Antibiotic Sensitivity of Pathogenic Bacteria Associated with Subclinical Mastitis of Dairy Cows in the Poro Region of the Ivory Cost

ABSTRACT

Subclinical mastitis is the most considered pathology in dairy farming and is responsible for economic losses in cows. The condition remains asymptomatic in dairy cows and treatment is mainly done without laboratory analysis. The objective of this work is to research bacterial causes of subclinical mastitis in dairy cows and to study their sensitivity to certain antibiotics. Thus, this study was carried out in four departments (Korhogo, Sinématiali, Dikodougou and M'bengué) in the Poro region (Ivory Coast) from May to August 2022 in traditional farms on 288 neighborhood milk samples taken from dairy cows with subclinical mastitis. The milk samples were analyzed using standard bacteriological isolation and identification techniques. *Staphylococcus aureus* were isolated on Baird-Parker potassium tellurite agar and mixed with egg yolk*, Klebsiella spp* isolated on MacConkey agar, *Escherichia coli* isolated on MacConkey, *Pseudomonas aeruginosa* isolated on TSA and King A agar, *Micrococcus spp* isolated on Chapman agar; then incubated at 37°C for 24 to 48 hours. The identification of bacteria was carried out by standard methods (appearance of colonies, Gram staining, catalase test, coagula-associated oxidase test, etc.). The biochemical characteristics were studied using the API Bio Mérieux galleries (API Staph and API20E etc.) The sensitivity of the main germs isolated was tested against ten (10) antimicrobials including those used in the treatment of mastitis by veterinary clinicians in the region. from Poro.The bacteriological analysis were identified 43% of Gram-positive cocci in minority, with particulary 18% of *Staphylococcus aureus*. In majority, Gram-negative bacilli were detected at 57% with particulary 38% of Enterobacteria (*Klebsiella spp* and *Escherichia coli*). The antibiogram showed excellent sensitivity to *Staphylococcus aureus* to Gentamycin 100%, good sensitivity to chloramphenicol and neomycin at 90% then acceptable sensitivity to the Trimethoprim-Sulfamethoxazole combination, to cefalexin of 80%. The coagulase negative staphylococcus (SCN) group revealed good sensitivity to Cefalexin and Gentamicin of (87.5%) and acceptable sensitivity to Chloramphenicol, Neomycin of (75%) each. On the other hand, Enterobacteria showed excellent resistance to Ampicillin, Spiramycin and Penicillin 100% and acceptable resistance to Gentamycin (95.84%). Remarkable resistance has been observed on the following antibiotics : Ampicillin, Penicillin and Colistin. This remarkable resistance was noted in Staphylococci (100% for *Staphylococcus aureus* and 87.5% for SCN) and Enterobacteria. Given these results, suggestions were made for the treatment and prevention of subclinical mastitis on farms in the Poro region (northern Ivory Coast).

Keywords: Dairy cows; subclinical mastitis; pathogenic germs; antibiotics; Poro region.

1. INTRODUCTION

Mastitis is considered one of the most important, frequent and costly pathologies affecting dairy cows **[1,2]**, and the most penalizing for dairy farms **[3]**. In addition to the regular economic losses associated with the disease, it has major zoonotic potential and has been associated with the increasing development and rapid emergence of multi-drug resistant strains globally **[4,5]**. The health implications of this disease are serious and have been highlighted in reports from several countries. Mastitis, inflammation of the mammary gland, usually a consequence of adhesion, invasion and colonization of the mammary gland by mastitis pathogens, exists in three forms: clinical, subclinical and chronic mastitis **[6]**. Among these forms, subclinical mastitis is more common and results in a reduction in milk production without observable clinical signs or

milk abnormalities **[7,8]**. For this reason, it is difficult to diagnose and persists longer in the herd **[1]**. Subclinical mastitis (SCM) is the main form of this disease in dairy herds worldwide **[9,10]** and results in increased numbers of somatic cells in the milk produced and changes in its physical and chemical qualities **[11]**. The etiology of mastitis includes contagious microorganisms that survive and proliferate on theskin andteat wounds,as well as environmental microorganisms that are not retained on the teat **[6,7]**. Current studies have reported a shift in pathogens from major to minor pathogens, such as coagulase negative, Staphylococcus and other bacilli **[8,12]**. These studies have shown that these minor pathogens may play an important role in the pathogenesis of mastitis and vary between herds **[13,14]**. The primary treatment for mastitis is commonly administered by intramammary infusion or parenteral administration of antibiotics **[15]**. Antibiotics are widely used in livestock systems for prophylaxis, or as feed additives or animal growth factors **[16]**. This type of use induces changes in the digestive flora of animals leading to the emergence of resistant strains **[17]**. Also, failure to respect waiting times after treatments leads to the presence of antibiotic residues in animal products including milk **[18]**. Effective treatment of the disease depends on the antimicrobial susceptibility of the pathogens, the type of mastitis, the breed of cattle and the therapeutic technique **[3]**. The emergence of drug resistance is a major challenge for disease control, as resistance profiles are often herd-specific **[19]**. Combining more than one synergistic antimicrobial agent can be more effective than using a single drug and can achieve a high cure rate **[20,14,21]**. Rapid identification and understanding of the diversity of pathogens associated with mastitis is essential for effective prevention and control **[14]**. However, treatment is expected to become problematic in the near future due to the rapid increase in antibiotic-resistant pathogens **[14]**. Transmission of antimicrobial-resistant mastitis pathogens and foodborne pathogens to humans could occur if unpasteurized milk is consumed **[5,1,22]**. The widespread use of antibiotics in the control of mastitis significantly increases the risk of establishing and transmitting antibiotic resistance to consumers. Such a possibility is constantly under the attention of animal health and public health authorities, requiring a scientifically based redefinition of antibiotic therapies taking into account the intersection of animal welfare with social concerns **[23,24]**. The aim of this study was to estimate the distribution of pathogens associated with subclinical mastitis and to determine their resistance to antimicrobials, in a random selection of dairy farms in the northern part of Côte d'Ivoire at the regional level of Poro. To the authors' knowledge, there is a lack of data on regional differences in the prevalence of different mastitis pathogens and their antimicrobial resistance in Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Study Area

The study took place in four departments (Korhogo, Sinématiali, Dikodougou and M'bengué) in the Poro region (northern Ivory Coast) from May 5 until the end of August 2022 on traditional farms. In collaboration with veterinary technicians working at MIRAH (Ministry of Animal and Fisheries Resources from the different Korhogo departments); MIRAH was informed and milk samples were taken from each farm (farm) and transported to the LANADA laboratory (National Agricultural Development Support Laboratory) for confirmation of infection Fig. 1.

2.2 Materials

2.2.1 Biological materials

It is obtained from the exploitation of the teats of cows with mastitis from the different sites studied. Fresh cow's milk is collected every day from each site in each district.

2.2.2 Culture medium

Baird-Parker potassium tellurite agar,MacConkey agar, TSA or King A agar, Chapman agar.

2.2.3 Chemicals and reagents

Gram staining, catalase test, coagula-associated oxidase test, etc.

2.2.4 Technical materials

Marker, Racks, Autoclave, Water bath, Petri dish etc.

