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Research Article

Bacterial Pathogens Involved in Bovine Mastitis and Their Antibiotic Resistance Patterns in the Adamawa Region of Cameroon

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Abstract

Data on the sensitivity pattern of bacteria are scarce in sub-Saharan Africa, especially in Cameroon. This paper reports the prevalence of bovine mastitis and major bacterial pathogens associated with the disease and their antimicrobial profiles in the Adamawa Region of Cameroon. It was conducted to investigate the sensitivity pattern of bacteria isolated from mastitis cases that could be helpful in the application of appropriate therapeutic measures. For this study, 224 lactating cows were examined. A high average prevalence (59.8%) in subclinical mastitis was recorded as compared to clinical mastitis (3.6%; χ^2=163.7, P=10^{-4}). Out of the 135 clinical and subclinical mastitis cases recorded, bacteria were cultured from 115 milk samples (85.2%, n=135). In all, 14 different bacterial pathogens were isolated including: coagulase negative Staphylococci (27.5%), *Staphylococcus aureus* (23.3%), *Escherichia coli* (11.3%), *Streptococcus agalactiae* (7.1%), *Streptococcus dysagalactiae* (4.2%), *Enterococcus faecalis* (2.8%), *Klebsiella pneumoniae* (2.8%), *Enterobacter aerogenes* (2.1%), *Pseudomonas aeruginosa* (2.1%), *Corynebacterium* spp. (1.4%), *Proteus* spp. (1.4%), *Brucella* spp. (1.4%), *Mycoplasma* spp. (0.7%), and *Mycobacterium* spp. (0.7%). A major variation in the sensitivity of isolated bacteria against 14 different antibiotics was noticed. Overall the sensitivity test revealed that Enrofloxacin, Gentamicin, and to a lesser extent Oxacillin and Amoxicillin/Clavulanic acid, were most efficacious. The study gives a significant contribution to the epidemiology and contributes to reducing the lack of knowledge about the antibiotic resistance patterns of major bacterial mastitis in Cameroon. The application of these antibiotics could be beneficial in resolving the cases of bovine mastitis in dairy herds.

Keywords: Antibiotic resistance; Cameroon: Cattle; Mastitis; Pathogenic bacteria

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Introduction

Mastitis, an inflammatory mammary gland condition, is the most common, troublesome and the most expensive disease of dairy ruminants worldwide as it is responsible for heavy economic losses in terms of reduction in milk yield, profit margins, and quality of milk and milk products [1-4]. Although physical and chemical injuries may cause inflammation of the mammary gland, infections most often caused by bacteria or other microorganisms (fungi, viruses, algae) are the primary cause of mastitis [5]. Thus, based on etiopathological investigations, it is usually classified as subclinical, acute, subacute, chronic or gangrenous [6,7].

The causative organisms are well adapted to survive in the mammary glands and in most cases, establish mild subclinical infection of long duration during which pathogens of public health significance might be shed into milk from the infected quarters [8]. Furthermore, mastitis is associated with a number of zoonotic diseases including Tuberculosis, Brucellosis, Campylobacteriosis and streptococcal sore throat in which milk acts as a vehicle of infection [7,9]. Public hazards associated with the consumption of antibiotic contaminated milk and products cause allergic responses, changes in intestinal flora and development of antibiotic resistant pathogenic bacteria [10,11].

The dairy industry in Cameroon is rudimentary [12] and mastitis is becoming a significant constrained in its development. Gram positive and Gram negative bacteria are involved as major pathogens causing mastitis worldwide, such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp., *Klebsiella* spp. [13]. *S. aureus* and *E. coli* are the most commonly isolated pathogen from clinical mastitis [14]. *Staphylococcus* spp. is a major pathogen causing various forms of subclinical and clinical mastitis in cattle [15]. Coagulase negative staphylococci remain the most frequently isolated pathogens from the subclinical mastitis in dairy cows [14].

