ORIGINAL RESEARCH ARTICLE

A pilot Study: The diversity and resistance profiles of mastitis-causing bacteria found in dairy goats within Manyatta in Embu County, Kenya

Arthur I. Wafula [1,4], Mercy Y. Akinyi[2], David K. Mwaura[1], Julius N. Mugweru[3], Moses M. Njire [4], Peter G. Mwethera[1]

[1]*Department of Reproductive Health and Biology, Kenya Institute of Primate Research, Nairobi, Kenya*

[2]*Department of Animal Science, Kenya Institute of Primate Research, Nairobi, Kenya*

[3] *School of Pure and Applied Sciences, University of Embu, Kenya*

[4] *School of Biological Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

Corresponding author email: arthurisrael22@gmail.com

Abstract

Mastitis is a leading disease in dairy animals such as goats across the world. It is a unique disease because it can be caused by diverse types of bacteria. This study was carried out in Manyatta Sub-County in Embu County, a region where goat farming thrives. Farmers in Manyatta Sub-County have often been plagued by mastitis resulting in heavy financial losses and negatively impacting public health. The aim of this pilot study was to identify some of the common bacteria associated with mastitis in dairy goats within Manyatta Sub-County and testing their resistance profiles to generate preliminary data for combating mastitis in the region. A total of 17 goats representing the most common breed in the region were sampled for the study. Bacteria were isolated and identified using conventional culture method and 16S rRNA gene sequencing of DNA extracted from enriched broth cultures. Notably, culturing detected several Gram-positive bacteria that were absent in sequencing. Select resistance genes were screened through PCR. Some of the bacteria identified from the study include: *Staphylococcus aureus*, non-*aureus Staphylococcus*, *Streptococcus uberis*, *Escherichia coli*, Pseudomonas spp. Klebsiella, Aeromonas and Serratia spp. Among isolates identified by 16S rRNA sequencing of enriched cultures, Serratia spp. was the most frequently detected genus. Resistance genes detected include; bla_{SHV}, *aad*A, *tet*A and *tet*E. The high levels of resistance observed highlights the severity of mastitis in Manyatta. Our results revealed several bacteria species were responsible for mastitis cases in Manyatta. The results also revealed some antibiotics were no longer effective in treatment of mastitis in Manyatta due to the high level of resistance developed. The data obtained suggests intensive research should be conducted to determine effective ways of controlling the infections while shelving ineffective antibiotics.

Keywords: Mastitis, prevalence, resistance, resistance genes, sequencing.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

1.0. Introduction

In dairy goat farming, mastitis continues to be a serious problem among farmers due to its devastating effects on dairy goat farming (Kabui *et al*., 2024). Intra-mammary infections (IMA) caused by bacteria are considered to be the main cause of mastitis (Cheng & Han, 2020). The major risk factors associated with mastitis includes physical damage to the skin through injury, bites, scratches and bruises which break the skin barrier exposing tissues to pathogens (Abebe *et al*., 2023). The Staphylococcus genus is the most prevalent causative agent in bacterial mastitis in goats. However, mastitis is also caused by other bacteria species such as: *Streptococcus uberis*, *Escherichia coli,* Pseudomonas spp. *Clostridium perfringens* (Sahoo *et al*., 2023). The fact that mastitis can be caused by more than one etiological agent contributes significantly to making it a difficult disease to manage by antibiotics.

Mastitis in dairy goats is classified into two main categories but a third category may occur in some cases i.e., clinical mastitis and sub-clinical mastitis. However, with lack of proper intervention, two categories of mastitis are capable of developing into chronic mastitis (Sahoo *et al*., 2023). Goat milk is normally considered nutritious and a lot of health advantages are associated with the milk. It has similar constituent elements to cow milk with major differences arising from the protein structure (Stergiadis *et al*., 2019). Some of the health advantages goat milk has over cow milk include it is easier to digest due to its smaller fat globules. Goat milk also contains more fat and calories while still having more protein, vitamins and minerals compared to cow milk. Some of these differences have resulted in the recommended use of goat milk by people who are allergic to cow milk (Kabui *et al.,* 2024*)*.

The close similarity of goat milk to cow milk makes it appealing to consumers seeking alternatives to cow milk. Recently, dairy products from goat milk have gained prominence among these consumers (Stergiadis *et al*., 2019). However, mastitis continues to pose a serious challenge to dairy goat farming. These effects are exerted on both the farmers who incur heavy financial losses, animals whose health is affected as well as human health which may be compromised through the consumption of contaminated products (Schadt, 2023). Low quality dairy products result in low market value which is challenge to small scale farmers who depend on dairy farming as the main source of sustenance (Novac & Andrei, 2020).

