation of pathogenic germs thus, an important source of microbial infection for the consumers without pasteurization [1,5,6]. The milk contamination may be due to intrinsic and extrinsic factors. It can come from poor hygiene and health status of the cow, mastitis prevalence, production environment, and milking room and milk conserving practices in dairy farm [6-8].

In Maroua, raw milk is produced by cows raised in poorly maintained pens and where basic hygiene practices are not respected. These conditions generally affect the physico-chemical and microbiological quality of milk [9]. The milks produced in these conditions contain several opportunistic and potentially pathogenic germs such as *Salmonella, Streptococcus* and *Staphylococcus* [10]. It was shown that the microbiological quality of dairy products reflects good hygienic practices during the dairy milking process; raw milk contamination could occur in diseased or infected cows with environmental bacteria [11].

Microbiological analyzes of some samples of raw and fermented milks in Maroua have revealed that the majority were not of good quality [9,12]. These milks have a high level of opportunistic and pathogen bacteria, among which there is *Staphylococcus* and particularly the species *aureus*. In humans *Staphylococcus aureus* is responsible of food poisoning, skin diseases and in some cases of sepsis which can cause death [13,14]. Several strains of *Staphylococcus* show a high level of resistance to antibiotics [15,16]. Information on the antibiotic resistance level of *Staphylococcus* present in food could contribute greatly to the control of transmission's chain in bacterial resistance to humans. The objective of this study is to identify staphylococci in raw milks from Maroua (Cameroon) and evaluate their resistance to antibiotics.

Materials and Methods

Study zone and sampling

Raw milk samples were collected from farmers during milking time (between 5 a.m. and 6 a.m.) in five different quarters of the city of Maroua in the Far North Region of Cameroon: Domayo, Hardé 1, Missingueleo, Harde 2 (Table 1). This region have a favorable environment for the development of livestock farming, milk and its products are thus an integral part of the diet. The milk produced by cows is used for domestic consumption and the left over is sometimes sold for the needs of the whole family. The milk samples were from manual milking in the morning before the animals leave the sheepfold. A total, 30 raw milk samples were collected from five sedentary farmers in the city of Maroua from January to March 2018. Six samples by producers were collected: for physicochemical analyzes, 4 samples were used to determine the temperature and the pH of the milk at the outlet of the udder and to measure the density of milk in the laboratory; 2 other samples were used for microbiological analysis in laboratory. The milk samples collected were collected in sterile 500ml glass bottles, labeled and then sent to the laboratory at 4°C for further analysis in a cooler containing carboglaces.

Sites	Samples	Geographic coordinates
Kakataré	6	N 10. 59980 E 014. 32036 ALT : 398m
Hardé 1	6	N 10. 58792 E 014. 32609 ALT : 399m
Domayo	6	N 10. 35155 E 014. 19357 ALT : 400m
Missinguelow	6	N 10. 60763 E 014. 29580 ALT : 409m
Hardé 2	6	N 10. 58790 E 014. 32616 ALT : 388m

Table 1: Sampling sites.

Physico-chemical analysis

Immediately after milking, the temperature and pH at the outlet of the cow's udder were measured in situ using a pH meter (HANNA, HI9124) and a digital electronic thermometer (Matter Flo, 250513). Electrodes of these devices were immersed in 50ml of raw milk and the values increased after stabilization. This opera-

tion was repeated three times. The density was measured using the technique described by [17]. The samples were homogenized and then poured into a 500ml test tube, avoiding the formation of foam and taking care to fill it. The thermolactodensimeter (Gerber) was then gently immersed in the milk, holding it in its descent to near its equilibrium position and taking care to let it float freely. A slight rotational movement was then administered to the thermolactodensimeter. The gross density and the temperature were then read. When the temperature was different from 20°C, corrections were made by adding 0.0002 per degree if the temperature is above 20°C or by reducing 0.0002 on the gross density if it is lower than the standard temperature.

Microbiological analysis

Samples preparation

The microbiological methods used are those described by [18]. After homogenization of the raw milk samples using a vortex, series of decimal dilutions were carried out by adding 1 mL of each pure sample to a test tube containing 9 ml of peptone water (Roseto Abruzzi, Italy). This operation was repeated ten times in test tubes until the tenth dilution was obtained. The dilutions were homogenized and then added to culture media based on the desired microorganism.

