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Research Article

Profile of pathogenic bacteria isolated from cow's milk in N'Djamena: associated risk factors and antibiotic resistance

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Abstract

Raw milk and its derivatives are foodstuffs vulnerable to contamination by microorganisms including pathogenic bacteria and failure to comply with hygiene rules.

The objective of this study was to determine the profile of pathogenic bacteria isolated from cow's milk and to evaluate the effectiveness of antibiotics commonly used in veterinary and human medicine against these bacteria in Chad.

This was a prospective and analytical study based on bacteriological examination including 180 milk samples collected in 10 districts of N'Djamena, ranging from November 2021 to December 2022. The isolation, identification and testing of sensitivity of isolated bacteria to antibiotics were carried out under standard food bacteriology conditions.

Among 180 milk samples which were screened by bacteriological examination, 71 (34.44%) cases were positive. The bacterial strains isolated were: *Staphylococcus aureus* (38.03%), *Streptococcus agalactiae* (18.31%), *Staphylococcus hyicus* (11.27%), *Streptococcus uberis* (7.04%), *Escherichia coliO157H7* (7.04%), *Streptococcus pyogenes* (5.63%), *Aeromonas hydrophila* (5.63%), *Listeria monocytogenes* (4.22%) and *Mycobacterium tuberculosis* (2.81%). The risk factors most frequently associated with milk contamination were hand milking (100%) followed by unsanitary environment (65%), 35% of milks were sold in reused bottles and 58% of vendors and producers were uneducated.

The antibiogram of the 69 isolated bacterial agents showed reduced sensitivities to beta-lactams and aminoglycosides, and varied resistance to cyclins and fluoroquinolones. Streptomycin remains an aminoglycoside most sensitive to all strains of pathogenic bacteria with an average level of 95.7%. Staphylococci (*Staphylococcus aureus* and *Staphylococcus hyicus*) developed an average resistance of 80.15% to methycillin, 80.1% to vancomycin and all bacteria showed an average resistance of 85% to metronidazole. Streptococci strains (*Streptococcus agalactiae*, *Streptococcus uberis* and *Streptococcus pyogenes*) were very sensitive (76.3%) to vancomycin. The isolated *Listeria monocytogenes* strains were 100% sensitive to aminoglycosides and beta-lactams.

The two strains of *Mycobacterium tuberculosis* detected by GeneXpert had a profile of 50% sensitivity and 50% resistance to rifampicin.

This study not only made it possible to know the high rate and frequency of pathogenic bacteria in cow's milk as well as the associated risk factors, but also showed a reduction in sensitivity of bacteria to aminopenicillins and aminoglycosides, a strong resistance of *Staphylococcus aureus* to methicillin and vancomycin and a high sensitivity of streptococci to vancomycin.

Keywords: Cow's milk, pathogenic bacteria, antibiotic resistance, risk factor, N'Djamena.

INTRODUCTION

The assessment of the microbiological quality of a food consists of the search for germs of hygienic interest, germs of fecal contamination, pathogenic and toxigenic germs as well as germs of technological interest [1]. Milk and its derivatives constitute an excellent culture medium for these microorganisms which can proliferate there [2, 3].

In developing countries of which Chad is a part, the insufficient hygienic quality of food can lead to infectious diseases (cholera, typhoid, etc.), foodborne illness and poisoning (TIAC) or temporary problems among consumers (diarrhea, gastroenteritis, etc.). This is particularly true for raw milk and its traditional derivatives which are fragile and perishable foodstuffs, rich in water and nutrients [4, 5].

Food poisoning in Chad is poorly estimated because many cases are not reported. Among the implicated foods, milk and dairy products were at the forefront and the etiological agents

responsible for reported TIACs were frequently *Staphylococcus aureus*, *Salmonella* and *Clostridium Perfringens* [6, 7, 8].

Antibiotic resistance has become a major global public health issue in recent years. Indeed, the emergence and increasing spread of bacteria resistant to antibiotics calls into question the effectiveness of treatments in both humans and animals. This phenomenon is strongly correlated with the misuse and overconsumption of antibiotics; it is aggravated by the lack of innovation in the field for two decades, which has led to a reduction in the therapeutic arsenal. Due to its scale and its harmful consequences on the treatment of infections in humans and animals, the phenomenon of bacterial resistance raises serious concerns throughout the world because human and veterinary medicine use the same families of antibiotics [9, 10, 11].

The objective of this work was to determine the prevalence of pathogenic bacteria in different types of cow's milk as well as the risk factors associated with contamination, and to evaluate the effectiveness of antibiotics commonly prescribed in human and veterinary medicine in Chad.

MATERIAL AND METHODS

Framework of the study and conduct of the research work.

This was an observational, cross-sectional and analytical study carried out on cow's milk in the city of N'Djamena and its surrounding areas.

Milk samples collected from the wives of breeders in the peripheral areas (ferricks) and from women resellers in the different markets of 10 districts of the city of N'Djamena for the search for pathogenic bacteria were transported to the Research Unit in Food Sciences and Nutrition from the Research, Diagnostics and Scientific Expertise Laboratory (Labo-ReDES), Faculty of Human Health Sciences (FSSH), University of N'Djamena to be analyzed.