An important aspect in the appropriate control of infectious diseases is identification of the causative agents. Antimicrobial therapy aiming against infectious agents causing mastitis is usually recommendable [16]. The indiscriminate use of antimicrobial drugs without testing *in vitro* sensitivity, as commonly practice in the country, may be considered the primary cause of lack of success in treatment. Transmission of resistant pathogens to humans via bulk milk with subclinical mastitis is of major public health interest [17]. In addition,

the risk to human health for *Mycobacterium avium* subsp. *paratuberculosis* [18], *Mycobacterium bovis*, the causal agent of Tuberculous, mastitis, and other milk zoonoses is of great concern particularly in developing countries where there is an increase in the consumption of untreated milk [19]. Therefore, it is important to investigate the sensitivity pattern of the different bacteria isolated from mastitis as well as apply the appropriate therapeutic measures. Such data are very scarce in sub-Saharan Africa, especially in Cameroon.

In this context this study was carried out to identify the causative bacterial agents of bovine mastitis in Adamawa region of Cameroon as well as evaluate their antibiotic susceptibility profiles. The investigation also attempt to provide epidemiological data which are key to the formulation of antimicrobials therapeutic measures against bovine mastitis in the country.

Materials and methods

Study design and sampling population

In this study, 224 lactating cows from 16 different smallholder dairy farms located in the Adamawa Region of Cameroon were examined to determine the prevalence of mastitis, and to identify the major bacterial pathogens associated with the disease and their antimicrobial patterns. The cows enrolled were randomly chosen from farms practicing the semi intensive husbandry system and included 64 Holstein-Friesian breed, 50 Adamawa Gudali hybrid breed, 32 Adamawa Gudali breed, 34 White Fulani breed, 24 Red Fulani breed, and 20 Banyo Gudali breed. Of the total number of cows sampled, 103 cows were less than or equal to 5 years of age and 124 were more than 5 years of age.

Detection of mastitis

To determine clinical and subclinical mastitis in the lactating cows, clinical examination of the udder was performed [7,20]. Screening was done using the California mastitis test (CMT) (ImmuCell® CMT, Portland, USA) as previously described [12,20].

Microbiological analysis

Collection of milk samples

Before milk collection from the CMT positive animals, the teats of the udders were wiped thoroughly with 70% ethyl alcohol, with particular attention to the teat orifice. The first streams of milk were discarded and sterile test tubes were used in collecting the milk in a strictly aseptic manner. Approximately 10 ml of milk were collected per cow. The samples were delivered to the microbiology laboratory in an ice-cooled box within 4 hours and processed immediately for the isolation, characterization and identification of bacteria.

Direct microscopy

The milk samples were centrifuged and the obtained pellet was swiped on a slide and then stained. A Gram- and Ziehl Neelsen stains were used routinely [20].

Bacteriological culture

The bacteriological culture was carried out following standard microbiological technique and microbiological procedures for the diagnosis of bovine mastitis infection [20]. Briefly, a loop full of milk streaked on 7% sheep blood agar plates are checked for growth after 24, 48 and up to 72 hours to rule out slow growing microorganisms. A sample was considered negative if there is no growth after 72 hours. Suspected bacteria were sub-cultured onto different selective/differential bacteriological media and incubated at 37°C for 24 hours. Pure cultures were achieved as per procedures described by [21,22].

Colony morphology, hemolytic characteristics, Gram staining, catalase test, motility test, triple sugar iron reaction, CAMP test, IMViC (Indole, Methyl red, Voges-Proskauer, Citrate), coagulase and cytochrome oxidase tests were conducted to identify the isolates according to the procedures adopted by Quinn *et al.* [20]. Furthermore, biochemical identifications by commercial kits were carried out (Integral System Enterobacteria, Integral System Staphylococci, Integral System Streptococci, Liofilchem®, Abruzzo, Italy).

Standard specific culturing techniques were applied in the suspected cases of Paratuberculosis, Tuberculosis, Brucellosis and CBPP (Contagious Bovine Pleuropneumonia) for the isolation of *Mycobacterium* spp., *Brucella* spp., and *Mycoplasma* spp., respectively.

Antimicrobial susceptibility testing

Selected bacterial isolates were tested for susceptibility to different antimicrobials using in vitro disk diffusion (Kirby-Bauer) method as described by Quinn *et al*. [20]. Cultured broth was cross-checked with McFarland standard before applying on Mueller Hinton agar and disk application. Fourteen different antimicrobial disks obtained from commercial sources (Oxoid Ltd, Baring-stoke, Hampshire, England, and Liofilchem®, Abruzzo, Italy) were selected for the testing and they included: Enrofloxacin (5µg), Amoxicillin (10µg), Streptomycin (10µg), Erythromycin (15µg), Ampicillin (10µg), Gentamicin (30µg), Doxycycline (30µg), Oxytetracycline (30µg), Penicillin G (10 IU), Trimethoprim/sulphamethoxazole (1.25/23.75µg), Neomycin (30µg), Amoxicillin/clavulanic acid (20/10µg), Ceftiofur (15µg), and Oxacillin (1µg).