Attempts to control mastitis have seen an increased use of antibiotics in dairy goat farming. This surge in antibiotic use has contributed to the emergence and spread of antimicrobial resistance (Fazel *et al*., 2019; Lianou & Fthenakis, 2022). The rise of antimicrobial resistance is a serious challenge on global health with a very high number of rising deaths projected in the coming years as a direct result of antimicrobial resistant pathogens. By the year 2050, AMR is projected to cause over 10 million deaths globally if no effective interventions are introduced to curb the current rate (Kabui *et al*., 2024). The consumption of milk and other dairy products containing antibiotic resistance genes lead to a gradual build-up of these genes in the body which significantly contributes to the development of antibiotic resistance in these individuals (Sahoo *et al*., 2023). Apart from aiding the spread of antibiotic resistance genes, mastitis also threatens human health through zoonosis with some pathogens capable of being fatal to

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

human beings for instance the methicillin resistant *Staphylococcus aureus* strain (Schadt, 2023). Among bacteria that can cause mastitis, Staphylococcus genus is responsible for the highest number of infections.

Regular monitoring of goats allows farmers to identify wounds on udders and teats which are susceptible to infection. Wounds and cuts on the skin provide an entry points for bacteria to colonize the animal (Jabbar *et al*., 2020). Treatment and dressing of wounds as soon as they happen helps in keeping pathogens such as bacteria out (Cheng & Han, 2020). Detection in milk can be carried out through different assays. This includes, the California mastitis test, the somatic cell count, bacteriological examination of milk as well use of molecular diagnostic techniques (Yang *et al*., 2019). Despite the Somatic cell count and California mastitis tests being the most commonly used tests, they can be easily influenced by host animal factors such as elevated levels of white blood cells resulting in inaccurate results (Huang & Kusaba, 2022). This leaves bacteriological analysis through culturing and molecular diagnosis as the most reliable way of detecting mastitis in goats and other animals which are capable of acquiring mastitis (Matuozzo *et al*., 2020).

In Kenya, goat farming similarly faces challenges caused by mastitis. To our knowledge there is no study that has reported and characterized the causative agents of mastitis in Manyatta Sub-County in Embu, yet farmers have often been plagued by mastitis resulting in heavy financial losses and negatively impacting public health. The aim of this study was to identify bacterial species that are responsible for causing mastitis within Manyatta Sub-County in Embu through culturing and 16S rRNA sequencing.

This study aimed to characterize the diversity of mastitis-causing bacteria in dairy goats within Manyatta Sub-County in Embu County, Kenya and to determine their antimicrobial resistance patterns. Specifically, the study sought to identify bacterial species associated with mastitis using culture-based methods and 16S rRNA gene sequencing, and to assess their susceptibility to commonly used antimicrobials.

2.0. Materials and methods

2.1. Study area

The study was conducted in Embu County (Figure 1) which lies at 1350 meters above sea level. The region experiences weather which supports Dairy goat farming. The region receives an average of 1450 mm of rain annually while temperatures average at 19 ^{0}C.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