A total number of 71 non-duplicate pathogenic bacterial isolates were collected from 180 cow's milk samples (curd, raw milk and industrial milk products) to determine the resistance profile to antibiotics commonly used in human and veterinary medicine. in Chad.

Cow's milk and dairy products from all sources (breeders' wives, milk sellers and resellers, and industrial dairy products) were collected from November 2021 to December 2022 in N'Djamena.

Isolation and identification of pathogenic bacteria

A quantity of 25 mL of dairy products (raw milk, curdled milk, pasteurized milk, yogurt) was homogenized in 225 mL of buffered peptone water (EPT) (Liofilchem, Italy). The homogeneous mixture thus obtained was incubated at 37°C.

- ***Escherichia coli*** isolation and identification procedure (**ISO 16649-2**)

Escherichia coli were isolated on TBX (Tryptone-bile-glucuronate) agar. 1mL of the 10-1 and 10-2 dilutions were taken and introduced into the Petri dishes then 10 to 15 mL of the TBX medium had been poured into its dishes and the mixture was homogenized by a circular movement. After solidification, 5 mL of this medium was poured again to avoid any contamination. Incubation was done at 44°C for 24 to 48 hours. After 24 to 48 hours, most Escherichia coli strains possessing a β-D-glucuronidase acting by cleavage of BCIG (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid), causing the color colonies in blue to blue green with the exception of enterohemorrhagic serotype O157 which presents white colonies, was subcultured in Mueller-Hinton (MH) medium for serological identification tests.

• ***E.coli* O157 Antiserum Test**

The colonies transplanted on MH were agglutinated with the *E.coli* O157 antiserum on a microplate for the presumptive identification of the antigen of E.coli, serotypes O157H7.

- **Isolation and identification of *Staphylococcus aureus* and (*Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus uberis*)**

• **The ISO 6888-2:1999 method** was used for the isolation of *Staphylococcus aureus* on the selective Chapman-Mannitol medium. The surface spreading technique of 0.1 mL of the corresponding dilution on the medium was used. Then the Petri dishes were incubated at 37°C for 24 hours according to the Standard. At the end of this period, the colonies of *Staphylococcus aureus* appear surrounded by a yellow halo. Two phases of confirmation of S. aureus testing were used.

• **The ISO 6888 –2:1999 method** was also used for the isolation of *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus uberis*. The surface spreading technique of 0.1 mL of the corresponding dilution was carried out on fresh blood agar (GSF) and cooked blood agar (GSC) media supplemented with poly vitex. The Petri dishes were incubated for 24 to 48 hours in an oven at 37°C. The translucent colonies were subcultured in the same agar plates for serological and biochemical identification.

• **Search for catalase**

After isolation on Chapman medium for 24 hours, a distinct colony was taken and placed on a slide, a drop of hydrogen peroxide H2O2 was added to the colony. The appearance of foam attesting to the presence of catalase.

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide (H_2O_2) into H_2O and ½ O_2. Staphylococci were positive for catalase. Streptococci were catalase negative.

• **Coagulase testing**

Free coagulase is present in *S. aureus*, but can also be produced by *S.intermedius* or *S. hyicus*. This test consists of detecting the coagulase released into the external environment. The detection of this coagulase is carried out by adding 0.5 mL of human plasma and 0.5 mL of a 24-hour culture of staphylococci in broth to a hemolysis tube. The mixture was placed in an oven at 37°C and incubated for 24 hours. Strains of S. aureus cause plasma clotting most often in the first three hours. A positive test results in the formation of a coagulum. The STAPH and STREP galleries (BioMérieux) were also used for the biochemical identification of staphylococci and streptococci.

- **Procedure for the selective isolation and identification of *Listeria monocytogenes* (ISO ISO 11290-1/2)**

The surface spreading technique of 0.1 mL of the corresponding dilution was carried out on fresh (GSF) and cooked (GSC) blood agar media supplemented with poly vitex. The Petri dishes were incubated for 24 to 48 hours in an oven at 37°C. The translucent colonies were subcultured in the same agar plates for serological and biochemical identification.

• Confirmation of the genus, then the species: by means of appropriate biochemical, physiological and morphological tests (catalase, Gram staining, mobility; then hemolysis, utilization of carbohydrates).

- Procedure for isolating *Aeromonas hydrophila*

The surface spreading technique of 0.1 mL of the corresponding dilution was carried out on Thiosulfate Bile Sucrose (TCBS) agar. The Petri dishes were incubated for 24 hours in an oven at 37°C. The translucent yellow colonies were subcultured on MH agar for biochemical tests: the API 20 E and API 20NE

galleries (BioMérieux), by means of these biochemical tests, the *Aeromonas hydrophila* were identified.

Detection of sensitivity and resistance to rifampicin with the GeneXpert automated system

Principle: it is based on amplification of a fragment of the rpoB gene containing the central region at 81 base pairs and fragments of the target sequences of the IS1081 and IS6110 insertion elements with multiple copies by primers.