2.3 Methods

2.3.1 Collection of milk samples

With California Mastitis Test (CMT), all the lactating cows were selected on farm during the study. A total of 360 lactating cows were sampled on 45 farms (traditional livestock farms)

in the Poro region. Milk samples are taken directly from the udder, before evening milking. Two samples are taken from each quarter in sterile 10 ml tubes: the first will be used to detect mastitis on the cow's feet with the California mastitis test (CMT). The second is intended for bacteriological analysis and will only concern milk samples detected positive by the CMT. For the second sample, the milk is collected in a sterile bottle after washing with water and disinfection of the teats with 70° alcohol and elimination of the first streams. In fact, disinfection begins with the furthest district and ends in the nearest district, whereas sampling is the other way around. All these samples are identified and sent to the National Agricultural Development Support Laboratory (LANADA) in Korhogo under strict refrigeration conditions (4°C) where analyzes of microbiological parameters are carried out.

2.3.2 Sampling

Of the forty-five farms visited per locality, 288 samples were infected with subclinical mastitis after the CMT test in the Poro region. However, these samples were used for microbiological analyzes in the laboratory. In order to determine the cow's milk production departments most contaminated by major and minor pathogenic strains. These are the departments of Korhogo, Sinématiali, M'Bengué and Dikodougou.

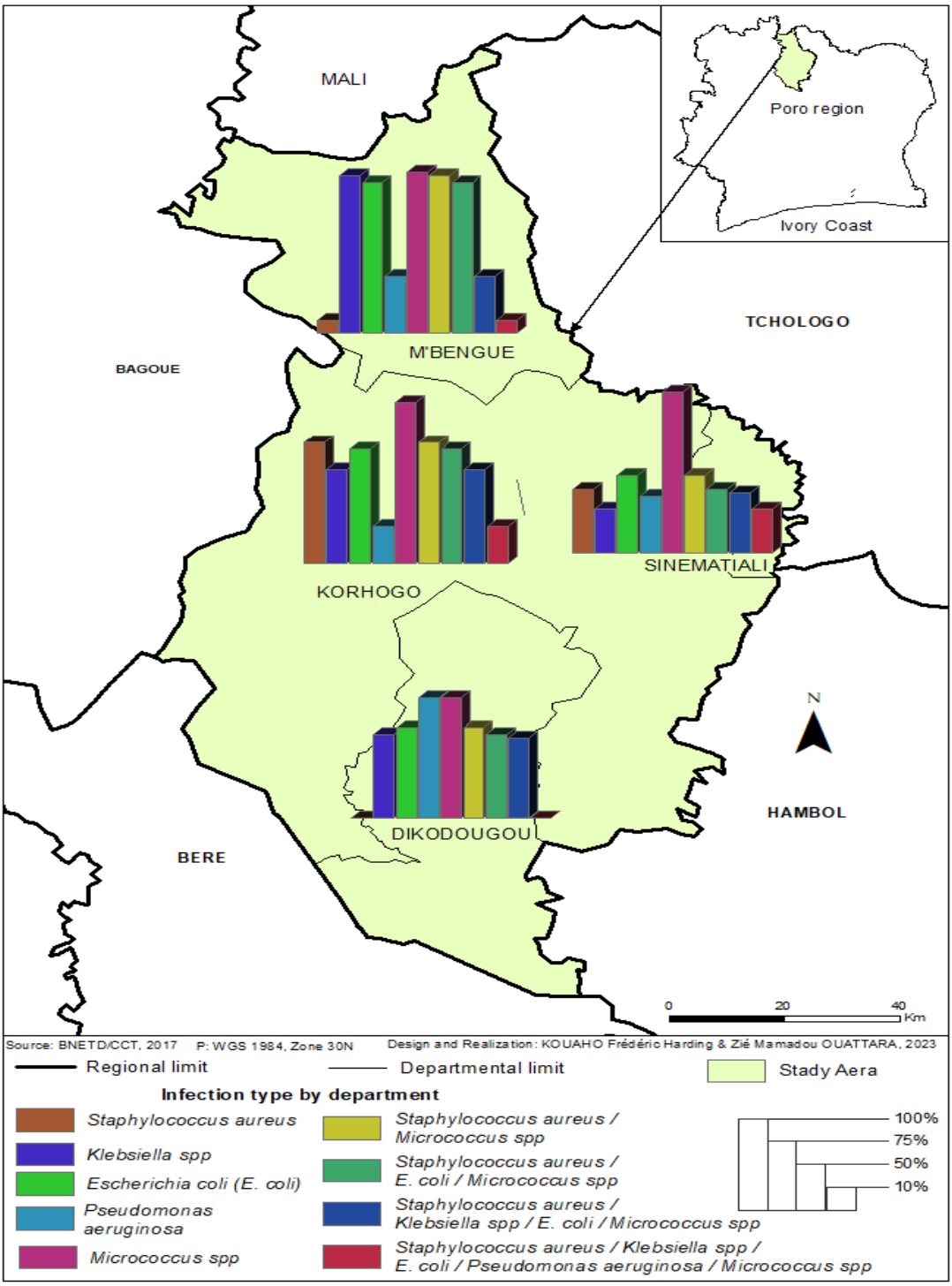

Fig. 1. Map presenting the types of bacterial infection by department visited in the Poro region

2.3.4 Bacteriological analyzes

The milk samples were analyzed by standard bacteriological isolation and identification techniques. Inoculation of CMT positive samples was carried out on Baird-Parker agar, MacConkey agar, TSA agar, Chapman agar and on Hektoen agar incubated at 37°C for 24–48 hours. The identification of bacteria was carried out by conventional methods (appearance of colonies, Gram staining, catalase test, oxidase test associated with coagulas, etc.) **[25]**. The biochemical characters were studied using the API Bio Mérieux galleries (API Staph and API20E etc.) allowing the characterization of bacterial species within the same genus: if at

least five bacterial colonies are present, the isolated germ is considered responsible for mastitis **[26]**. The sensitivity of the main germs isolated was tested against ten (10) antimicrobials including those used in the treatment of mastitis by veterinary clinicians in the Poro region. The antibiotic discs used are: Ampicillin (AM), Colistin (CS), Gentamicin (GM), Cefalexin (CEF), Chloramphenicol (CHL), Neomycin (N), Penicillin (P), Spiramycin (SP), Trimethoprim-sulfamethoxazole (SXT) and Tetracycline (TE). The classic agar diffusion method was used and the interpretation was made according to the criteria of the European committee on antimicrobial susceptibility testing-EUCAST (2023).

2.3.5 Statistical analyzes

Data analysis and processing were carried out using the Excel 2016 spreadsheet.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Global observation on cases of mastitis

After the CMT test carried out on 360 dairy cows in the Poro region, the diagnosis determined 288 affected cows subclinical mastitis and 32 cows affected by clinical mastitis including 30 Mérés, 2 N'damas; with dominance of the Mérés and N'damas breeds followed by the other breeds (Table 1).

3.1.2 Livestock system on study farms

The majority of dairy cows are raised in makeshift enclosures. The diet of dairy cows by breeders in this region consists mainly of pasture at 75.6% for years. Health monitoring and vaccination are less practiced in the Poro region; most animals had calved several times.