In all, 12 isolated bacteria were subjected to antimicrobial susceptibility testing with the exception of *Mycoplasma* and *Mycobacterium* species. *Brucella* species were tested for antimicrobials susceptibility using five antimicrobial agents [Enrofloxacin (5µg), Streptomycin (10µg), Gentamicin (30µg), Doxycycline (30µg), Oxytetracycline (30µg)]. Based on the susceptibility to antimicrobials, the bacteria were categorized into three groups: sensitive, intermediate and resistant. For statistical analysis, the intermediate group was considered as resistant.

The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute [23].

Statistical analysis

The qualitative data were analyzed using Statistical software STATA version 13 (STATA Corporation, College Station, Texas, USA). Univariate analyses on prevalence percentages were performed. Statistical differences were calculated by Chi Square test and *P*-values less than 0.05 were considered statistically significant.

Results

Clinical and subclinical mastitis prevalence

Out of the 224 lactating cows examined, a high average prevalence in subclinical mastitis (59.8%) was recorded as compared to clinical mastitis (3.6%; χ^2=163.7, P=10^{-4}) (Table 1).

Breed	Number of cows examined	Age of cows		Clinical		Subclinical	
		< 5 years	> 5 years	Test positive	Percentage (%)	Test positive	Percentage (%)
(i) Holstein-Friesian	64	25	39	3	4.7	46	71.9[A,B,C]
(ii) Adamawa Gudali hybrid	50	40	10	2	4.0	35	70[D,E]
(iii) Adamawa Gudali	32	0	32	0	0	14	43.7[A,D]
(iv) White Fulani	34	23	11	1	2.9	19	55.9
(v) Red Fulani	24	11	13	2	8.3	11	45.8[B]
(vi) Banyo Gudali	20	4	16	0	0	9	45[C,E]
Total	224	103	121	8		134	

Table 1: Distribution of bovine clinical and subclinical mastitis in Adamawa Region of Cameroon according to breed.

[A]: $\chi^2 = 7.20$, P = 0.007; [B]: $\chi^2 = 5.19$, $P = 0.0227$; [C]: $\chi^2 = 4.87$, $P = 0.0273$; [D]: $\chi^2 = 5.59$, $P = 0.018$; [E]: $\chi^2 = 3.82$, $P = 0.0505$

The subclinical and clinical mastitis were most represented in the bovine population aged less than or equal to 5 years, 69.9% and 5.8% (n=103), respectively. In relation to age, a significant difference was observed only for subclinical mastitis (69.9% vs 51.2%, n=121; $\chi 2$=8.06, P=0.0045). In relation to the farm, prevalence rate ranged from 25.0% to 81.8%, for subclinical mastitis and from 0% to 9.1% for clinical mastitis. The farms with the highest prevalence rates for subclinical mastitis also showed the highest prevalence rates for clinical mastitis.

Bacteria isolates

From the 135 clinical and subclinical mastitis cases recorded, bacteria were successfully cultured from 115 milk samples (85.2%, n=135). In one hundred and four samples (77.0%, n=135) grew pure cultures. Eleven samples (8.1%, n=135) had mixed growth, of which one isolate per sample was considered for further analyses based on medical/veterinary importance judgment taking into consideration the morphology of the colonies. Twelve samples presented no growth (8.9%, n=135), four samples (3.0%, n=135) were contaminated with manure at the site of collection hence were discarded, and fungi grew in four other samples (3.0%, n=135), so they were not included in the analyses. Mastitis of viral origin or uncultivable bacterial species may be responsible for the negative cultures.

In all, 14 different bacterial pathogens were isolated (Table 2).