Using a Pasteur pipette, 2 mL of prepared milk (curd, raw milk and industrial cow's milk products) and 4 mL of the reagent were taken and mixed in another sterile jar. The mixture was vortexed and then incubated at room temperature for 10 minutes. The pot was vortexed again and incubated at room temperature for 5 minutes. Then, 2 mL of the liquefied mixture was aspirated and transferred into the Xpert® MTB/RIF ULTRA cartridge and the test was run for 1 hour 30 minutes.

Study of the sensitivity of isolated bacteria to antibiotics

Choice of antibiotics

Antibiotics were chosen based on their prescription in both veterinary and human medicine.

Table 1: Antibiotics chosen for the sensitivity test [12, 13,14].

Category	Family	Antibiotic/dose	Diameter (mm)		
			Sensitive	Intermediate	Resistant
	Aminosides	Gentamicin (15 µg)	>16	14-16	<14
		Kanamycin (30 µg)	>24	18-24	<18
		Streptomycin (10 µg)	>22	14-22	<14
Antibiotic		Penicillin G (6 µg)	>37	26-37	<26
(Bio-Rad, CLSI)		Ampicillin (10 µg)	>14	12-14	<12
	B-Lactams	Amoxicillin (25 µg)	>23	19-23	<19
		Methicillin (5 µg)	>16	12-16	<12
		Cefotaxim (30 µg)	>31	25-31	<25
		Ceftriaxone (30 µg)	>22	19-22	<19
	Cyclins	Tetracyclin (30 µg)	>31	23-31	<23
		Doxycyclin (30 µg)	>31	23-31	<23
		Oxytetracyclin (30 µg)	>29	21-29	<21
	Fluoroquinolones	Levofloxacin (5µg)	>29	23-29	<23
		Ciprofloxacin (5µg)	>27	21-27	<21
		Ofloxacin (10 µg)	>27	21-27	<21
	Macrolides	Erythromycin (15 µg)	>29	23-29	<23
		Clarithromycin (15 µg)	>23	26-32	<26
		Azithromycin (15 µg	>26	21-26	<21
	Rifamycin	Rifampicin (30 µg)	>39	34-39	<34
	Glycopeptides	Vancomycin (30 µg)	>23	17-23	<17
	Nitroimidazoles	Metronidazole (75 µg)	>15	12-25	<15
	8 Families	21 antibiotics			

Quality control was carried out using the reference strains: *E. coli* ATCC 25922; *Staphylococcus aureus* NCTC 12493; *Enterococcus faecalis* ATCC 29212.

Antibiogram

The antibiogram was carried out using the Kirby-Bauer technique, which is the method by diffusion of disks impregnated with antibiotics in MH agar medium flooded with suspended bacterial inoculum. The identification of ultra-resistant bacteria was carried out following the recommendations of the Antibiogram Committee of the French Society of Microbiology and the European Committee on Antimicrobial Susceptibility Testing (CA-SFM, EUCAST; 2016-2022), of the Clinical Laboratory Standards Institute (CLSI) and the Food and Drug Administration (FDA) of the United States of America.

Detection of extended-spectrum beta-lactamase (ESBL) production

The ESBL was detected on Muller-Hinton agar using the double disk synergy test method according to the procedure of Jarlier et al. (1988). Discs of cefotaxim (30 µg), ceftazidime (30 µg), cefepim (30 µg), and aztreonam (30 µg), were placed 30 mm (center to center) from an amoxicillin/acid disc clavulanic acid (20/10 µg) then incubated at 35 - 37 °C. After 18 to 24 hours of incubation, the production of the ESBL by the tested organism was based on the partial inhibition of the ESBL by the acid clavulanic. The existence of even a weak synergy between Cefotaxim, ceftazidim, cefepim, aztreonam and clavulanic acid is characterized by an image in the shape of a champagne cork.

Reading

- Double disk method:

C3G-AMC-C3G, spaced 25 mm apart

✓ If an image is formed in a champagne cork, BLES+

- Combine disk method

C3G-CLAV-C3G, spaced 30 mm apart

Calculation of the difference of 2 diameters

✓ If < 5 mm ESBL-

✓ If > 5 mm ESBL+

Written and signed informed consent

Sir/Madam**,**

We would like to sample the cow's milk that you produce or sell, this is a procedure usually done to look for pathogenic bacteria in the milk likely to contaminate producers, consumers, and sellers/resellers.

The sample will be used to identify the pathogen(s) responsible for the food poisoning which may affect the health of producers, consumers and sellers. The results obtained will allow the authorities of the Ministries of Livestock and Public Health and Prevention to take corrective measures in order to prevent the risk of food poisoning and undoubtedly to know better about the cause of diseases.

You will be informed of any change in the purpose of the research on the samples and you can object to it.

Sir/Madam, agree to provide the milk sample from your milking cow or marketed dairy products is essential for the completion of this study which will contribute to improving the health of breeders and consumers around the world and particularly in the Chad.

Data processing and analysis

Using Microsoft Office Excel and Microsoft Office Word 2019, we analyzed the results and wrote the report. The chi-square test was used to study the relationships between variables with a margin of error limited to 5%.

Resistance phenotype analysis: The proportion of resistant for each antibiotic was calculated as the sum of resistant antibiotics relative to the sum of susceptible and resistant. The proportion of resistant antibiotic class represented the average resistance of all antimicrobial agents belonging to that class.