3.2 Observation of the CMT Test Analysis

The analysis made it possible to note acute subclinical mastitis in 51.11% (184/360) of cases and chronic subclinical mastitis in (25%) (90/360) of cases (Table 1). Acute subclinical mastitis is characterized by the association of local signs (edema, heat, pain, redness, etc.) and/or general signs (anorexia, depression, hyperthermia, etc.) **[27]**. On the other hand, subclinical mastitis results only in an immune reaction evidenced indirectly by an increase in the concentration of somatic cells in milk **[28**].

3.3 Bacteriological Property of Milk

The bacteriological examination made it possible to isolate and identify the different pathogenic germs. Out of a total of 360 dairy cows, 288 mixed milk samples from the cows tested positive for CMT, 32 samples were found to be negative. The 91.11 % were culture positive and various bacterial genera were isolated (Fig. 1).

3.4 Bacterial Infection of Milk

There was a significant difference in the presence of bacterial infection in fresh milk ($P<0.05$) from each farm in the studied localities. Of all the milk samples analyzed, no *Streptococcus digalactiae*, *agalactiae* and *Streptococcus uberis* were isolated. In milk from different districts taken from the udders of cows in each locality, 75% and 68% of the samples contained *Staphylococcus aureus* and *Escherichia coli* in the locality of Korhogo followed by bi-infections and penta-infections. The locality of Sinématiali is the second locality which had less microbial infection of *Staphylococcus aureus* of 39.58% of cases and 46% of *Escherichia coli* followed by bi-infections and penta-infections observed. However, in the locality of M'Bengué and Dikodougou we noted a reduction in *Staphylococcus aureus* of 8.33% and 90% of *E. coli* followed by bi-infections and penta-infections while in the locality of Dikodougou we observed an absence of *S. aureus* and a reduction of *E. coli* of 54% with bi-infections and tetra-infections. In quarter milk, the frequencies of *E. coli* present by locality were higher than those of *S. aureus*. The number of samples containing the germs studied fluctuate by locality observed in the Poro region in the milk collected from the different quarters of dairy cows Fig. 1.

Table 1. Prevalence of mastitis according to cow breeds after the CMT test

Scores	Mother	N'dama	Baoulé	Zebus	Metis	Frequency %	Types of mastitis
0	38	0	2	0	0	40 (11.11%)	None (no mastitis)
1	30	2	0	0	0	32 (8.88%)	Clinical
2	12	0	0	2	0	14 (3.88%)	Simple subclinical
3	164	12	2	2	4	184 (51.11%)	Acute subclinical
4	78	6	4	2	0	90 (25%)	Chronic subclinical

Total	322	20	8	6	4	360 (100%)

3.5 Relationship between CMT scores and isolation of *Staphylococcus aureus* and *Escherichia coli*

Table 2 shows that 30% *S. aureus* were isolated in milk samples with a score of "4" less than that of "3" higher by 50%. We see that the more the scores evolve, the lower the number of *S. aureus* and *E. coli* isolated.

From this Table 3, it appears that the most identified pathogenic germs were observed in the department of Sinématiali 32.58% of cases, followed by Korhogo 26.96%, M'Bengué 25.84% and Dikodougou 14.60% of cases which were the least isolated out of the 89 germs. On the other hand, the major pathogenic germs were observed in the department of Korhogo 6/14 of *S. aureus*, 5/19 of *E. coli* i.e. 42.85 % and 26.31% of cases followed by the department of Sinématiali 5/14 S. aureus, 4/19 *E. coli* or 35.71% and 21.05% of cases. However, we observe a low rate of *S. aureus* 3/14 or 21.42% then a significant quantity of *E. coli* 42.85 % of cases in the department of M'Bengué and a complete absence of *S. aureus* in the Dikodougou department followed a presence of *E. coli* of 28.57 and 42.85 % of isolated cases.

3.6 Prevalences of Pathogenic Germs Observed and Identified by Locality

From this table, it appears that Gram-positive cocci were the least isolated (43%) followed by Gram-negative bacilli 57% (non-Enterobacteria (16%) and 38% Enterobacteria) which predominate (Fig. 2).

Table 2. CMT score, *Staphylococcus aureus* isolation and *Escherichia coli* isolation

CMT score	Isolation of *S. aureus*	Frequency (%)	Isolation of *E. coli*	Frequency (%)
2	2	**20%**	5	**33.33%**
3	5	**50%**	6	**40%**
4	3	**30%**	4	**26.67%**
Total	10	**100%**	15	**100%**

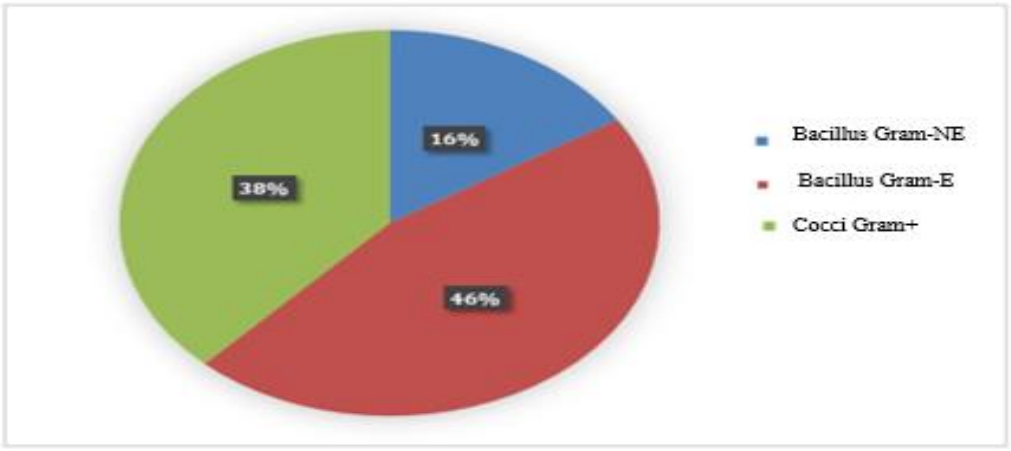

Fig. 2. Gram staining of identified germs

NE= Non Enterobacteria (Pseudomonas aeruginosa), E= Enterobacteria (E. coli, Klebsiella spp)

Table 3. pathogenic germs isolated in the different localities

Departments		Pathogenic germs							Total	
		S. aureus	***E.coli***	***Klebsiella spp***	***P. aeruginosa***	***Micrococcus spp***	***S. lentus***	***S. xylosus***	**Number**	**Frequency %**
Korhogo	Karakro	2	2	1	2	1	0	0	8	33.33%
	Korhogo	1	1	2	1	1	2	0	8	33.33%
	Napiéoledougou	2	0	0	0	0	1	0	3	12.5 %
	Kombolodougou	1	2	1	1	0	0	0	5	20.83%
Total		6	5	4	4	2	1	0	24	100%
M'bengue	Bougou	2	2	1	2	1	0	0	8	38.09%
	Katiali	0	1	2	0	0	0	0	3	14.28%
	M'bengué	1	2	0	1	2	0	0	4	19.04%
	Katogo	0	1	2	2	1	0	0	6	28.57%
Total		3	6	5	5	4	0	0	21	100%
Senematiali	Bahouakaha	2	1	2	1	2	1	0	9	32.14%
	Sinematiali	0	1	1	0	0	0	1	3	10.71%
	Kagbolodougou	1	2	0	2	1	1	1	8	28.57%
	Sediego	2	0	2	1	1	0	2	8	28.57%
Total		5	4	5	4	4	0	0	28	100%
Dikodougou	Boron	0	1	1	0	1	0	0	3	23.07%
	Dikogougou	0	1	0	2	1	0	0	4	30.76%
	Guiembe	0	2	2	1	1	0	0	6	46.15%
Total		0	4	3	3	3	0	0	13	100%
General Total		14	19	17	16	13	6	4	89	
Frequencies %		15.73	21.34	19.1	17.97	14.6	6.74	4.49		100