Bacteria isolated	Frequency	Prevalence rate (%)
Coagulase Negative Staphylococci (CoNS)	39	27.5
Staphylococcus aureus	33	23.3
Escherichia coli	16	11.3
Streptococcus agalactiae	10	7.1
Streptococcus dysagalactiae	6	4.2
Enterococcus faecalis	4	2.8
Klebsiella pneumoniae	4	2.8
Enterobacter aerogenes	3	2.1
Pseudomonas aeruginosa	3	2.1
Corynebacterium spp.	2	1.4
Proteus vulgaris	2	1.4
Brucella spp.	2	1.4
Mycoplasma spp.	1	0.7
Mycobacterium spp.	1	0.7
Fungi	4	2.8
Negative samples	12	8.4
Total	142	100

Table 2: Frequency of occurrence of the isolated bacteria.

CoNS (Coagulase negative Staphylococci) had the highest prevalence (39 cases) overall, followed by *Staphylococcus aureus* (33 cases), *Escherichia coli* (16 cases), *Streptococcus agalactiae* (10 cases), *Streptococcus dysagalactiae* (6 cases), *Enterococcus faecalis* (4 cases), *Klebsiella pneumoniae* (4 cases), *Enterobacter aerogenes* (3 cases), *Pseudomonas aeruginosa* (3 cases), *Corynebacterium* spp. (2 cases), *Proteus* spp. (2 cases), *Brucella* spp. (2 cases), *Mycoplasma* spp (1 case), and *Mycobacterium* spp. (1 case). *Brucella* and *Mycoplasma* species were cultured from clinical mastitis cases while *Mycobacterium* spp. was cultured from a case of subclinical mastitis, and were all isolated from milk samples gotten from the local indigenous cattle.

Therefore, the predominant bacteria involved in clinical and subclinical mastitis in the Adamawa Region of Cameroon, were identified as Coagulase Negative Staphylococci, *Staphylococcus aureus*, *Escherichia coli*, and Streptococci.

Antimicrobial susceptibility testing

The *in-vitro* antimicrobial susceptibility assays showed high resistance patterns (Figure 1).

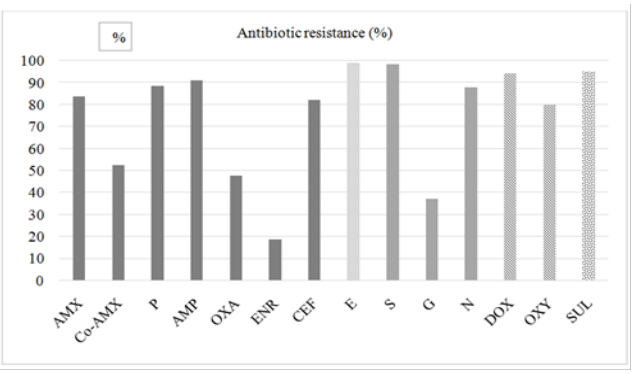

Figure 1: *In-vitro* resistance patterns (%) observed for each antibiotic tested.

AMX = Amoxicillin (10 µg), Co-AMX = Amoxicillin/Clavulanic acid (20 / 10 µg), P = Penicillin G (10 IU), AMP = Ampicillin (10 µg), OXA = Oxacillin (1 µg), ENR = Enrofloxacin (5 µg), CEF = Ceftiofur (15 µg), E = Erythromycin (15 µg), S = Streptomycin (10 µg), G = Gentamicin (30 µg), N = Neomycin (30 µg), DOX = Doxycyclin (30 µg), OXY = Oxytetracycline (30 µg), SUL= Trimethoprim/Sulphamethoxazole (1.25 / 23.75 µg).

The resistance percentages ranged from 18.5% (n=124) for Enrofloxacin (5µg) to 99.0% (n=122) for Erythromycin (15µg).

Fluoroquinolones resistance rate was the lowest recorded, and significant differences were observed between Enrofloxacin *vs* Streptomycin (98.4%; χ^2=162.7, *P*=10^{-4}), Enrofloxacin *vs* Sulfamethoxazole plus Trimethoprim (95.1%; χ^2=146.6, *P*= 10^{-4}), Enrofloxacin *vs* Doxycycline (94.3%; χ^2=144.9, *P*= 10^{-4}).