Total mesophilic aerobic flora (TMAF) and *Staphylococcus* counts

For the enumeration of the total mesophilic aerobic flora (TMAF), 100 µl of each dilution was inoculated on the sterilized PCA agar (Plate Count Agar, Roseto Abruzzi, Italy). After spreading the inoculum, the petri dishes were incubated at 30°C for 72 hours. The colonies obtained were counted and the results were given in Colony-forming Unit per ml of milk (CFU/ mL) [19].

For the enumeration of *Staphylococcus*, 100 µL of each dilution was inoculated on the surface on the sterile Chapman agar (Humeau, France). The inoculated petri dishes were then incubated at 37 ° C for 24 hours.

Characterization and identification of *Staphylococcus*

The colonies obtained were characterized by several tests. First, Gram staining was carried out on the different colonies and only the Gram + coccus arranged in clusters were retained. The catalase test was also carried out on the colonies obtained. The search for DNase (Deoxyribonuclease) was done by cultivating a presumptive *Staphylococcus* on the DNase agar (Difco, Detroit), the petri dishes were incubated at 37°C for 24 hours. After this the dishes were flooded with hydrochloric acid and the excess liquid is poured out. The presence of a clear halo around the colony shows the activity of DNAse and the bacteria is categorized *Staphylococcus aureus*. These tests permitted to differentiate *Staphylococcus aureus* (Coccus Gram +, Catalase + and DNase +) from the other *Staphylococcus* (Coccus Gram +, Catalase + and DNase-) [20]. DNAse negative bacteria were identified as *Staphylococcus spp*.

Antibiogram and resistance percentage of *Staphylococcus*

The disk diffusion method was used to confirm the susceptibility of *Staphylococcus* to antibiotics Standardized disks, according to the recommendations of the European antibiogram committee [21]. In this method, the isolated *Staphylococcus* was suspended and standardized through a turbidity test. An inoculum of 0.5 opacity on the Mc Farland scale was made in a test tube containing distilled water. The previous suspension was then inoculated onto the solidified Mueller-Hinton (Biokar, France) agar, and the antibiotic-treated paper was tapped on the inoculated plate. The dishes were incubated at 35 °C for 24 hours to allow the discs containing antibiotics to diffuse through the solidified agar resulting in the formation of an inhibition zone. After this, the size of the inhibition zone formed around the paper disc was then measured and then interpreted according to the method of [21,22]. The inhibition size zone corresponds to the concentration of the antibiotic. The resistance percentages of *Staphylococcus* against different families of antibiotics were then calculated. The antibiotics used are listed in table 2.

Statistical analysis

The variance of the different repetitions was obtained by using Microsoft Office Excel 2007 software. On Statgraphics Centurion 17.1.06. Software, ANOVA with one factor, followed by the Duncan test was performed to compare the different samples of raw milk from each site.

Family	Antibiotic	Load	Symbol
Beta-lactams	Penicillin G	10UI	P
	Oxacillin	5 µg	OX
	Cefotaxime	30/10 µg	CTX
	Meropenem	10 µg	MRP
	Daptomycin	30 µg	DAP
	Moxalactam	30 µg	MX
Glycopeptides	Teicoplanin	30 µg	TEI
	Vancomycin	10 µg	VA
Aminosides	Gentamycin	10 µg	GEN
	Tobramycin	30 µg	TOB
	Amikacin	30 µg	AK
	Netilmycin	30 µg	NET
	Isepamicin	30 µg	ISE
Macrolides and related	Erythromycin	30 µg	E
	Lincomycin	10 µg	L
	Clindamycin	2UI	CD
	Spiramycin	30 µg	SPR
	Pristinamycin	15UI	RP
	Telithromycin	15 µg	TEL
Quinolones and Fluoroquinolones	Ofloxacin	5 µg	OF
	Levofloxacin	5 µg	LEV
Phenicols	Chloramphénicol	30 µg	C
Sulfamides	Cotrimoxazol	25 µg	COT
Nitrated products	Nitrofurantoin	100 µg	NIT
Others	Rifampicin	30 µg	RIF

Table 2: Antibiotics used for Staphylococcus antibiograms.