NB: S= Staphylococcus, P= Pseudomonas

Table 4. Pathogenic germs identified in the localities

Region	Workforce	Group/Gram	Pathogenic germs	Number of isolated	Frequency %
PORO	56 (100%)	Gram negative bacilli	*Pseudomonas aeruginosa*	8	14%
			E.coli	15	27%
			Klebsiella spp	9	16%
		Gram-positive cocci	*Staphylococcus aureus*	10	18%
			Micrococcus spp	6	11%
			SCN	8	14%

SCN= Coagulase-negative staphylococci

3.7 Sensitivity of Major Pathogenic Germs Isolated to the Ten Antibiotics Tested

The antibiogram was carried out on the main germs isolated from samples from subclinical mastitis areas (*S. aureus*, SCN, Enterobacteria (*E. coli*, *Klebsiella spp*) in order to determine in vitro sensitivity to antibiotics (ten antibiotics used). *Staphylococcus aureus* showed excellent sensitivity to gentamycin 100%, good sensitivity to chloramphenicol and neomycin at 90% then acceptable sensitivity to trimethoprim-sulfamethoxazole, to cefalexin 80%. The group of coagulase negative staphylococcus (SCN) revealed good sensitivity to cefalexin and gentamycin of (87.5%) and acceptable sensitivity to chloramphenicol, neomycin of (75%) each. Enterobacteriaceae showed excellent resistance to ampicillin, Spiramycin and penicillin 100% and acceptable resistance to Gentamycin (95.84%).

Basically, remarkable resistances have been observed on the following antibiotics: ampicillin, penicillin and colistin. This remarkable resistance has been noted in Staphylococci and Enterobacteria. *Staphylococcus aureus* and coagulase negative staphylococci (SCN) showed high resistance to the colistin profile (100% for *Staphylococcus aureus* and 87.5% for SCN). Enterobacteriaceae showed negligible resistance to colistin (25%) (Table 5).

3.8 Resistance Profile of the Different Pathogenic Germs Identified

3.8.1 Resistance profile of identified Enterobacteriaceae

The strains of Enterobacteria present a resistance rate equal to 100% or 24/24 concerning the following antibacterials: ampicillin, penicillin, spiramycin, we also note excellent effective sensitivity on our isolated strains with a rate of 1% resistance on the colistin. For gentamicin, cefalexin, neomycin we note a resistance rate of 95.83%, 91.66%, 70%; then we observe a clear resistance 66.66% and 58.33% to tetracycline, trimethoprim + sulfamethoxazole and chloramphenicol Fig. 3.

Table 5. Antibiogram of the different pathogenic germs isolated and identified

Tested antibiotics	*Enterobacteriaceae spp*		*Staphylococcus spp*		*Staphylococcus aureus*	
	S	R	S	R	S	R
Cefalexin	2 (8.44%)	22 (91.66%)	7 (87.5%)	1 (12.5%)	8 (80%)	2 (20%)
Chloramphenicol	4 (16.66%)	14 (58.34%)	6 (75%)	2 (25%)	9 (90%)	0 (0%)
Ampicilin	0 (0%)	24 (100%)	2 (25%)	6 (75%)	7 (70%)	3 (30%)
Colistin	18 (75%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	10 (100%)
Neomycin	7 (29%)	17 (71%)	6 (75%)	2 (25%)	9 (90%)	0 (0%)
Penicillin	0 (0%)	24 (100%)	2 (25%)	6 (75%)	6 (60%)	4 (40%)
Gentamicile	1 (4.16%)	23 (95.84%)	5 (87.5%)	1 (12.5%)	10 (100%)	0 (0%)
Spiramycin	0 (0%)	24 (100%)	5 (62.5%)	1 (12.5%)	6 (60%)	1 (10%)
Tretracycline	3 (12.5%)	16 (66.66%)	4 (50%)	3 (37.5%)	6 (60%)	4 (40%)
Trimethoprim - Sulfamethoxazole	5 (20.84%)	14 (58.33%)	5 (62.5%)	2 (25%)	8 (80%)	2 (20%)

S= sensitive; R= resistance

3.8.2 Resistance profile of identified SCN

The results of the antibiogram show a resistance of 87.5% for colistin and also a resistance of 75%

is observed for ampicillin and penicillin. On the other hand, we observe a sensitivity of 87.5% for cefalexin and an effective sensitivity of 75% for chloramphenicol and neomycin. And an average sensitivity rate of 62.5% to spiramycia, gentamicin and trimethoprim + sulfamethoxazole was noted during antibiogram. Then a rate of 50% was noted for the antibacterial tetracycline concerning the SCN strains Fig. 4.

S. aureus strains have a resistance rate equal to 100% or 10/10 regarding the antibacterial colistin. On the other hand, we note excellent sensitivity on our isolated strains with a rate of 100% sensitivity to gentamicin Fig. 5. And a rate of 90%, 80% and 70% for chloramphenicol, cephalexin, tetracycline, trimethoprim + sulfamethoxazole and ampicillin. An average rate of 60% was observed with the fllowing antibiotics: spiramycine, tetracycline, penicillin.

3.8.3 Resistance profile of identified *Staphylococcus aureus*

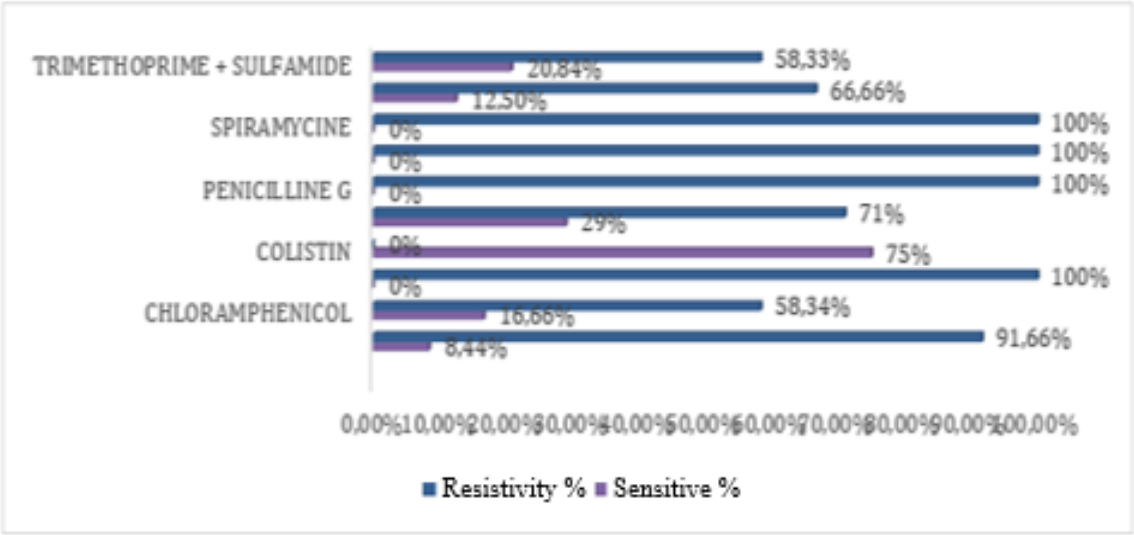

Fig. 3. Resistance of Enterobacteriaceae (*E. coli* and *Klebsiella spp*) to the antibiotics tested