Significant differences were observed between classes of antibiotics, in particular between Aminoglycosides (99.2%, n=122) *vs* Fluoroquinolones (19.3%, n=124; χ^2=161.9, *P*=10^{-4}), and within the same class of antibiotics: Amoxicillin (83.6%) *vs* Amoxicillin-Clavulanic acid (52.5%; χ^2=27.2, *P*=10^{-4}), Amoxicillin *vs* Oxacillin (47.5%; χ^2=38.1, *P*=10^{-4}), Penicillin (88.3%) *vs* Ampicillin (91.0%; χ^2=5.6, *P*=0.0176), Penicillin *vs* Amoxicillin-Clavulanic acid (χ^2=21.2, *P*=10^{-4}), Penicillin *vs* Oxacillin (χ^2=28.4, *P*=10^{-4}), Ampicillin *vs* Amoxicillin-Clavulanic acid (χ^2=44.6, *P*=10^{-4}), Ampicillin *vs* Oxacillin (χ^2=54.1, *P*=10^{-4}), Streptomycin (98.4%) *vs* Gentamicin (37.1%; χ^2=106.6, *P*=10^{-4}), Streptomycin *vs* Neomycin (87.7%; χ^2=10.9, *P*=0.001), Gentamicin *vs* Neomycin (χ^2=67.0, *P*=10^{-4}), Doxycycline (94.3%) *vs* Oxytetracycline (79.8%; χ^2=11.6, *P*=0.0007).

In relation to the Gram affinity, Gram positive bacteria showed a significant higher resistance rate (86,2%) only for Cephalosporins (χ^2=4.9, *P*=0.0289). Gram negative bacteria revealed a high resistance rates for Beta-Lactam antibiotics (100%), Macrolides (92.9%), and Tetracyclines (100%), but the differences were not significant (*P*>0.05) (Figure 2).

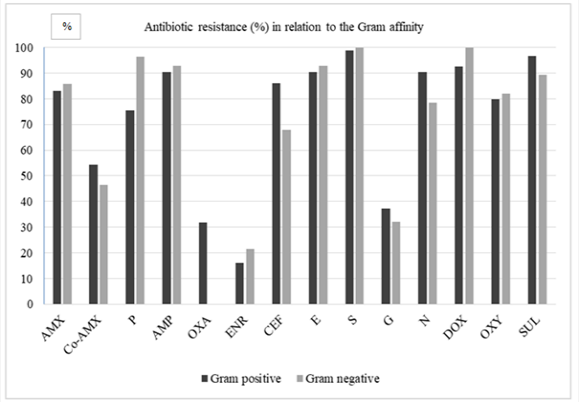

Figure 2: Antibiotic resistance patterns (%) observed for each antibiotic in relation to the Gram affinity.

AMX =Amoxicillin (10 µg), Co-AMX = Amoxicillin/Clavulanic acid (20 / 10 µg), P = Penicillin G (10 IU), AMP = Ampicillin (10 µg), OXA = Oxacillin (1 µg), ENR = Enrofloxacin (5 µg), CEF = Ceftiofur (15 µg), E = Erythromycin (15 µg), S = Streptomycin (10 µg), G = Gentamicin (30 µg), N = Neomycin (30 µg), DOX = Doxycyclin (30 µg), OXY = Oxytetracycline (30 µg), SUL= Trimethoprim/Sulphamethoxazole (1.25 / 23.75 µg).

Table 3 shows the antibiotic resistance (%) profiles recorded for each isolated bacterium.

Isolates	N°	ENR	AMX	S	E	AMP	G	DOX	OXY	PEN	SUL	N	Co-AMX	CEF	OXA
SA	33	9.1[1]	87.9	100[4]	90.9	97.0	24.2[8,9]	100 [11,13]	78.8 [16,19]	75.8[26,29,30]	93.9	90.9[40]	57.6[41]	93.7	12.1[45,47,49,51]
CoNS	39	17.9	84.6	100 [2,3]	94.9[5]	97.4[6]	30.8[7]	97.4 [10,12]	94.9 [16,17,18,21,23,24,25]	94.9[26,27,28,33]	100[36,37,38]	89.7[39]	69.2 [41,43]	84.2	20.5[44,46,48,50]
EC	16	12.5	93.7	100	87.5	87.5	31.2	100 [14,15]	87.5 [20,22]	100[30,31,32,34,35]	93.7	62.5[39,40]	50	56.2	-
STREP. A.	10	20	70	90[2]	80	80[6]	70 [7,8]	60[10, 11,14]	60[17]	50 [27,31]	90[36]	90	20[41,42]	80	80[44,45]
STREP. D.	6	16.7	66.7	100	66.7[5]	83.3	66.7[9]	66.7 [12,13, 15]	33.3 [18,19, 20]	33.3[28,29,32]	100	83.3	16.7[43]	83.3	66.7 [46,47]
EF	4	50[1]	75	100	100	0	50	100	50[23]	50 [33,34]	100	100	25	50	100 [48,49]
KP	4	50[1]	75	100	100	100	25	100	100	75[35]	100	100	50	75	-
EA	3	0	66.7	100	100	100	33.3	100	33.3 [21,22]	100	66.7[37]	100	33.3	66.7	-
PA	3	33.3	66.7	100	100	100	33.3	100	100	100	66.7[38]	100	33.3	100	-
C	2	0	100	100	100	100	100	100	100	0	100	100	50	100	100 [50,51]
PV	2	50	100	100	100	100	50	100	50[24]	100	100	100	50	100	-
B	2	100	-	50[3,4]	-	-	100	100	50[25]	-	-	-	-	-	-
Isolates	**N°**	**ENR**	**AMX**	**S**	**E**	**AMP**	**G**	**DOX**	**OXY**	**P**	**SUL**	**N**	**Co-AMX**	**CEF**	**OXA**