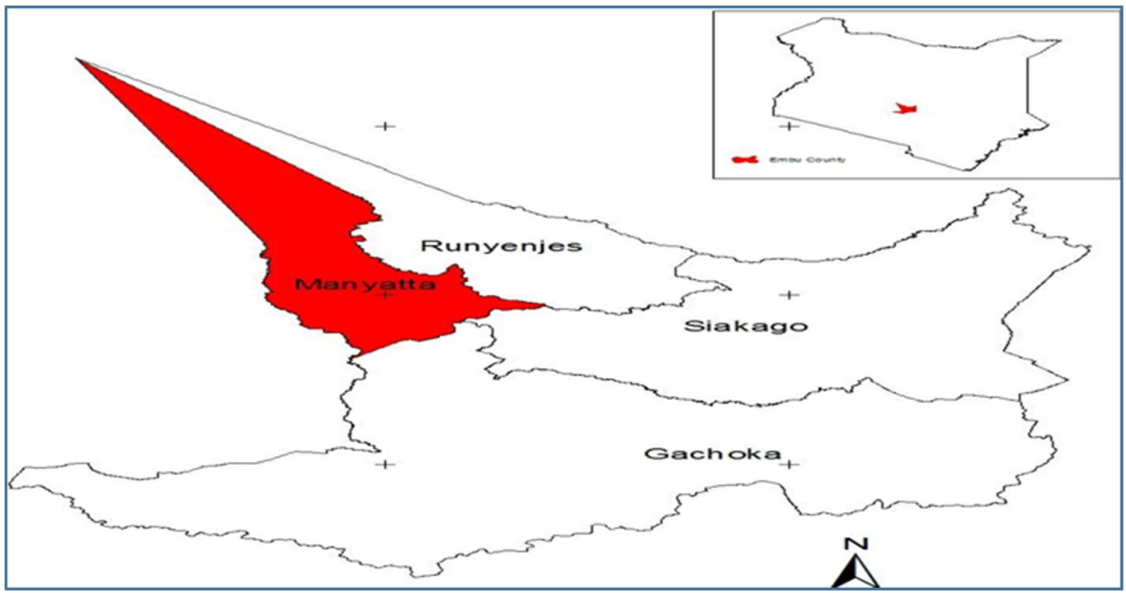

Figure 1. Manyatta in Embu County Source: Semantic Scholar.

2.2. Study design and sample size calculation

A cross-sectional study was done for this study between the months of August to December 2023. The sample size was determined using Fisher's Formula as referenced by Dahoo et al (2003) as follows; $n = (Z^2*P*Q)/E^2$, where n is the sample size, Z^2 is the Z score for the confidence level, P is the standard deviation, Q is 1-P and E is the margin of error. The calculated sample size for this study was 68 goats. However, only 17 goats were ultimately sampled due to strict inclusion criteria and logistical constraints. Specifically, sampling was limited to goats that tested positive for mastitis at the time of field visits and to farmers who voluntarily consented to participate. Additionally, the absence of a comprehensive registry of dairy goat farmers in the region necessitated the use of snowball sampling to identify eligible participants. This was facilitated by a local veterinary officer. As a result, fewer mastitis-positive goats were available during the study period than initially anticipated, leading to a reduced final sample size.

2.3. Milk sampling and California mastitis test (CMT)

With the study focusing on identifying some of the most common bacteria involved in causing mastitis, goats manifesting clinical symptoms of mastitis were sampled (Keane *et al*., 2013). Goats under antibiotic treatment as well as those suffering from other diseases were excluded from sampling. A total of 17 goats were used for the study. Visual and physical examination was performed on the goats to check for any signs of clinical mastitis by a veterinarian. Some of these signs include, swelling of the udder, swelling and reddening of the teats, sunken eyes and difficulty in movement. Milk samples were obtained through direct milking. Before the start of milking, the teats and udders were cleaned and disinfected using pre-milking iodine solution and then dried by dry paper towels. The teats were wiped with cotton swabs dipped in 70% ethanol.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

California mastitis test (CMT) was done before collection of milk samples. Milk was drawn with the first 3-5 squirts drawn to the ground. Three (3mL) of milk from each quarter of the udder was then collected in a CMT paddle and an equal volume of commercial CMT reagent added to it. The paddle was gently shaken and gel formation was observed after 20 seconds. The results were scored on a scale of 3 following the manufacturer's guidelines. During CMT, a score of 0, shows there is no reaction between the milk and the CMT reagent. A score of 1-3 shows a positive reaction. As the score increases from 1-3, the severity of the infection increases with a score of 3 showing the most severe cases. From the positive samples, ten (10mL) sterile falcon tubes were used to collect the milk. The samples were kept at 4 ^{0}Cand transported to the microbiology and antibiotic resistance laboratory at the Kenya Institute of Primate Research for processing.

2.4. Bacteriological analysis

Standard microbiological techniques were used to determine the identity of the possible mastitis causing bacteria. Due to the high diversity in the causative agents, three different culture media were used (Song *et al*., 2024). 60µl of milk samples was inoculated by streaking on MacConkey agar (HiMedia ®), Mannitol Salt Agar (HiMedia ®), and 5% Sheep Blood agar (OXOID ®), for isolation. The plates were incubated for 24-48hrs. Culture plates that had mixed cultures were sub-cultured onto Nutrient Agar (Neogen ®) to obtain pure cultures. Gram staining was performed on pure isolates. The Isolates were then subjected to a series of biochemical tests including; citrate utilization test, indole test, catalase test, urease test, methyl red test, voges' Proskauer test and growth in Triple Sugar Iron agar. Classification and identity of possible mastitis causing bacteria was carried out through analysis of gram staining results, morphological characteristics and biochemical tests (Giuliano *et al*., 2019).