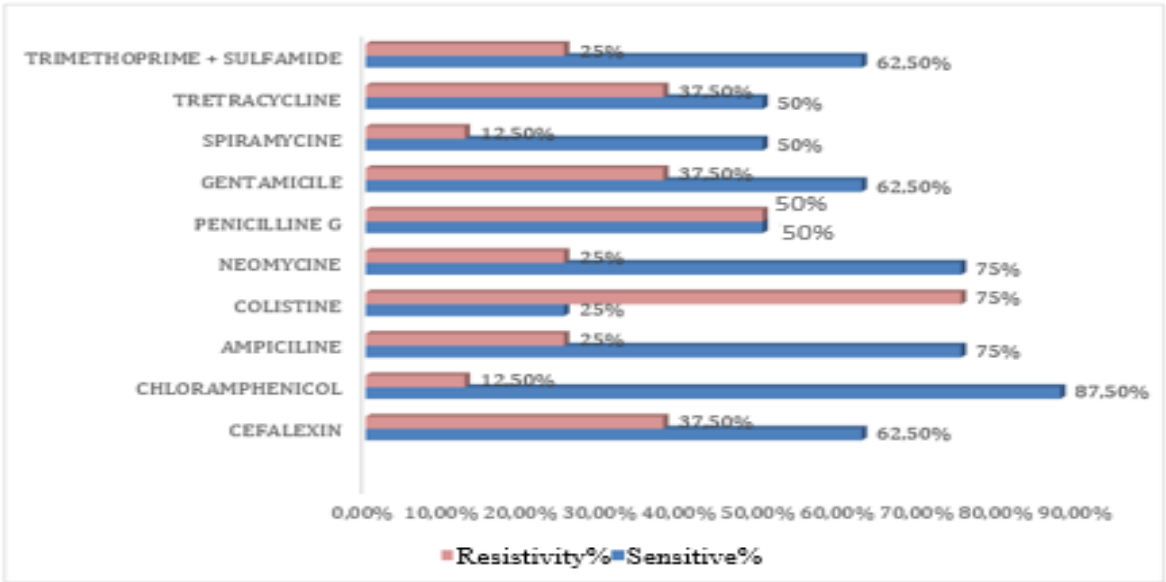

Fig. 4. Resistance profile of SCN (*S. lentus* and *S. xylosus*) to the antibiotics tested

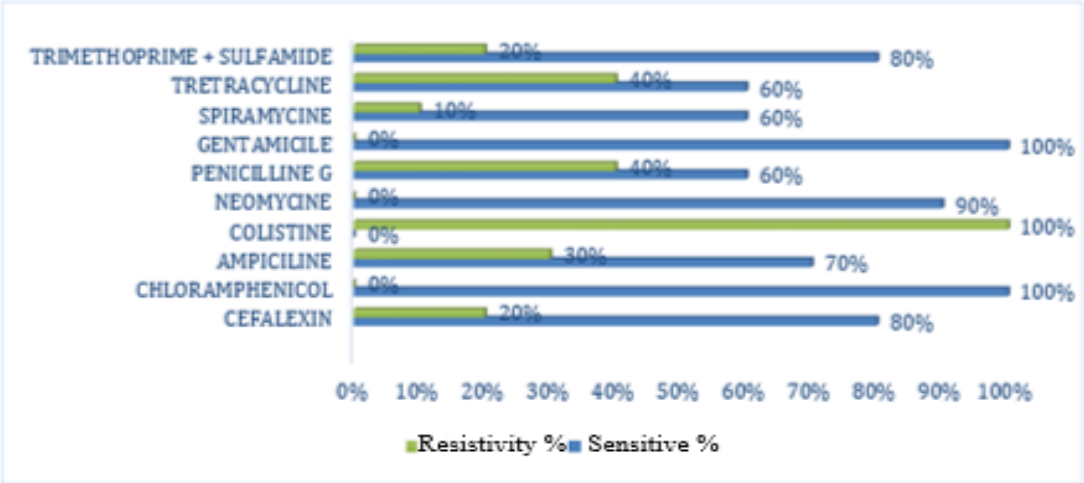

Fig. 5. Resistance profile of *S. aureus* to the antibiotics tested

3.8.4 Resistance profile of identified Staphylococcus

The 32 strains isolated from Staphylococcus (*S. aureus* and SCN) were found to be resistant to at least one antimicrobial agent. The isolated strains showed multiple resistance. Resistance was observed for colistin (17/32) or 53.12 %. There was low resistance found for ampicillin (9/32) i.e. 28.12 %, tetracycline (7/32) i.e. 21.87 % and penicillin (6/32) i.e. 18.75 %. On the other hand, very low resistance was observed for trimethoprim + sulfamethoxazole (4/32) or 12.5 %, spiramycin (2/32) or 6.25 %, neomycin (2/32) and gentamicin (1 /32) or 3.12. No resistance was found for Chloramphenicols (Table 6). It is important to mention that all *S. aureus* strains that were tested with colistin were resistant to this antimicrobial agent.

Table 6. Antibiotic resistance profile of identified Staphylococcus strains

Tested Antibiotics	*S. aureus*	SCN	
		S. lentus	*S. sylosus*
Cefalexin	2	0	1
Chloramphenicol	0	0	0
Ampicilin	3	4	2
Colistin	10	2	5
Neomycin	0	1	1
Penicillin g	0	2	4
Gentamicile	0	0	1
Spiramycin	1	1	0
Tetracycline	4	1	2
Trimethoprim +Sulfamide	2	0	2

3.8.5 Resistance profile of the different Enterobacteriaceae identified

The isolated Enterobacteriaceae (*E. coli* and *Klebsiella spp*) strains showed 100% resistance to nine antimicrobials out of the 24 tested; none of the isolates were resistant to colistin. In the present study, the 24 strains tested showed the phenomenon of multiple resistance, as follows: fifteen strains (100%) to four antimicrobials in *E. coli*, five strains (100%) in *Klebsiella spp*, thirteen strains (86 .66%), eleven strains (73.33%), ten strains (66.66%), eight (53.33%) antimicrobials all in *E. coli*. On the other hand, the phenomenon of multiple resistance to the antimicrobials tested was also observed in the strains of *Klesiella spp* six strains (66.66%), seven strains (77.77%), and four strains (44.44%) then a strain (11.11%).