Table 3: *In-vitro* antibiotic resistance patterns (%) observed for each bacterial isolates.

SA = *Staphylococcus aureus*, CoNS = Staphylococci coagulase negative, *EC* = *Escherichia coli*, *STREP. A.* = *Streptococcus agalactiae*, *STREP. D.* = *Streptococcus dysgalactiae*, *EF* = *Enterococcus faecalis*, *KP* = *Klebsiellapneumoniae*, *EA* = *Enterobacter aerogenes*, *PA* = *Pseudomonas aeruginosa*, *C* = *Corynebacterium* spp., *PV* = *Proteus vulgaris*, B = *Brucella* spp., ENR = Enrofloxacin (5 µg), AMX =Amoxicillin (10 µg), S = Streptomycin (10 µg), E = Erythromycin (15 µg), AMP = Ampicillin (10 µg), G = Gentamicin (30 µg), DOX = Doxycyclin (30 µg), OXY = Oxytetracycline (30 µg), P = Penicillin G (10 IU), SUL= Trimethoprim/Sulphamethoxazole (1.25 / 23.75 µg), N = Neomycin (30 µg), Co-AMX = Amoxicillin/Clavulanic acid (20 / 10 µg), CEF = Ceftiofur (15 µg), OXA = Oxacillin (1 µg).

[1](χ^2 = 5.11, *P* = 0.024); [2](χ^2 =4.0, *P* = 0.046); [3](χ^2 = 20.0, *P* = 10^{-4}); [4](χ^2 = 17.0, *P* = 10^{-4}); [5](χ^2 = 5.1, *P* = 0.0238); [6](χ^2 = 4.2, *P* = 0.0402); [7](χ^2 = 5.2, *P* = 0.0231); [8](χ^2 = 7.1, *P* = 0.0078); [9](χ^2 = 4.3, *P* = 0.0383); [10](χ^2 = 12.2, *P* = 0.0005); [11](χ^2 = 14.5, *P* = 0.0001); [12](χ^2 = 7.9, *P* = 0.0049); [13](χ^2 = 11.6, *P* = 0.0007); [14](χ^2 = 7.6, *P* = 0.0060); [15](χ^2 = 5.9, *P* = 0.0154); [16](χ^2 = 4.2, *P* = 0.0398); [17](χ^2 = 9.0, *P* = 0.0027); [18](χ^2 = 17.0, *P* = 10^{-4}); [19](χ^2 = 5.2, *P* = 0.0228); [20](χ^2 = 6.4, *P*= 0.0111); [21](χ^2 = 12.2, *P* = 0.0005); [22](χ^2 = 4.5, *P* = 0.0347); [23](χ^2 = 8.7, *P* = 0.0033); [24,25](χ^2 = 5.6, *P* = 0.0175); [26](χ^2 = 5.5, *P* = 0.0195); [27](χ^2 = 13.1, *P* = 0.0003); [28](χ^2 = 17.0, *P* = 10^{-4}); [29](χ^2 = 4.3, *P* = 0.0383); [30](χ^2 = 4.6, *P*= 0.0313); [31](χ^2 = 9.9, *P* = 0.0016); [32](χ^2 = 13.0, *P* = 0.0003); [33](χ^2 = 8.7, *P* = 0.0033); [34](χ^2 = 8.9, *P* = 0.0029); [35](χ^2 = 4.2, *P* = 0.0402); [36](χ^2 = 4.0, *P* = 0.0460); [37,38](χ^2 = 13.3, *P* = 0.0003); [39](χ^2 = 5.7, *P* = 0.0174); [40](χ^2 = 5.8, *P* = 0.0160); [41](χ^2 = 8.0, *P* = 0.0047); [42](χ^2 = 4.3, *P* = 0.0373); [43](χ^2 = 6.1 *P* = 0.0134); [44](χ^2 = 12.8 *P* = 0.0003); [45](χ^2 = 17.6 *P* = 10^{-4}); [46](χ^2 = 5.7 *P* = 0.0173); [47](χ^2 = 9.3 *P* = 0.0023); [48](χ^2 = 11.4 *P* = 0.0007); [49](χ^2 = 16.3 *P* = 0.0001); [50](χ^2 = 6.5 *P* = 0.0107); [51](χ^2 = 10.2 *P* = 0.0014). The superscripts highlighted italic numbers (1- 51) represent significant values.