2.5. DNA extraction

DNA extraction was performed using a modified boiling technique (Yamagishi *et al*., 2016). The 17 milk samples were cultured overnight in nutrient broth (Neogen ®) The enrichment in nutrient broth was done in order to increase the bacterial load thus increasing the number of bacterial cells subjected to DNA extraction (Jarvis *et al*., 2015*)*. 1000µl of nutrient broth containing bacterial cells was centrifuged at 14,000 ×g for 10 minutes. The cells were collected and dissolved in 100µl of nuclease free water. The suspension was then transferred to a heat block and allowed to boil for 10 minutes at 95 ^{0}C. The suspension was then cooled on ice for 5-10 minutes then centrifuged at 12,000 ×g for 5 minutes. The supernatant containing bacterial DNA of interest was collected and transferred to sterile Eppendorf tubes. The DNA was stored at -20 ^{0}C awaiting sequencing.

2.6. 16. S rRNA sequencing

DNA extracted from the 17 milk samples was shipped out to Inqaba Biotech in South Africa for sequencing. Sanger sequencing that targeted the conserved 16S rRNA bacterial gene was done using the universal 27F forward primer and the universal 907-R reverse primer. Data from the sequencing was obtained as chromatograms. Sequence analysis was conducted using BioEdit

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

version 7.2 and consensus sequences (contigs) were formed from the forward and reverse sequences of each sample. A BLAST search was carried out on the NCBI database to determine the identity of the bacteria (http://www.ncbi.nlm.nih.gov).

2.7. Antibiotic susceptibility testing

Pure isolates were subjected to antibiotic susceptibility testing on Mueller Hinton Agar (HiMedia) following the Kirby Bauer disc diffusion protocol (Michira *et al.*, 2023). Commonly used antibiotics for the treatment of mastitis in Manyatta were selected for antibiotic susceptibility testing. These included; amoxicillin (10 µg/L), tetracycline (10 µg/L), chloramphenicol (30µg/L), streptomycin (10 µg/L), penicillin (10 µg/L), Cloxacillin (30µg/L). Discs containing antibiotics were introduced to the plates and then incubated at 37 ^{0}C for 24 hours. The respective zones of inhibition that formed were measured and results interpreted according to the Clinical & Laboratory Standard institute (CLSI, 2020). The bacteria were then classified either as resistant(R), intermediate (I) or susceptible(S).

2.8. Molecular detection of antibiotic resistance genes

DNA extraction was carried out using the modified boiling technique (Yamagishi *et al.*, 2016). DNA amplification for different resistance genes (Table 1) was done using specific primers to screen for genes coding for resistance towards penicillin, amoxicillin, cloxacillin, streptomycin and tetracycline. These genes were specifically selected for the study taking into account the types of antibiotics that are commonly used in managing mastitis in Manyatta sub-county. The genes are also available in most coliforms which were predominant in this study, raising public health issues that could arise when transferred to human beings through consumption of contaminated dairy products. A total of 17 DNA samples were used for the detection which targeted 5 specific resistance genes. The PCR conditions for a 35 cycle PCR for the TEM gene were as follows: initial denaturation at 95 ^{0}C for 3 minutes, denaturation at 95 ^{0}C for 30 seconds, annealing at 55 ^{0}C for 1 minute, extension at 72 ^{0}C for 30 seconds and final extension at 72^{0}C for 5 minutes. These conditions varied in regards to the other genes that were screened. The PCR amplicons were then subjected to gel electrophoresis on 1.5% agarose gel and visualized using a UV transilluminator (LABNET). PCR products were then purified and sent for sequencing at Inqaba biotech in South Africa to ascertain the presence of the resistance genes (Gundran *et al.*, 2019).

Table 1. Resistance genes screened during PCR and their respective primers.