RESULTS

Mapping of the localities of N'Djamena and its surrounding areas surveyed

Figure 1 illustrates the ten (10) districts of N'Djamena where the collection of cow's milk samples takes place. Chad is located between 7th and 24th degrees North latitude and 13th and 24th degrees East longitude. It covers an area of 1,284,000 km^2; it is the fifth largest country in Africa after Sudan, Algeria, Zaire and Libya. From North to South, it extends over 1,700 km and, from East to West, over 1,000 km. It shares its borders with Libya to the north, Sudan to the east, the Central African Republic to the south and Cameroon, Nigeria and Niger to the west. The province of N'Djamena has a total of 64 neighborhoods, and is divided into 10 districts (figure 1). The country belongs politically and economically to Central Africa, but due to the similarities in climatic conditions, it is also linked to the Sahelian countries. The two mainstays of Chad's economy are made up of agriculture and livestock, including cow's milk and its derivatives which constitute the center of this study. The geolocation of Chad would certainly have contributed to the transhumance of breeders from neighboring countries and therefore to the transmission of pathogenic microorganisms in milk including cases of pathogenic bacteria (*Mycobacterium tuberculosis*, *Escherichia coli* O157H7 etc.).

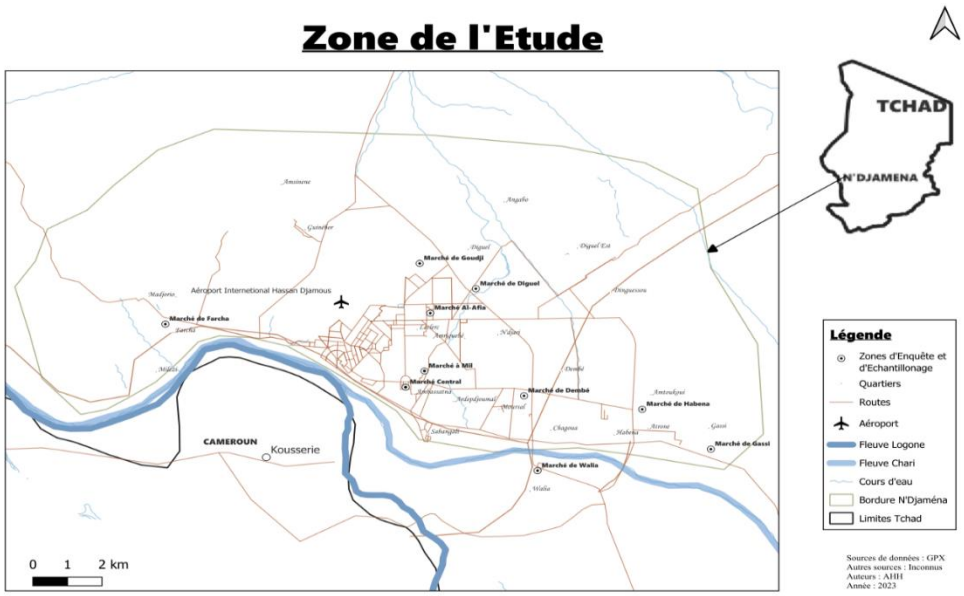


Figure 1: Mapping of the localities surveyed

Overall prevalence of pathogenic bacteria in milk

A total of 180 samples of cow's milk (curdled milk, raw milk and industrial dairy products) were included in the study during the 12-month period. The result of the research showed that 71 (34.44%) cultures were positive, and 109 (60.55%) cultures were sterile (p = 0.30: non-significant difference in favor of negative culture).

Distribution of positive samples according to educational level

Figure 2 illustrates the distribution of samples according to educational level. Those not in school come first, followed by primary, secondary and higher education with the proportions of 41 (58%), 16 (22%), 9 (13%) and 5 (7%) respectively.

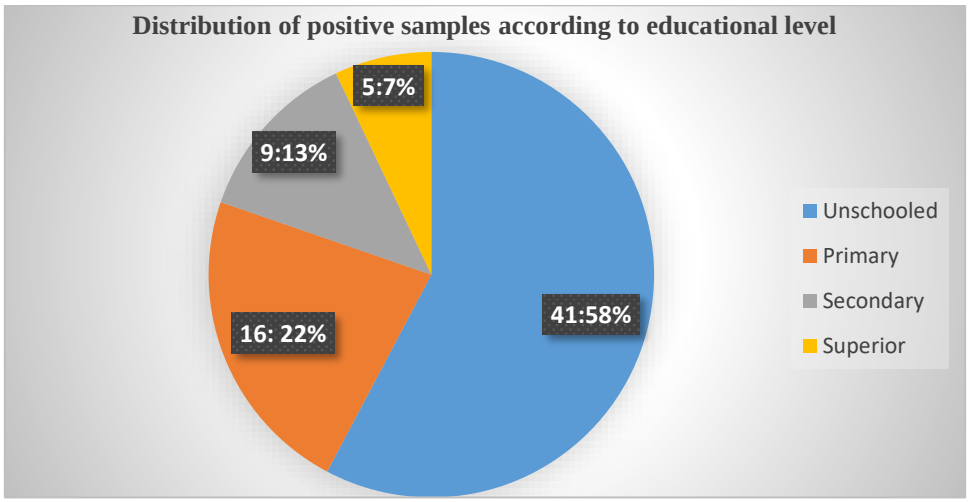


Figure 2: Distribution of positive samples according to educational level

Distribution of positive samples according to the 10 districts

Table 2 shows the distribution of positive samples according to the Districts. The 9th District has the highest number of positive samples (4.8%) followed by the 8th (3.6%) and 7th (2.8%) Districts.