High resistance to Beta-Lactam antibiotics was recorded from Gram negative (100%, n=28) nevertheless no significant difference resulted towards Gram positive (96.8%, n=94; χ^2=0.9 *P*=0.3385). In particular, a significant difference was demonstrated for Penicillin (96.4%, n=28, *vs* 75.5%, n=94; χ^2=6.0 *P*=0.0146).

The 16.7% of *Staphylococcus* species (n=72) showed Methicillin resistance phenotypically, and no significant differences were recorded between *Staphylococcus* spp. coagulase negative (20.5%) and *S. aureus* (12.1%).

Oxacillin resistant Streptococci isolates were 75% (n=16), while 81.2% were Ampicillin resistant (*vs* penicillin: χ^2=4.8, *P*=0.0285; *vs* Amoxicillin and Clavulanic acid: χ^2=12.5, *P*=0.0004), 68.7% were Amoxicillin resistant, 43.7% were Penicillin-resistant, 18.7% were Amoxicillin and Clavulanic-acid resistant (*vs* Oxacillin: χ^2=10.2, *P*=0.0014; *vs* Amoxicillin: χ^2=8.1, *P*=0.0044

Discussion

In most sub-Saharan countries including Cameroon, sub-clinical mastitis received little or no attention and efforts are focused on the treatment of clinical cases while high productive and economic losses could come from sub-clinical mastitis. In the present study, there were overwhelming cases of sub-clinical mastitis (59.8%) compared to clinical mastitis (3.6%). Our findings are similar to those of many studies [12,24]. In the current study, fourteen different bacterial pathogens were isolated from milk samples collected from 135 mastitis cows. The isolated bacteria were Coagulase negative Staphylococci (27.5%), *Staphylococcus aureus* (23.3%), *Escherichia coli* (11.3%), *Streptococcus agalactiae* (7.1%), *Streptococcus dysagalactiae* (4.2%), *Enterococcus faecalis* (2.8%), *Klebsiella pneumoniae* (2.8%), *Enterobacter aerogenes* (2.1%), *Pseudomonas aeruginosa* (2.1%), *Corynebacterium* spp. (1.4%), *Proteus* spp. (1.4%), *Brucella* spp. (1.4%), *Mycoplasma* spp (0.7%), and *Mycobacterium* spp. (0.7%). The study showed that *Staphylococcus* spp, *Escherichia coli*, and *Streptococcus* spp, are the major cause of mastitis in Adamawa Region Cameroon. This finding is in agreement with those of many studies carried out in many parts of the world [7,25-28].

The *in vitro* antibiotic susceptibility testing of twelve different types of bacterial isolates to 14 different antibiotics such as Enrofloxacin, Amoxicillin, Streptomycin, Erythromycin, Ampicillin, Gentamicin, Doxycycline, Oxytetracycline, Penicillin G, Trimethoprim/sulphamethoxazole, Neomycin, Amoxicillin/Clavulanic acid, Ceftiofur, and Oxacillin showed overall effective drug therapy against isolated pathogens, in the following order: Enrofloxacin, Gentamicin, and to a lesser extent by Oxacillin and Amoxicillin/Clavulanic acid was observed but resistance of most of the isolates to the other antibiotics were noticed. The variation in the sensitivity of common antibiotics could be the result of extensive and indiscriminate use of these in the treatment of udder infection.