Oligo Name	5` - Oligo Seq - 3`	Targeted Antibiotic
TEM-F1	ATGAGTATTCAACATTTCCG	Penicillin, Amoxicillin, Cloxacillin
TEM-R1	GACAGTTACCAATGCTTAATCA	Penicillin, Amoxicillin, Cloxacillin
Bla-SHV-F1	CTTTACTCGCCTTTATCG	Penicillin, Amoxicillin, Cloxacillin
Bla-SHV-R1	TCCCGCAGATAAATCACCA	Penicillin, Amoxicillin, Cloxacillin
Tet(A)-F	GTGAAACCCAACATACCCC	Tetracycline
Tet(A)-R	GAAGGCAAGCAGGATGTAG	Tetracycline
Tet(E)-F	AAACCACATCCTCCATACGC	Tetracycline

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

Tet(E)-R	AAATAGGCCACAACCGTCAG	Tetracycline
aadA-F	ATTTGCTGGTTACGGTGACC	Streptomycin
aadA-R	CTTCAAGTATGACGGGCTGA	Streptomycin

3.0. Results

3.1. Identification and characterization of Mastitis-Causing Bacteria

Total colony count was done after culturing (**Table 2 and Figure 2 & 3**). The alpine breed showed a consistently high count across all media, especially on MacConkey (MCA). Saanen showed high count on mannitol salt agar (MSA) and moderate count on sheep blood agar (SBA). Toggenburg showed variable growth with some pure breeds (sample 8) showing very high MSA counts, while others were more balanced. The Kenya alpine produced mixed results with one sample (11) showing high counts across all media, while another (16) had a much lower growth on MSA and SBA.

Table 2. Mean and standard deviation of Colony count after bacterial culture.

Sample	Breed	MacConkey Agar	Mannitol Salt Agar	Sheep Blood Agar (5%)
1	British Alpine	133.0 ± 43.7	69.7 ± 14.5	248.3 ± 35.1
2	Alpine	580.3 ± 75.2	274.7 ± 48.1	194.7 ± 5.0
3	Toggenburg	_	252.3 ± 104.2	209.3 ± 13.0
4	Alpine	196.7 ± 35.7	78.0 ± 20.8	102.0 ± 19.7
5	Saanen	304.0 ± 23.8	455.7 ± 67.6	230.0 ± 34.9
6	Cross Alpine	392.7 ± 33.8	218.3 ± 34.9	235.3 ± 25.3
7	Saanen Pure	203.0 ± 8.7	168.3 ± 14.6	115.7 ± 30.9
8	Toggenburg Pure	500.0 ± 28.0	438.7 ± 9.8	104.3 ± 15.6
9	Alpine	404.7 ± 34.2	186.0 ± 10.4	144.0 ± 14.1
10	Toggenburg	478.7 ± 62.3	312.0 ± 49.0	229.0 ± 36.5
11	Kenya Alpine	513.0 ± 75.0	412.0 ± 16.1	219.0 ± 33.7
12	Kenya Alpine	227.0 ± 17.2	90.7 ± 6.9	181.3 ± 19.5
13	Alpine	252.3 ± 36.1	205.3 ± 8.5	196.7 ± 8.9
14	Toggenburg	208.7 ± 12.9	160.7 ± 4.6	274.3 ± 10.1
15	Saanen	329.7 ± 97.4	158.3 ± 12.6	147.3 ± 21.0
16	Kenya Alpine	400.3 ± 32.9	53.7 ± 19.7	157.3 ± 42.6
17	Alpine	386.7 ± 47.5	292.7 ± 23.2	283.3 ± 22.7

Table 2 shows total colony count of bacteria colonies observed on different culture media. Culturing was done in triplicates across the three media classes.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