Table 2: Distribution of positive samples according to the 10 districts

Borough	Raw milk (n =40) (%)	Rotten milk (n =20) (%)	Yogurt (n =11) (%)
1er	4 (1.6)	2 (0.4)	1(0.11)
2ème	1 (0.4)	1(0.2)	2 (0.22)
3ème	2 (0.8)	1(0.2)	2 (0.22)
4ème	1(0.4)	1(0.2)	1(0.11)
5ème	1(0.4)	1(0.2)	1(0.11)
6ème	1(0.4)	1(0.2)	1(0.11)
7ème	**7(2.8)**	3(0.6)	1(0.11)
8ème	**9 (3.6)**	2 (0.4)	1(0.11)
9ème	**12 (4.8)**	5 (1)	0 (0)
10ème	2 (0.8)	3 (0.6)	1(0.11)
Total	40 (100)	20 (100)	11(100)

Frequency of pathogenic bacteria isolated from cow's milk

The different germs identified during analysis are distributed in table 3. *Staphylococcus aureus* were predominant with a rate of 27 (38.03%) followed by *Streptococcus agalactiae* and then *Staphylococcus hyicus* with rates of 18.31% and 11. 27% respectively.

Table 3: Frequency of pathogenic bacteria isolated in cow's milk

Bacterial specie	Effective	Percentage (%)
Staphylococcus aureus	27	38.03%
Staphylococcus hyicus	8	11.27%
Streptococcus agalactiae	13	18.31%
Streptococcus uberis	5	7.04%
Streptococcus pyogenes	4	5.63%
Escherichia coli O157	5	7.04%
Aeromonas hydrophila	4	5.63%
Listeria monocytogenes	3	4.22%
Mycobacterium tuberculosis	2	2.81%
Total	71	100%

Distribution of pathogenic bacteria according to the types of cow's milk

Table 4 shows the distribution of pathogenic bacteria according to the types of cow's milk. Raw milks 42 (59.15%) were the most contaminated by pathogens followed by curdled milks 21 (29.57%) and yogurts 8 (11.27%).

Table 4: Distribution of pathogenic bacteria according to the types of cow's milk

Bacterial specie	Raw milk (%)	Rotten milk (%)	Yogurt (%)	Raw milk (%)
Staphylococcus aureus	27	17 (63)	7 (26)	3 (11.11)
Staphylococcus hyicus	8	5 (62.5)	3 (37.5)	0 (0)
Streptococcus agalactiae	13	8 (61.53)	4 (31)	1 (7.7)
Streptococcus uberis	5	3 (60)	2 (40)	0 (0)
Streptococcus pyogenes	4	1 (25)	2 (50)	1 (25)
Escherichia coli O157	5	3 (60)	1 (20)	1 (20)
Aeromonas hydrophila	4	2 (50)	2 (50)	0 (0)
Listeria monocytogenes	3	1 (33.33)	0 (33.33)	2 (66.66)
Mycobacterium tuberculosis	2	2 (100)	0 (0)	0 (0)
Total (%)	71	42 (59.15)	21 (29.57)	8 (11.27)

Result of the field survey on the risk factors associated with the contamination of cow's milk

According to the field survey, milk milking was carried out 100% by the producers. The unsanitary environment (markets, streets) of the sale was estimated at 65% and the sale of cow's milk with reused plastic bottles was estimated at 35% (table 6: photos (a, b, c)).

Susceptibility and resistance profile of two (2) *Mycobacterium tuberculosis* complexes to rifampicin

The two *Mycobacterium tuberculosis* detected by Gene Xpert had a profile of 50% sensitivity and 50% resistance to rifampicin.

Evaluation of the effectiveness of antibiotics against 69 pathogenic bacteria isolated from cow's milk

The ever-increasing frequency of pathogenic bacteria in foodstuffs and in particular in cow's milk and the rapid emergence of strains resistant to most antibiotics, now requires testing of sensitivity to antibiotics used in human therapy. animal, for each isolated strain of bacteria. Generally speaking, the 69 isolated bacteria developed reduced sensitivities to beta-lactams and aminoglycosides (Table 5). Streptomycin remains an aminoglycoside most sensitive to the 69 strains of pathogenic bacteria isolated in milk with an average sensitivity level of 95.7%. Staphylococci (*Staphylococcus aureus* and *Staphylococcus hyicus*) have developed an average resistance of 80.15% to methycillin and 80.1% to vancomycin. Streptococci strains (*Streptococcus agalactiae*, *Streptococcus uberis* and *Streptococcus pyogenes*) were very sensitive (76.3%) to vancomycin. The isolated *Listeria monocytogenes* strains were 100% sensitive to aminoglycosides and beta-lactams. The 69 strains developed varying resistances and sensitivities to cyclins and fluoroquinolones (Table 5).