In the past two decades, a significant increased of antimicrobial resistance among Gram-positive bacteria has been observed, including multidrug-resistant staphylococci, penicillin-resistant streptococci, and among Gram-negative bacteria, including the emergence and spread of resistance in Enterobacteriaceae. *Klebsiella pneumoniae* and *Enterobacter* spp. infections now involve strains not susceptible to third-generation cephalosporins. Such resistance in *K. pneumoniae* to third-generation cephalosporins is typically caused by the acquisition of plasmids containing genes that encode for extended-spectrum *β*-lactamases (ESBLs), and these plasmids often carry other resistance genes as well. ESBL-producing *K. pneumoniae* and *Escherichia coli* are now relatively common in healthcare settings and often exhibit multidrug resistance. ESBL-producing *Enterobacteriaceae* have now emerged in the community as well [29].

In the currently study, bacteria of the family *Enterobacteriaceae* recorded 100% resistance to Beta-Lactams. Moreover, *Enterobacter aerogenes* showed over 66.7% to the third generation Cephalosporins. Resistance of *Enterobacter* spp. to third-generation Cephalosporins was the most typically caused by overproduction of AmpC *β*-lactamases, and treatment with third-generation cephalosporins may select for AmpC-overproducing mutants. Some *Enterobacter cloacae* strains are now ESBL and AmpC producers, conferring resistance to both third- and fourth-generation cephalosporins [30].

Fluoroquinolones resistance *Enterobacteriaceae* was 17.4% (n=23). Quinolone resistance in *Enterobacteriaceae* is usually the result of chromosomal mutations leading to alterations in target enzymes or drug accumulation. More recently, however, plasmid-mediated quinolone resistance has been reported in *K. pneumoniae* and *E. coli*, associated with acquisition of the *qnr* gene [30].

Oxacillin-resistant *Staphylococcus aureus* (MRSA) represents an important problem worldwide, and its prevalence may vary significantly in human and veterinary medicine. Most MRSA isolates show resistance to virtually all Beta-lactams by production of penicillinase and a low-affinity penicillin-binding protein (PBP) called PBP 2a [31].

Since its detection in Papua New Guinea and Australia, Penicillin resistance in *Streptococcus* spp. has now been reported worldwide [32]. In the present study the Penicillin resistance rate observed for *Streptococci* isolates was 43.7% (n=16), lower when compared to other beta-lactams, in particular to Ampicillin (81.2%, n=16; χ^2=4.8 *P*=0.0285).

Further investigations will be needed to study the beta lactamase production by Gram negative isolates, and Oxacillin/Methicillin resistance from *Staphylococcus* genus.

In a summary, the different bacteria isolated from sub-clinical and clinical mastitis cases in this study showed that Staphylococci were the most common, followed by *Streptococcus* species and *Escherichia coli*. Thus, for effective treatment of bovine mastitis, medicinal formulations should contain antibiotics with good inhibition spectrum of against most species of bacteria. In this context, it is interesting to note that Enrofloxacin especially, and to a lesser extent, Gentamicin, Oxacillin and Amoxicillin/Clavulanic acid showed the highest sensitivity among almost all of the bacteria isolates in this study and should considered among the choice antibiotics for effective treatment of bovine mastitis in the study area to yield the best possible result. Other studies [33,34] have shown similar susceptibility pattern regarding the use of Fluoroquinolones against bovine mastitis pathogens.

Finally, due to logistical reasons we were unable to perform the antibiotic susceptibility test for the isolated *Mycobacterium* and *Mycoplasma* species. Nevertheless, this will be carryout in subsequent studies when the situation will have been resolved.

In conclusion, potential drug resistant pathogens in otherwise normal dairy herd may be a serious concern for public health. Current findings suggest further studies with the isolated strains of bacteria. This study revealed the existence of alarming levels of resistance of *Staphylococcus* spp., Gram negative bacteria and to a lesser extent, *Streptococcus* spp. to commonly used antimicrobial agents. The results suggest a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. Thus, it is very important to implement a systemic application of an in vitro antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections.

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Conflict of Interest

The author declares that there is no conflict of interests regarding the publication of this paper.

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