Results and Discussion

Physical quality of raw milk

The physicochemical characteristics (pH, temperature and density) of raw milk analyzed are presented in the table 3. The pH values vary between 5.18 ± 0.32 (S2) and 5.80 ± 0.2 (S4) with 100% of the samples that do not respect the standards value. The temperature of the milk measured directly at the outlet of the udder is between 34.3 ± 0.282 ° C (S2) and 36 ± 0.212 ° C (S1). The density ranges from 1.025 ± 0.0007 (S2) to 1.030 ± 0.0021 (S5) with 80% of raw milk samples having a density below the standard (1.028 < normal density > 1.033. There was a significant difference between the samples for the three parameters studied (P > 0.05).

Sites	P^H	Density	T (°C)
Norms	**6,6-6,8**	**1,028-1,033**	
S1= Kakataré	$5,60 \pm 0,4^{ab}$	$1,025 \pm 0,0014^{a}$	$36 \pm 0,212^{d}$
S2= Hardé 1	$5,18 \pm 0,32^{a}$	$1,025 \pm 0,0007^{a}$	$34,3 \pm 0,282^{a}$
S3= Domayo	$5,70 \pm 0,3^{ab}$	$1,025 \pm 0,0014^{a}$	$35,2 \pm 0,141^{bc}$
S4= Missinguelow	$5,80 \pm 0,2^{b}$	$1,025 \pm 0,0014^{a}$	$34,4 \pm 0,353^{ab}$
S5= Hardé 2	$5,49 \pm 0,^{21}ab$	$1,030 \pm 0,0021^{b}$	$35,3 \pm 0,070^{c}$

Table 3: Paramètres physico-chimiques du lait cru Values followed by different letters are significantly different (P < 5%).

The normal pH of raw milk should range between 6.6 and 6.8. However, all the milk samples analyzed (100%) have values below 6.0 and do not respect the standards of AFNOR. It is well known that value of a sample depends on when the milk was produced by the cow, any processing done to the milk, and how long it has been packaged [23]. The samples of this study were collected in dry season. This variability in raw milk could be linked to the climate, the lactation stage, the food availability, the water intake, the health status of the cows and the milking conditions [24]. The average values of the milks analyzed are lower than those obtained by Labioui [25] in Morocco.

Average density values were lower compared to the norm, with 80% of samples of which the density is less than 1.028. Below the density value of 1.028, the raw milk is considered as wet [9]. In the environment of this study, this frodulous practice is used by farmers and sellers of raw milk with the aim of increasing the quantity of their product and, by extension, their income [26,27]. The density depends on the dry matter, fat content, the increase in temperature and food availability. Although it is known that water was added in our samples, It was also proved that with increasing temperature, the density and dynamic viscosity of cow milks lowered [28].

The results revealed higher average temperature values. Theses temperatures were closer to the ambient temperature during the study period Insofar as in dry season the temperature was varying from 38 to more than 42°C. In fact, Some studies [5,29] revealed that the temperature of milk varies according to the ambient temperature with low temperatures of milk in the rainy and cold season against temperatures which can go up to 44°C in hot and dry season in N'Djamena in Chad. With higher temperatures, preserv-

ing raw milk becomes difficult due to the proliferation of potentially pathogenic germs capable of altering the quality of the milk [29] and causing illness to consumers [30].

Microbiological quality of raw milk

Abundances of TMAF and *Staphylococcus*

The microorganism's concentration of the samples examined varied in function of different farmers (Table 4). Aerobic plate counts or TMAF ranged from (9 ± 0.9) 10^3 (S4) to (7.2 ± 0.9) 10^9 CFU/mL (S3). The difference between the samples S1, S2, S4 and S5 was not significant but significant between all the samples and S3. About 80% of samples conforming to standards (105 CFU/ml), only site S3 has a microbial load higher than the standard. The concentration *Staphylococcus* ranged from (1.2 ± 0.3)10^3 (S4) and (1.03 ± 0.08) 10^4 CFU / mL (S5). The difference was significant between all the samples analyzed. The samples in all the locations did not respected the French reference (AFNOR) range (*Staphylococcus* <10^2CFU/g). The concentration of *Staphylococcus* represents more than 13% of the TMAF in 80% of the sites.