Table 7. Antimicrobial resistance profile of isolated strains of Enterobacteriaceae

Tested antibiotics	Enterobacteria (n=24)	
	***E. coli* n= 15**	***Klebsiella spp* n= 9**
Cefalexin	13	9
Chloramphenicol	8	6
Ampicilin	15	9
Colistin	0	0
Neomycin	10	7
Penicillin g	15	9
Gentamicile	15	9
Spiramycin	15	9
Tretracycline	11	4
Trimethoprim + Sulfamide	13	1

3.8.6 Resistance model of identified strains of SCN, Enterobacteriaceae and *Staphylococcus aureus* based on antibiotic families

All 34 isolates studied belonging to several bacterial genera were 100% resistant to three families of antibiotics (Table 8). The phenomenon of resistance has manifested itself for several antibiotics, such as penicillin, spiramysin, colistin, tetracycline, neomycin, ampicillin, and gentamicin, antibiotics frequently used in the treatment of mastitis in cows in several countries; and which remains dominated by five large families of antibiotics which are: Beta-lactams, Aminosides, Macrolides, Tetracyclines, Cephalosporins and Polymyxins (Table 8).

4. DISCUSSION

Bacteriological results were positive for 288 samples. Thus, the culture of certain positive quarter milk samples turned out to be negative, despite attempts to subculture the samples. These results confirm those of (Serieys, 1985b) **[29]**, which stipulate that a high cellular concentration is not necessarily associated with a bacterial infection. Several hypotheses were formulated by Bouchot et *al.* **[30]** to explain the problem of sterile samples. Furthermore, according to Boutet et *al.* **[31]**, it is possible that the quarters from which these sterile milk samples come present real inflammation but not of bacterial origin. Finally, according to authors cited by Boutet et *al*. **[31**], another hypothesis that could explain this observation is based on the power of certain germs, such as *S. aureus*, to penetrate and survive in mammary epithelial cells and the macrophages. A high proportion was observed for mono-infection subclinical mastitis in the Korhogo department, 75% higher than the other departments Sinématiali 38%, M'Bengué 8% and Dikodougou 0% and bi-infections were observed in these different departments with a proportion of 75%, 46%, 94% and 54%. (61.86%) and bi-microbial (37.11%). However, the cases where three, four and five germs were isolated could be explained by the colonization of the neighborhoods by different bacteria. Indeed, for this cow, the CMT was positive for all four quarters. Numerous studies carried out on

Table 8. Identified resistance model of SCN (n= 8), Enterobacteriaceae (n= 24) and *Staphylococcus aureus* (n=10) depending on the antibiotic families tested

Pathogenic germs	Number identified	Resistance profile observed (antibiotic families)	
Enterobacteria	21 (87.5%)	AM, N, GM	Aminoglycosides
	24 (100%)	P	Beta-lactams
	24 (100%)	SP	Macrolides
	16 (66.66%)	TE	Tetracyclines
	14 (58.33%)	CHL	Phenicolates
	6 (25%)	CS	Polymyxins
	22 (91.66%)	CEF	Cephalosporins
	14 (58.33%)	SXT	Sulfanamides + Trimethropime
Staphylococcus aureus	3 (30%)	AM, N, GM	Aminoglycosides
	4 (40%)	P	Beta-lactams
	1 (10%)	SP	Macrolides
	4 (40%)	TE	Tetracyclines
	0 (0%)	CHL	Phenicolates
	10 (100%)	CS	Polymyxins
	2 (20%)	CEF	Cephalosporins
	2 (20%)	SXT	Sulfanamides + Trimethropime
SCN	3 (37.5%)	AM, N, GM	Aminoglycosides
	6 (75%)	P	Beta-lactams
	1 (12.5%)	SP	Macrolides
	3 (37.5%)	TE	Tetracyclines
	2 (25%)	CHL	Phenicolates
	7 (87.5%)	CS	Polymyxins
	1 (12.5%)	CEF	Cephalosporins
	1 (12.5%)	SXT	Sulfanamides + Trimethropime

NB: Ampicillin (AM), Colistin (CS), Gentamicin (GM), Cefalexin (CEF), Chloramphenicol (CHL), Neomycin (N), Penicillin (P), Spiramycin (SP), Trimethoprim-sulfamethoxazole (SXT) and Tetracycline (TE)

mastitis show that the major pathogens are mainly represented by *St. Uberis*, *St. Agalactiae*, *St. Digalactiae* and *S. aureus*. In this study, no Streptococcus strains were isolated. This observation would be mainly linked to our isolation method, because according to Bouchot et *al*. **[30]**, certain Streptococci are difficult to isolate on blood agar. But this absence was also noted by Boutet et al. **[30]** in a study on the germs responsible for subclinical bovine mastitis. On the other hand, *S. aureus* represents 18% of the strains isolated and identified, which is clearly different from that (Skyaka, 2007) **[31]** which is 22.22%; the prevalence is lower than that observed by certain authors, (Kudinha and Simango 2002), **[32]** who found 34.2% and 36.63% respectively. Similarly, a frequency of 15% was noted for this pathogen during a study in mixed-race and local cattle from the semi-intensive production systems of Kaolack and Fatick in Senegal **[33]**. *S. aureus* is part of a group of contagious bacteria that are passed from one cow to another during milking. This bacterium is present in the majority of herds and most often causes chronic infections responsible for an increase in SCC and which appear throughout lactation. Sometimes, an *S. aureus* infection can progress differently, causing a peracute illness such as gangrenous mastitis. Enterobacteriaceae (*E. coli and Klebsiella spp*) were isolated and identified with respective frequencies of 27% and 16%. This result is much higher than those of (Ranard, 1985) **[34]** and (Skyaka, 2007) **[31]** by 2.4% on both sides of the seeds. For them, the percentage of neighborhoods infected with coliforms is commonly 15 to 30 times lower than for Staphylococci or Streptococci, which is not the case in our study. Concerning minor pathogenic germs, the most frequently identified were coagulase negative staphylococci (CNS) with a prevalence of 14%. This prevalence is lower than those observed by Bada-Alambedji et *al.* **[32]** in Niger and **[30]**, which are respectively 22.5 and 24.6% in conventional breeding. Also, two studies carried out in France and reported by Bouchot et *al.* **[30]**, revealed respective frequencies of 12.7% and 14.8% which is in agreement with the results of our study. The proportion observed for these germs is a major problem because, even if these pathogens are not the cause of a real pathological process, just by their presence within the udder, they can disturb the quality of the milk by increasing the somatic cell counting of quarter milk **[34]**. This observed prevalence could be related to unsatisfactory good hygiene practices on farms, especially during the rainy season. On the other hand, Antibiotic resistance is a significant problem in cow mastitis. Antimicrobial resistance helps bacteria stay alive after treatment with antibiotics, and some of the resistance mechanisms include the presence of antimicrobial resistance genes that can spread by horizontal transfer from bacteria to bacteria with mobile genetic elements such as plasmids, phages and pathogenicity islands, or by random mutations when bacteria are under stress **[35,36]**. In cases of mastitis, the prevalence of antimicrobial-resistant bacteria appears to be increasing, at least for some antimicrobials. Studies have reported that more than 50% of isolates that because mastitis were resistant to beta-lactams or penicillin **[37]**. Some authors noted that in the treatment of mastitis caused by Staphylococci sensitive to penicillins, it is recommended to administer β-lactam antimicrobials (especially penicillin G), and as an alternative treatment, cloxacillin, macrolides and lincosamides can be used. The same authors advise against the use of fourth generation cephalosporins as a therapeutic alternative, as they can generate strains resistant to broad-spectrum β-lactams **[38,39]**. The antimicrobial results were found to be sensitive and more effective to four families of antibiotics in Staphylococci (*S. aureus* and SCN) which are; aminoglycosides, phenicols, cephalosporin and Sulfanamides + Trimethropime. Indeed, with 100%, 90% and 80% effectiveness on the evolution of *S. aureus* and 87.5%, 75% for SCN, gentamicin, chloramphenicol, neomycin, cefalexin and trimethoprim + sulfamethoxazole, constitute antibiotics of choice for treating subclinical mastitis due to this pathogen. These results are similar to those obtained by Houssa, **[40]** and Bouchot et al. [30] who obtained excellent sensitivity of Staphylococci to chloramphenicol, gentamicin, neomycin, cefalexin and trimethoprim-sulphametoxazole. Our results are consistent with those of (Hama, 2006**)** **[41]** who qualify the effectiveness of gentamicin as excellent against Staphylococci. On the other hand, **[29]** obtained a lower sensitivity for chloramphenicol. The sensitivity of Staphylococci that we have noted with chloramphenicol could be explained by the fact that the use of the latter in animals has been prohibited for years, because of the bone marrow aplasia that it is likely to cause. Good sensitivity of Staphylococci to chloramphenicol was also noted by Bada-Alambedji et *al.* **[32]** and Houssa, **[40]**. An unacceptable resistance of Staphylococci to Polymyxin s, Beta-lactams, Aminosides and Tetracyclines was observed for penicillin, ampicillin, Tetracyclines and colistin. This observation is similar to that made by Boutet et *al*. **[31]**. This percentage of resistance

