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%; χ²=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 dysgalactiae (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

Introduction
Mastitis, an inflammatory mammary gland condition, is the most common, troublesome and the most expensive disease of dairy rumenants 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 attempt also 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, 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), Cefotiofur (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%; *χ²=163.7, P=10⁻⁴*) (Table 1).

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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 bacteria were successfully cultured from 115 milk samples (85.2%, n=135). In one hundred and four samples (77.0%, n=135) grew pure bacteria were successfully cultured from 115 milk samples (85.2%, n=135).

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number of cows examined</th>
<th>Clinical cases</th>
<th>Subclinical cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Holstein-Friesian</td>
<td>64</td>
<td>25</td>
<td>39</td>
</tr>
<tr>
<td>(ii) Adamawa Gudali</td>
<td>50</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>(iii) Adamawa Gudali</td>
<td>32</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>(iv) White Fulani</td>
<td>34</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>(v) Red Fulani</td>
<td>24</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>(vi) Barney Gudali</td>
<td>20</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>224</td>
<td>103</td>
<td>121</td>
</tr>
</tbody>
</table>

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

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

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

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

\[\chi^2 = 7.20, P = 0.007; \chi^2 = 5.19, P = 0.0227; \chi^2 = 4.87, P = 0.0273; \chi^2 = 5.59, P = 0.018; \chi^2 = 3.82, P = 0.0505\]

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

### Table 2: Frequency of occurrence of the isolated bacteria.
Fluoroquinolones resistance rate was the lowest recorded, and significant differences were observed between Enrofloxacin vs Streptomycin (98.4%; $\chi^2$=162.7, $P=10^{-4}$), Enrofloxacin vs Sulphamethoxazole plus Trimethoprim (95.1%; $\chi^2$=146.6, $P=10^{-4}$), Enrofloxacin vs Doxycycline (94.3%; $\chi^2$=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%, $n=124$; $\chi^2=161.9, P=10^{-4}$), and within the same class of antibiotics: Amoxicillin (83.6%) vs Amoxicillin-Clavulanic acid (52.5%; $\chi^2=27.2$, $P=10^{-4}$), Amoxicillin vs Oxacillin (47.5%; $\chi^2=38.1$, $P=10^{-4}$), Penicillin (88.3%) vs Ampicillin (91.0%; $\chi^2=5.6$, $P=0.0176$), Ceftriaxone vs Amoxicillin-Clavulanic acid ($\chi^2=21.2$, $P=10^{-4}$), Penicillin vs Oxacillin ($\chi^2=28.4$, $P=10^{-4}$), Ampicillin vs Amoxicillin-Clavulanic acid ($\chi^2=44.6$, $P=10^{-4}$), Ampicillin vs Oxacillin ($\chi^2=54.1$, $P=10^{-4}$), Streptomycin (98.4%) vs Gentamicin (37.1%; $\chi^2=106.6$, $P=10^{-4}$), Streptomycin vs Neomycin (87.7%; $\chi^2=10.9$, $P=0.001$), Gentamicin vs Neomycin ($\chi^2=67.0$, $P=10^{-4}$), Doxycycline (94.3%) vs Oxytetracycline (79.8%; $\chi^2=11.6$, $P=0.0007$).

In relation to the Gram affinity, Gram positive bacteria showed a significant higher resistance rate (86.2%) only for Cephalosporins ($\chi^2=144.9$, $P=10^{-4}$), Gram negative bacteria revealed a high resistance rate for Beta-Lactam antibiotics (100%), Macrolides (92.9%), and Tetracyclines (100%), but the differences were not significant ($P=0.05$) (Figure 2).

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

<table>
<thead>
<tr>
<th>Isolates</th>
<th>N</th>
<th>ENR</th>
<th>AMX</th>
<th>S</th>
<th>AMP</th>
<th>G</th>
<th>DOX</th>
<th>OXY</th>
<th>PEN</th>
<th>SUL</th>
<th>N</th>
<th>Co-AMX</th>
<th>CEF</th>
<th>OXA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>33</td>
<td>9.14</td>
<td>87.9</td>
<td>100</td>
<td>90.9</td>
<td>97.0</td>
<td>24.24</td>
<td>100</td>
<td>78.8</td>
<td>94.4</td>
<td>75</td>
<td>89.6</td>
<td>99.6</td>
<td>57.6</td>
</tr>
<tr>
<td>CoNS</td>
<td>39</td>
<td>17.9</td>
<td>84.6</td>
<td>100</td>
<td>94.9</td>
<td>97.4</td>
<td>30.82</td>
<td>100</td>
<td>94.9</td>
<td>98</td>
<td>100</td>
<td>90.0</td>
<td>49.8</td>
<td>69.2</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>16</td>
<td>12.5</td>
<td>93.7</td>
<td>100</td>
<td>87.5</td>
<td>87.5</td>
<td>31.2</td>
<td>100</td>
<td>87.5</td>
<td>91</td>
<td>100</td>
<td>93.7</td>
<td>84.2</td>
<td>56.2</td>
</tr>
<tr>
<td>Streptococcus dysgalactiae</td>
<td>10</td>
<td>20</td>
<td>79.1</td>
<td>83.3</td>
<td>66.7</td>
<td>66.7</td>
<td>33.3</td>
<td>13.3</td>
<td>33.3</td>
<td>66.7</td>
<td>100</td>
<td>93.7</td>
<td>62</td>
<td>80</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td></td>
</tr>
</tbody>
</table>

**SA** = Staphylococcus aureus, **CoNS** = Staphylococci coagulase negative, **EC** = Escherichia coli, **STREP. D.** = Streptococcus dysgalactiae, **EF** = Enterococcus faecalis, **KP** = Klebsiellapneumoniae, **EA** = Enterobacter aerogenes, **PV** = Proteus vulgaris, **B** = Brucella spp., **ENR** = Enrofloxacin (5 µg), **AMX** = Amoxicillin (10 µg), **G** = Gentamicin (30 µg), **S** = Neomycin (30 µg), **ENR** = Enrofloxacin (5 µg), **CEF** = Ceftriaxone (15 µg), **AMX** = Amoxicillin (10 µg), **S** = Streptomycin (10 µg), **G** = Gentamicin (30 µg), **DOX** = Doxycycline (30 µg), **SUL** = Sulphamethoxazole (1.25 / 23.75 µg), **PV** = Proteus vulgaris.

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

**Table 3:** Antibiotic resistance (%) profiles recorded for each isolated bacterium.
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; \( \chi^2=0.9 \), \( P=0.3385 \)). In particular, a significant difference was demonstrated for Penicillin (96.4%, n=28, vs 75.5%, n=94; \( \chi^2=6.0 \), \( P=0.0146 \)).

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: \( \chi^2=4.8 \), \( P=0.0285 \); vs Amoxicillin and Clavulanic acid: \( \chi^2=12.5 \), \( P=0.0004 \)). 68.7% were Amoxicillin resistant, 43.7% were Penicillin-resistant, 18.7% were Amoxicillin and Clavulanic-acid resistant (vs Oxacillin: \( \chi^2=10.2 \), \( P=0.0044 \); vs Amoxicillin: \( \chi^2=8.1 \), \( P=0.0044 \)).

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 dysgalactiae (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 Mucobacterium 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, Ceftriaxone, 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 \( \beta \)-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 current 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 \( \beta \)-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). Quinoline 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; \( \chi^2=4.8 \), \( P=0.0285 \)).

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 Enterobacteriaceae and Escherichia coli. Thus, for effective treatment of bovine mastitis, medicinal formulations should contain antibiotics with good inhibition spectrum 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 carried out 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 systematic 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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