The total aerobic mesophilic flora of the raw milk samples complies in 80% of cases with European standards (105 CFU/mL) [31,32]. These results are much lower than those found by other authors in several countries: in Algeria [32,33], Morocco [29], Cameroon [9]. This parameter is considered as a general indicator of the overall quality of the product because it reveals the conditions of production, more particularly hygienic practices during milking [34]. This lower bacterial contamination of the samples could be also explained by the fact that sometimes, some farmers treat sick cows themselves with the antibiotics available (at the market) and do not respect the time necessary before milking.

Sites	S1	S2	S3	S4	S5
Tf	(11 ± 1,2)10^{3a}	(12,2 ± 0,8)10^{3a}	(7,2 ± 0,9)10^{9b}	(9 ± 0,9)10^{3a}	(6,4 ± 2,1)10^{4a}
Staph	(1,6 ± 0,2)10^{3a}	(1,9 ± 0,65)10^{3ab}	(2,9 ± 1,03)10^{3b}	(1,2 ± 0,3)10^{3a}	(1,03 ± 0,08)10^{4c}

Table 4: TMAF and Staphylococcus counts Values followed by different letters are significantly different (P <5%).

Staphylococcus identification

Characteristics like morphology of the colony, Gram staining, the catalase test and the DNAse test permitted to identify 19 strains of *Staphylococcus* grouped into 13 strains of *Staphylococcus aureus* and 6 of *Staphylococcus* sp. (Figure1) *Staphylococcus aureus* was present in the samples of all the sites) with more than 50% on the site S3.

Figure 1: Repartition of different staphylococcus on the raw milks.

All the samples analyses were contaminates with *Staphylococcus.* I is known that *Staphylococcus* is a human commensal bacteria that are generally found in the nostrils, armpits, on the skin, etc. [35,36]. Their presence in raw milk therefore reflects the non-application of hygiene rules such as washing hands [33] and mastitis in cow [32,37]. Contamination of milk becomes a major public health problem with the presence of *Staphylococcus aureus* as a responsible for food poisoning. This pathogenic germ constitutes a real risk for public health because it is capable, under certain conditions, of producing thermostable enterotoxins which can resist to thermal treatments [38]. The presence of Staphylococci in 100% of our samples, with an average load of 1.7.103 CFU / ml is more worrying as the species S. aureus were present in 100% of our samples compared to the 0% found by Labioui [25], 9.8% of Yabrir [32] and 17.7% of Hamiroune [40]. The rate of the presence of S. Aureus was closer to: 70% found by Maïworé [9]; 81.93% found by Ghazi [33] and 100% found by Alexopoulos [39].

Antibiotic resistance and percentage of resistance

The antibiogram of the strains of *Staphylococcus* isolated from raw milk samples is shown in table 5. There is 94.74% resistance to β-lactams, 78% to Macrolides, 42.1% to Glycopeptides, 15.78% to Quinolones, 5.3% with Aminoglycosides, 10.52 with nitrofurantoin, 10.52% with fusidic acid, and 5.3% with cotrimoxazole. Levofloxacin and chloramphenicol show 100% antibacterial activity on the 19 strains of *Staphylococcus* isolated. The antibiogram and the resistance percentages are given in table 5.

Family	Antibiotics	Strains																		
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
Bêta-lactams	Pénicilline G	S	R	R	R	R	S	S	R	R	R	S	R	R	S	S	S	S	R	R
	Oxacilline	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	Cefotaxime	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	R
	Meropenème	S	S	R	S	R	S	S	S	R	R	S	S	S	S	S	S	S	R	S
	Daptomycine	S	S	R	S	R	S	S	R	R	R	S	S	S	S	S	S	S	S	R
	Moxalactam	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	R	S	S
Glycopeptides	Teicoplanine	S	S	R	S	S	R	S	R	R	S	R	R	S	S	S	S	S	S	S
	Vancomycine	S	R	S	S	S	R	R	R	R	S	R	S	S	S	S	S	S	S	S
Aminosides	Gentamycine	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
	Tobramycine	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
	Amikacine	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
	Netilmycine	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
	Isepamicine	S	S	S	S	S	R	S	S	R	R	S	S	S	S	S	S	S	S	S
Macrolides and related	Erythromycine	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
	Lincomycine	S	R	R	S	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S
	Clindamycine	S	R	S	S	R	S	S	S	R	I	S	S	S	S	S	R	S	S	S
	Spiramycine	S	R	I	S	I	I	S	I	I	S	I	R	I	I	I	S	S	I	I
	Pristinamycine	S	R	S	S	I	S	S	S	R	S	S	S	S	S	S	S	S	S	S
	Télithromycine	S	R	S	S	S	S	S	R	R	S	S	S	S	S	S	R	S	S	S
Quinolones and Fluoro-quinolones	Ofloxacine	S	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S
	Levofloxacine	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Phenicoles	Chloramphé-nicol	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Sulfamides	Cotrimoxazole	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Nitrated products	Nitrofuran-toine	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S
Others	Rifampicine	S	S	S	S	I	S	S	S	R	R	S	S	S	S	S	R	I	S	S
	Acide fusidique	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S