observed in Staphylococcus can be justified by the wide use of these antibiotics in the treatment of mastitis. Indeed, in the forty-five farms visited, Beta-lactams and Polymyxin are widely used in the treatment of mastitis. Staphylococci showed almost ineffective sensitivity to colistin; this sensitivity was zero for *S. aureus*. This result confirms that of Bouchot et al. **[29]**. The inappropriate use of antibiotics (insufficient doses, long treatment duration, etc.) are often the cause of resistance phenomena. However, it is important to report very poor effectiveness of all the antimicrobials tested against Enterobacteria. With the exception of Polymyxins (colistin), **[41,42,43]** no antibiotic has had an effectiveness frequency greater than 50% on this species of bacteria. This result is not fortuitous when we know that Enterobacteriaceae, because of its production of beta-lactamases (enzymes which inactivate antibiotics), is naturally resistant to beta-lactams and certain cephalosporins. This is the case observed for certain families of antibiotics such as; Macrolides, beta-lactamases, Cephalosporins, Aminosides and Tetracyclines which have shown very effective resistance to this type of bacteria **[44,45]**.

5. CONCLUSION

The data from our study revealed that acute and chronic subclinical mastitis predominates in dairy cows in the Poro region, located in the north of Côte d'Ivoire. Several pathogenic germs are responsible for these types of mastitis in cows in the region. Among the pathogenic germs isolated and identified, others (Enterobacteria) are caused by non-compliance with hygiene rules and some (Staphylococcus) have a negative impact on the health of humans and animals. While other antimicrobials remain effective against these pathogens, some do not. Staphylococcus strains exhibited remarkable multiple resistance to ampicillin, penicillin and colistin. Indeed, the good practice of hygiene rules now remains urgent for health, if we consider the reactions between animals, humans, the environment and animal products. The numerous resistance phenomena observed in Staphylococcus and Enterobacteria, in the isolates require discernment in the choice of mastitis treatment, taking into account both the health of the animal, productivity, but also the ease of transmission of bacteria from milk to humans. For this reason, antimicrobial susceptibility testing is highly recommended for breeders in the area. We concluded that Enterobacteriaceae (*E. coli* and *Klebsiella spp*) were more resistant and more frequent in the farms studied, due to exposure to a high number of antibiotics, but also due to the high frequency of isolation. of bacterial strains exhibiting exaggerated resistance to antimicrobials.

CONSENT

The consent of the Ministry of Animal and Fishery Ressources of the Poro region was obtained, in order to facilitate exchange between dairy cow breeders from the different departments of the region and allow the collection of milk.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abrahamén M, Persson Y, Kanyima BM, Båge R. Prevalence of subclinical mastitis on dairy farms in urban and peri-urban areas of Kampala, Uganda. Too much. Anim. Health Products. 2013; 46:99–105.
2. Abebe R, Hatiya H, Abera M, Megersa B, Asmare K. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* from dairy herds in Hawassa dairy shed, southern Ethiopia. BMC Veterinarian. Res. 2016; 12:270.
3. Barkema H, Schukken Y, Zadoks R. Invited review: The role of cow, pathogen, and treatment regimen in therapeutic success for *Staphylococcus aureus* bovine mastitis.J. Dairy Sci. 2006;89:1877–1895.
4. Pol M, Ruegg P. Relationship between antimicrobial drug use and antimicrobial