The most prescribed antibiotics in therapy (human and animal) were: gentamicin (aminoglycosides), beta-lactams (penicillin G, amoxicillin, ceftriaxone), ciprofloxacin (fluoroquinolones), tetracycline and doxycycline (Cyclins), erythromycin (macrolide)s and metronidazole (nitroimidazoles). The 69 strains of bacteria isolated developed an average resistance of 85% to metronidazole.

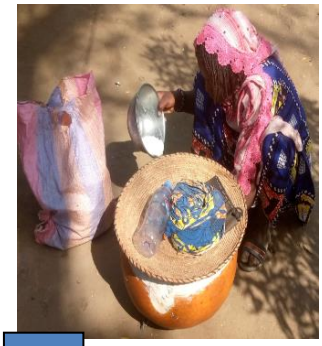
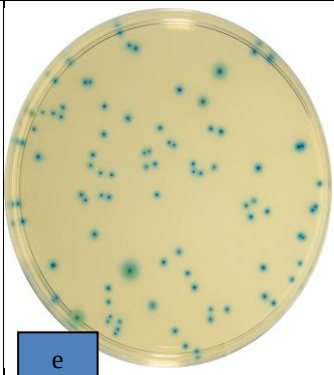
Table 5: Evaluation of the effectiveness of 69 pathogenic bacteria isolated from cow's milk with selected antibiotics

ATB	Bacterial specie															
	S.aureus (n=27)		*S.hyicus* (n=8)		*Str.agalactiae* (n=13)		*Str.uberis* (n=5)		*Str.pyogenes* (n=4)		*E.coli* O157 (n=5)		*A.hydrophyla* (n=4)		*L.monocytogenes* (n=3)	
	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)	S (%)	R+I (%)
GNN	17 (63)	10 (37)	6 (75)	2 (25)	9 (69.2)	4 (31)	4 (80)	1 (20)	2 (50)	2 (50)	3 (60)	2 (40)	3 (75)	1 (25)	3 (100)	0 (0)
KAN	21 (78)	6 (22)	7 (87.5)	1 (12.5)	11 (84.6)	2 (15.4)	5 (100)	0 (0)	3 (75)	1 (25)	4 (80)	1 (20)	4 (100)	0 (0)	3 (100)	0 (0)
STN	25 (92.6)	2 (7.4)	8 (100)	0 (0)	12 (92.3)	1 (7.7)	5 (100)	0 (0)	3 (75)	1 (25)	3 (60)	2 (40)	3 (75)	1 (25)	3 (100)	0 (0)
PG	6 (22)	21(72)	3 (37.5)	5 (62.5)	5 (38.5)	8 (61.5)	3 (60)	2 (40)	2 (50)	2 (50)	NR		NR		2 (66.7)	1 (33,3)
AMP	11 (41)	16 (59.2)	4 (50)	4 (50)	6 (46.1)	7 (54)	2 (40)	3 (60)	1 (25)	3 (75)	2 (40)	3 (60)	3 (75)	1 (25)	3 (100)	0 (0)
AML	13 (48.1)	14 (52)	3 (37.5)	5 (62.5)	9 (69.2)	4 (31)	3 (60)	2 (40)	2 (50)	2 (50)	3 (60)	2 (40)	3 (75)	1 (25)	3 (100)	0 (0)
MET	1 (3,7)	26 (98.3)	3 (37.5)	5 (62.5)	NR		NR		NR		NR		NR		NR	
CTX	25 (92.6)	2 (7.4)	8 (100)	0 (0)	10 (74)	3 (23.1)	5 (100)	0 (0)	4 (100)	0 (0)	4 (80)	1 (20)	4 (100)	0 (0)	3 (100)	0 (0)
CRO	24 (89)	3 (11.1)	7 (87.5)	1 (12.5)	9 (69.2)	4 (31)	3 (60)	2 (40)	2 (50)	2 (50)	2 (40)	3 (60)	3 (75)	1 (25)	3 (100)	0 (0)
TET	14 (52)	13 (48.1)	5 (62.5)	3 (37.5)	7 (54)	6 (46.1)	3 (60)	2 (40)	3 (75)	1 (25)	3 (60)	2 (40)	4 (100)	0 (0)	2 (66.7)	1 (33.3)
DXT	13 (48.1)	14 (52)	8 (100)	0 (0)	9 (69.2)	4 (31)	5 (100)	0 (0)	2 (50)	2 (50)	NR		NR		2 (66.7)	1 (33.3)
OXT	23 (85.2)	4 (15)	4 (50)	4 (50)	7 (54)	6 (46.1)	3 (60)	2 (40)	3 (75)	1 (25)	NR		NR		3 (100)	0 (0)
LEV	22 (81.5)	5 (18.5)	5 (62.5)	3 (37.5)	8 (61.5)	3 (60)	5 (100)	0 (0)	3 (75)	1 (25)	3 (60)	2 (40)	4 (100)	0 (0)	3 (100)	0 (0)
CIP	17 (63)	10 (37)	8 (100)	0 (0)	7 (54)	6 (46.1)	5 (100)	0 (0)	2 (40)	2 (50)	2 (40)	3 (60)	1 (25)	3 (75)	2 (66.7)	1 (33.3)
OFL	23 (85.2)	4 (15)	8 (100)	0 (0)	10 (74)	3 (23.1)	4 (80)	1 (20)	3 (75)	1 (25)	3 (60)	2 (40)	4 (100)	0 (0)	3 (100)	0 (0)
ERY	12 (44.4)	15 (55.5)	6 (75)	2 (25)	7 (54)	6 (46,1)	4 (80)	1 (20)	3 (75)	1 (25)	NR		NR		2 (66.7)	1 (33.3)
CLN	23 (85.2)	4 (15)	7 (87.5)	1 (12.5)	10 (74)	3 (23.1)	4 (80)	1 (20)	4 (100)	0 (0)	NR		NR		3 (100)	0 (0)
AZM	13 (48.1)	14 (52)	6 (75)	2 (25)	7 (54)	6 (46.1)	4 (80)	1 (20)	3 (75)	1 (25)	NR		NR		3 (100)	0 (0)
VAN	4 (15)	23 (85.2)	2 (25)	6 (75)	10 (74)	3 (23.1)	4 (80)	1 (20)	3 (75)	1 (25)	NR		NR		NR	
MTZ	2 (7,4)	25 (92.6)	2 (25)	6 (75)	3 (23.1)	10 (74)	1 (20)	4 (80)	1 (25)	3 (75)	0 (0)	5 (100)	1 (25)	3 (75)	1 (33.3)	2 (66.7)