Table 5: Antibiograms of Staphylococcus strains I : intermediate ; R : Resistant ; S : Sensitive; S1-S19 : Strain 1-Strain 19

The test for the antibiotic-resistant activity of *Staphylococcus* isolated from our samples reveals a heterogeneous degree of resistance depending on the antibiotics used. The study revealed a higher level of resistance to β-lactams (94.74%). This may be due to several factors: firstly, antibiotics of the β-lactam family are most used by farmers because their high availability on the informal market, their low cost and their easy administration (not requiring the presence of veterinary or health personnel) [41,42]. Secondly, on the cellular physiology, *Staphylococcus* produce penicillinases (enzymes which inactivate penicillin) and on the other hand they contain in their genome the mec A gene responsible for the synthesis of a protein which binds mutated penicillins (PLP2a) lacking affinity for β-lactams [43,44]. This result is similar to that of Abera [1], who showed 94.4% resistance of *S. aureus* to penicillin. Resistance to other families of antibiotics can be the result

of several mechanisms [45]: modification of the structure of the antibiotic for Aminoglycosides [46], ejection of the antibiotic from the bacteria and modification of the target for Quinolones [47,48], the decrease in membrane permeability and the modification of the target for Glycopeptides [48] and the modification of the target for Macrolides [49].

Levofloxacin and chloramphenicol have shown 100% antibacterial activity. According to Robert [45], levofloxacin has a bactericidal effect and acts by inhibiting nucleic synthesis in the bacteria, while chloramphenicol has a bacteriostatic effect by inhibiting protein synthesis in the ribosome [46]. Abera [1] in Ethiopia found similar results to ours with 100% chloramphenicol antibacterial activity against strains of *S. aureus* isolated from milk.

	Antibiotics tested										
Resistance	**β-lac.**	**Glyc.**	**Amin.**	**Macr.**	**Quin.**	**Chlo.**	**Cotr.**	**Nitr.**	**Fus. A.**	**Dapt.**	**Rifa.**
S.S	1	11	18	4	16	19	18	17	17	16	14
R.S	18	8	1	15	3	0	1	2	2	3	5
%R	94,7	42,1	5,3	79	15,8	0	5,3	10,5	10,5	15,8	26,3

Table 6: Resistance phenotypes of Staphylococcus isolated from milk

β-lac. : β-lactam ; Glyc.: Glycopeptides; Amin. : Aminosides ; Macr. : Macrolides ; Quin. : Quinolones ; Chlo. : Chloramphenicol ; Cotr: Cotrimoxazole ; Nitr. : Nitrofurantoine ; Fus A.: Fusidic A. : Dapt : Daptomycin ; Rifa. : Rifampicin

S.S = Sensitive Strains ; R. S = Resistant strains; %R = Percentage of resistance

Conclusion

The objective of this study was to identify *Staphylococcus* in raw milks from Maroua (Cameroon) and evaluate their resistance to antibiotics. The samples analyzed in all the sites were contaminated with *Staphylococcus*, especially the strain *aureus*. The most alarming is that these strains have a high rate of resistance to commonly used antibiotics. The concordance of our results with those of other researchers from various countries raises questions about the progress of bacterial resistance around the world. In order to ensure better quality food in the city of Maroua, Farmers should be made aware of the application of hygiene rules when milking on farms. In addition, regulation and monitoring of the administration of antibiotics to farm animals should be effective. Extending this study to other pathogenic bacteria in milk will also make it possible to assess their resistance to antibiotics.

Conflict of Interest

The authors declare that there is no conflict of interest relating to this manuscript.

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