susceptibility of Gram-positive mastitis pathogens. J Dairy Sci. 2007; 90:262–273.
5. Beyene TJ, Hayishe H, Gizaw F, Beyi AF, Abunna F, Mammo B, Ayana D, Waktole H, Abdi RD. Prevalence and antimicrobial resistance profile of Staphylococcus in dairy farms, slaughterhouses and humans in Addis Ababa, Ethiopia. BMC Res. Remarks, ten. 2017;171.
6. Ruegg PL. A 100-year review: detection, management and prevention of mastitis. J. Dairy Sci. 2017; 100:10381–10397.
7. Zeryehun T, Abera G. Prevalence and bacterial isolates of mastitis in dairy farms in selected districts of Harrarghe Eastern Zone, Eastern Ethiopia.J. Veterinarian. Med. 2017;6498618
8. Ndahetuye JB, Persson Y, Nyman A-K, Tukei M, Ongol deputy, Båge R. Etiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali, Rwanda. Too much. Anim. Health Products. 2019; 51:2037-2044.
9. Bradley AJ. Bovine mastitis: An evolving disease. Vet. J. 2002; 164:116-128.
10. Östensson K, Lam V, Sjogren N, Wredle E. Prevalence of subclinical mastitis and pathogens isolated from the udder in dairy cows in southern Vietnam. Too much. Anim. Health Products. 2012; 45:979–986.
11. Dos Reis CBM; Barreiro JR, Mestieri L, Porcionato MADF, dos Santos, MV. Effect of somatic cell number and mastitis pathogens on milk composition in Gyr cows. BMC Veterinarian. Res. 2013; 9:67.
12. Mpatswenumugabo JP, Bebora LC,Gitao GC, Mobegi Virginia, Iraguha B, Kamana O, Shumbusho B. Prevalence of subclinical mastitis and distribution of pathogens in dairy farms in Rubavu and Nyabihu districts, Rwanda. J. Veterinarian. Med. 2017;8456713.
13. Piessens V, Van Coillie E, Verbist B, Supré K, Braem G, Van Nuffel A, De Vuyst L, Heyndrickx, M De Vliegher S. The distribution of coagulase-negative Staphylococcus species in the milk and environment of dairy cows differs between herds. J Dairy Sci. 2011; 94:2933–2944.
14. Vakkamäki J, Taponen S, Heikkila AM, Pyörälä S. Bacteriological etiology and treatment of mastitis in Finnish dairy herds. Acta veterinarian. Scan. 2017;59,33.
15. Bhosale R, Osmani RA, Ghodake PP Sheikh, SM, Chavan SR. Mastitis: An Intensive Crisis in Veterinary Science. Int. J. Pharma Res. Health Sci. 2014; 2:96–103.
16. Zinedine A, Faid M, Benlemlih M. Detection of antibiotic residues in milk and dairy products by microbiological method. Journal of Industrial, Health and Environmental Microbiology. 2007 ;1 :1-9.
17. Gysi M. Antibiotics used in dairy production in 2003 and 2004. Revue Suisse Agriculture. 2006; 38:215-220.
18. Oliveira R, De Pietro A, Cass Q. Quantification of cephalexin as residue levels in bovine milk by high-performance liquid chromatography with on-line sample cleanup. Talanta. 2006; 71:1233–1238.
19. Silveira-Filho VM, Luz EST, Campos APF, Silva WM, Barros deputy, Medeiros E, Freitas MFL, Mota RA, Sena MJ, Leal-Balbino TC. Antibiotic resistance and molecular analysis of Staphylococcus aureusIsolated from cow's milk and dairy products in northeastern Brazil.J. Food Prot. 2014;77:583–591.
20. Olivier SP, Murinda SE, Jayarao BM. Impact of antibiotic use in adult dairy cows on antimicrobial resistance in veterinary and human pathogens: a comprehensive review. Foodborne pathogen. Dis. 2011;8: 337–355.
21. Laven, R.; Balcomb, C.; Tulley, W.; Lawrence, K. Effet of dry period duration on the effet of an intramamary teat sealant on the risk of mastitis in cattle treated with antibiotics at drying off. New Zealand veterinarian. 2014; J, 62, 214-220.
22. Oliveira L, Ruegg P. Treatments for clinical mastitis occurring in cows in 51 large Wisconsin dairy herds. J. Dairy Sci. 2014; 97:5426–5436.
23. Stevens M, Piepers S, De Vliegher S. Mastitis prevention and control practices and mastitis treatment strategies associated with (primarily important) antimicrobial consumption in dairy herds in Flanders, Belgium. J. Dairy Sci. 2016;99: 2896-2903.
24. Ruegg PL. Mastitis Management on Organic and* Dairy Farms Conventional 1.J. Anim. Sci. 2009;87:43–55.
25. Saoussene Smaali. Study of the bacterial etiology of subclinical mastitis sheep in eastern Algeria. Department of Natural and Life Sciences, L'Arbi Tébissi University -Tébessa, Algeria. Africa SCIENCE journal. 2014 ;10(4) ;225-231.
26. Rakotozandrindrainy R, Foucras G. Bacterial etiology of mastitis in dairy cows in the dairy triangle of the highlands of

Madagascar. Rev. Med. Vet. 2007;158: 106-110.
27. Remy D. Mastitis: hygiene, prevention, environment, 1st edn. Paris, France, Agricultural France. 2010:260.
28. Pauline, Louise Angoujard. Survey on the diagnosis and treatment of mastitis in dairy cows by field veterinarians in France in 2015. Thesis for the veterinary doctorate Presented and publicly defended before the Faculty of Medicine of Créteil. 2015: 121.
29. F. Serieys. Cellular concentration of individual cow's milk: influence of mammary infection status, number, lactation status and milk production. Annals of Veterinary Research,1985, 16(3), pp. 255-261.
30. Bouchot M, Catel J, Chirol C et *al*. Bacteriological diagnosis of bovine mammary infections. Rec. Med. Vet. 1985;161(6-7):567-577.
31. Boutet P, Detilleux J, Motkin M, Deliege M, Piraux E., Depinois A, Debliquy P, MainiLJ, Czaplicki G, Lekeux P. Comparison of the cellular rate and antimicrobial sensitivity of the germs responsible for bovine subclinical mastitis between the conventional and organic sectors. Ann. Med. Vet. 2005; 149:173-182.
32. Bada-Alambedji R, Kané Y Issa Ibrahim A, et al. Bacteria associated with subclinical mastitis in urban and peri-urban livestock farms in Niamey (NIGER). RASPA. 2005;3 (2):119-124.
33. Konté M. Study of the prevalence of mastitis in mixed-race and local cattle from the semi-intensive production systems of Kaolack and Fatick in: Proceedings of the workshop to report the results of the Procordel project in Senegal held on, at CESAG, Dakar; 2003:44-46.
34. Perrin-Coullioud I, Martel JL, Broullet P, Fedida M. Identification and antibiotic sensitivity of various species of staphylococci associated with inapparent and subclinical bovine mastitis. Rev. Med. Vet. 1991; 42:39-47.
35. Brussow H, Canchaya C, Hardt W-D. Phages and evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol. Mol. Biol. Tour. 2004; 68:560–602.
36. Pantosti A, Sanchini A, Monaco M. Antibiotic resistance mechanisms in Staphylococcus aureus.Futur Microbiol. 2007; 2:323–334.
37. Jamali, H.; Radmehr, B.; Ismail, S. Short communication: Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from clinical bovine mastitis. J Dairy Sci. 2014;97: 2226–2230.
38. Ondiek J, Kemboi F. Bovine mastitis prevalence, etiology, therapeutics and control in Tatton Farm Park, Egerton University.Int. J. Curr. Microbiol. Appl. Sci., Sep. 2018;3417–3426.
39. Kaczorek E, Małaczewska J, Oójcik R, Rekawek W, Siwicki A. Phenotypic and genotypic antimicrobial susceptibility pattern of *Streptococcus spp*. Isolated cases of clinical mastitis in dairy cattle in Poland. J. Dairy Sci. 2017; 100:6442–6453.
40. Houssa E. Evaluation of the prevalence and causes of subclinical mastitis in intensive dairy cattle farming, in the peri-urban area of Dakar (case of Wayembam and Niacoulrab farms). Thesis: Med. Vet: Dakar. 2006;111.
41. Ame S, gunIvez FLA, Fukuda Y, Tada C, Jiménez IL, Valley WFM, Nakai Y. Prevalence and etiology of mastitis in dairy cattle in El Oro Province, Ecuador. J. Veterinarian. Med. Sci. 2018; 80:861–868.
42. Delfosse C, Foidmont E, Curnel Y, Humblet MF, Hanzen C, Berotozzi C, Bartiaux-Thill N. Eco-pathological study of risk factors for mastitis in dairy farms in Wallonia. Renc. Search Ruminants. 2006; 13:440.
43. El Jakee J, Aref NE, Gomaa A, ElHariri M, Galal HM, Omar SA, Samir A. Appearance of coagulase-negative staphylococci as a cause of mastitis in dairy animals: A danger for the environment. Int. J. Veterinarian. Sci. Med. 2013; 1:74–78.
44. León-Galvunn MF, Barboza-Corona JE, Lechuga-Arana AA, Valencia-Posadas M, Aguayo DD, Cédillo-Pelunez C, Marchéjenez-Ortega EA, Gutierrez-Chavez AJ. Molecular detection and sensitivity to antibiotics and bacteriocins of pathogens isolated from bovine mastitis in family dairy herds in central Mexico. BioMed Res. Int.2015; 615153.
45. Paduch J-H, Krömker V. Colonization of teat skin and teat canal by mastitis pathogens in dairy cattle. Tierarztl. Prax.

Aug. G Grosstiere—Nutztiere. 2011;39: 71–76.