Legend: ATB=antibiotic; n=number; % (R+I) = resistance percentage; S = sensitivity percentage; NR= not required; % = percentage

Gentamicin (GNN); Kanamycin (KAN); Streptomycin (STN); Penicillin G (PG); (Ampicillin (AMP); Amoxicillin (AML); Methicillin (MET); Cefotaxim (CTX); Ceftriaxone (CRO); Tetracycline (TET); Oxytetracyclin (OXT); Levofloxacin (LVX); Ciprofloxacin (Cip); Ofloxacin (OFL) Erythromycin (ERY); Clarithromycin (AZM); Vancomycin (VAN); *S.aureus* (*Staphylococcus aureus*); *Str.uberis* (*Streptococcus uberis*); *Str.pyogenes* (*Streptococcus pyogenes*); *E.coli* O157 (*Escherichia coli* O157);

Table 6: macroscopic, microscopic and biotechnological steps in the characterization of pathogenic bacteria isolated in cow's milk

1	a): raw milks sold in unhygienic cans on ferricks b): curdled milk sold at the side of the street in large gourds c : yogurts sold in a grocery store in the 6th Borough.	 a	 b	 c
2	d: preparation of milk for bacteriological culture e: *Escherichia coli β-glucuronidase positive on TBX agar, characteristic colony: blue to blue green color* f : *Staphylococcus aureus* colonies on Chapman agar	 d	 e	 f
3	g :*Listeria monocytogenes* colonies on Chocolate+ polyvitex agar h: antibiogram for *Listeria monocytogenes* i : formation of (ESBL) by *Escherichia coli* resistant to third generation cephalosporins (C3G)	 g	 h	 i
4	j : biochemical identification of Escherichia coli O157H7	 j		

DISCUSSION

During 12 months of study, 180 samples of cow's milk (raw milk, curdled milk and yogurt) were included and taken for the search for pathogenic bacteria. Our research work focuses on the isolation, biochemical identification and phenotype detection of resistance to antibiotics used in veterinary and human medicine in Chad. Bacteriological analysis of milk samples detected 71 pathogenic bacteria, representing a prevalence of 34.44%. This prevalence is similar to that obtained by Hamiroune et al in 2016 in Algeria [15]. This series of studies noted a predominance of *Staphylococcus aureus* with a rate of 38.03% followed by *Streptococcus agalactiae* and then *Staphylococcus hyicus* with rates of 18.31% and 11.27% respectively. This prevalence is lower than that obtained by the Canadian food inspection agency which was 85.75% *Staphylococcus aureus* and a rate greater than 50% of pathogenic bacteria in raw milk cheese [1]. The high prevalence of *Staphylococcus aureus* and other pathogens in milk could be explained by fecal-oral contamination, milking of cow's milk by the dirty hands of unschooled producers (58%) identified by this series of studies, and the unsanitary environment for the sale of cow's milk in the districts of the city of N'Djamena (table 2). Furthermore, the same microbiological characteristics of raw milk and its derivatives have been reported [16].

This series noted the presence of *Escherichia coli* O157H7, *Aeromonas hydrophila* and *Listeria monocytogenes* in raw cow's milk with rates of 60%, 50% and 33.33% respectively. Furthermore, the presence of dangerous pathogenic bacteria

(e.g. *Salmonella* spp., *E. coli* O157) has been noted [2, 17]. The high presence of these pathogenic bacteria in cow's milk (raw milk, curdled milk and yogurt) could be explained by the fact that, on farms, the collection of milk from cows was carried out with 1 liter cans and half, 5 liters and/or 25 liters whose hygienic quality remains to be desired (table 6). No measures were taken to preserve the milk before processing in ideal conditions, which are to lower the temperature of the milk in order to avoid the proliferation of bacteria. Cold (refrigeration, freezing and deep-freezing) and heat (pasteurization and sterilization) are the elementary technological bases of the dairy industry [17]. The presence of two *Mycobacterium tuberculosis* complexes in raw cow's milk could be explained either by endogenous transmission (mastitis, animals carrying tuberculosis bacilli) or exogenous (unsanitary environments, people carrying tuberculosis bacilli, or by cross-contamination with bottles used by patients with relapses or undetected family carriers of tuberculosis).

Regarding the risk factors associated with the contamination of cow's milk, this study noted that milk milking was carried out 100% by the hands of producers in unsanitary enclosures. The unsanitary environment (markets, streets) of the sale was estimated at 65% and 35% the sale of cow's milk with reused plastic bottles. Furthermore, several authors have shown that the insufficient hygienic quality of food can lead to infectious diseases (cholera, typhoid, etc.), toxic infections and food poisoning or temporary problems among consumers, including producers (diarrhea, gastro- enteritis etc.). This is particularly true for raw milk and its traditional derivatives which are fragile and perishable foodstuffs [4, 5].

Speaking of antibiotic sensitivity, the two *Mycobacterium tuberculosis* complexes detected by Gene Xpert had a profile of 50% sensitivity and 50% resistance to rifampicin. Several authors have detected with geneXpert the resistance and sensitivity of the *Mycobacterium tuberculosis* complex to rifampicin [18, 19, 20].

Generally speaking, the 69 strains of bacteria isolated had developed reduced sensitivities to beta-lactams and aminoglycosides (Table 5). The resistance of the isolated strains could be explained by the production of beta-lactam inhibitor enzymes (penicillinases and cephalosporinases) of the strains in question. Furthermore, several authors have highlighted the

resistance phenotypes of enterobacteria and extended-spectrum beta-lactamase (ESBL)-producing staphylococci in clinical and environmental samples [21, 22, 23, 24]. Sèmanou et al in 2018 in Benin and Bagré et al (2014) in Ouagadougou in Burkina Faso confirmed the presence of residues of antibiotics from different families in raw and curdled cow's milk, indicators of bacterial resistance including beta-lactams [24, 25]. The acquisition of the varied resistance of strains of pathogenic bacteria isolated from cow's milk in this series of studies would be attributable to the release of large quantities of antibiotic residues and resistant bacteria in the feces of humans and animals in the environment. Through the use of these feces as fertilizer in agriculture and by various phenomena (runoff, crops, etc.), pastures and surface water reservoirs can be contaminated not only by resistant bacteria but also by residues. of medications [24, 26].

The coagulase-positive *Staphylococcus aureus* strains isolated in this series developed an average resistance of 80.15% to methycillin and 80.1% to vancomycin. On the other hand, this series noted that strains of streptococci (*Streptococcus agalactiae*, *Streptococcus uberis* and *Streptococcus pyogenes*) were very sensitive (76.3%) to vancomycin. Several authors have, through previous work, implicated the mecA and vanA resistance genes acquired by coagulase-positive *Staphylococcus aureus* to resist beta-lactams and glycopeptides [27, 28, 29].

Metronidazole developed an average resistance of 85% to all 69 strains of the bacteria isolated. Previous studies carried out by Sèmanou and Chadha showed enormous quantities of metronidazole detected in cow's milk residues and human feces attesting to this bacterial resistance [24, 26]. Plausible explanations for this assertion would be the cross-resistance of strains to this category of antibiotic and the misuse of antimicrobials in human and veterinary medicine. It would therefore be essential to set up a global coalition in order to limit the damage associated with antibiotic resistance and reduce the consumption of antibiotics for better use. The costs of these actions are estimated at 3-4 billion dollars annually [1, 2, 4, 30].

CONCLUSION

The present study made it possible to determine a high prevalence of pathogenic bacteria in cow's milk as well as the characterization of the resistance profile to antibiotics commonly prescribed in human and veterinary medicine for the treatment of people and animals in Chad. It appears from this study that pathogenic bacteria are more frequent and predominant in raw cow's milk than curdled milk and yogurt. This study also indicated that staphylococci are the most frequent and most incriminated germs in the contamination of raw milk followed by streptococci and *Escherichia coli* O157H7. The risk factors most associated with the contamination of cow's milk were the milking of milk by the hands of producers and the unsanitary environment for the sale of milk by sellers and resellers crowned by the level of education.

In view of these results, we recommend the involvement of the authorities of the Ministries of Health and Livestock of Chad for the supervision of breeders and sellers of cow's milk and advise the rational prescription of antibiotics in human medicine and veterinarian in our region.

Conflict of Interest

The authors declare no conflict of interest.

Contribution of Authors

All authors contributed significantly to the writing and editing of this manuscript. It has been seen and approved by all the authors. This manuscript has not been sent for publication elsewhere.

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