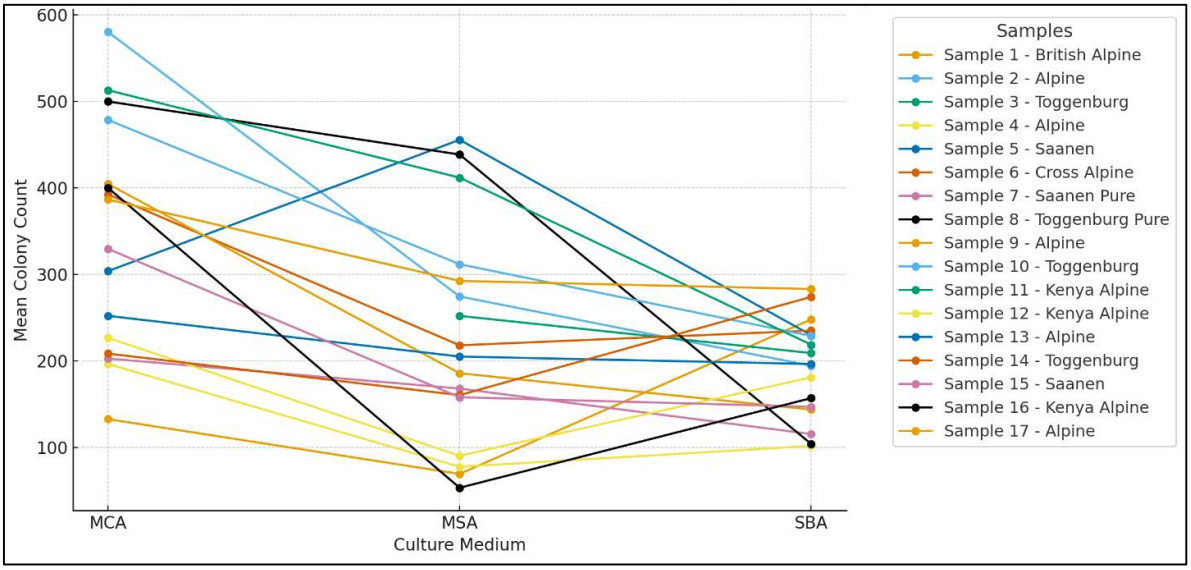

Figure 2. Illustration of how sample average colony count changes across the three media (MacConkey, Mannitol Salt, and Sheep Blood Agar).

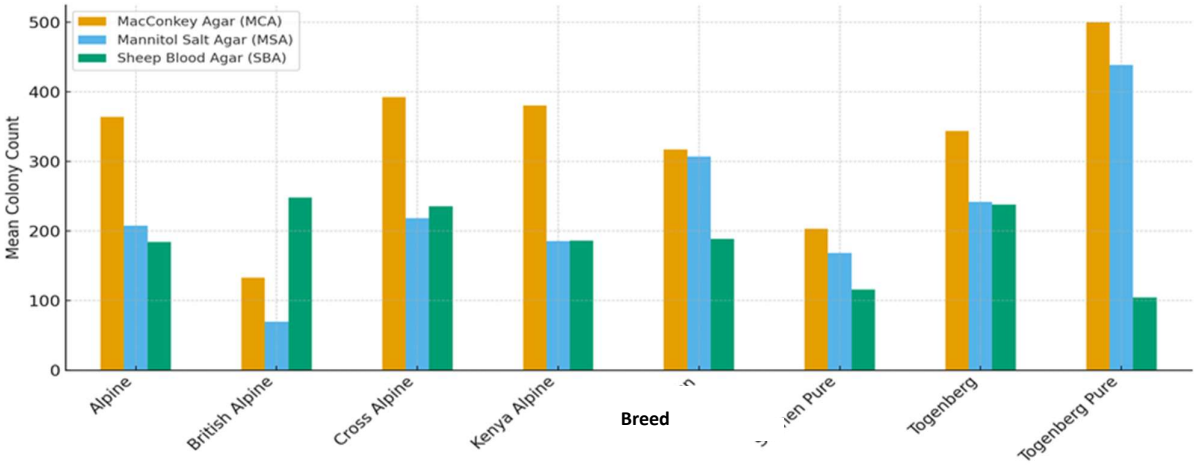

Figure 3. A comparison of average colony counts across goat breeds for each culture medium.

Different bacteria isolates were observed on the different culture media used. Colonies showed different characteristics further indicating the diversity that exists within mastitis causing-bacteria. Some of the important morphological parameters that were considered during isolation include colony size, color change, and colony margin. A total of 21 colonies identified by their unique morphological features were selected for the subsequent biochemical tests.

The identity of the isolated bacteria was established after a series of tests. Firstly, pure colonies were subjected to Gram staining procedure and results recorded. After Gram staining,

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

biochemical tests targeting specific but unique features of these organisms were performed. Gram-negative bacteria known for causing mastitis were prevalent in comparison to their Gram-positive counterparts.

After biochemical testing and recording of results, a list of possible putative organisms (Table 3) was established. They include: Staphylococcus aureus, non-*aureus* Staphylococcus, *Streptococcus uberis*, *Escherichia coli*, Pseudomonas spp. and *Clostridium perfringens*, Aeromonas and Serratia spp.

Table 3. Putative organisms after biochemical tests.

Gram staining	Catalase	Urease	Citrate Utilization	Starch Hydrolysis	Indole test	MR	VP	Gas production	Glucose Fermentation	Lactose Fermentation	Sucrose fermentation	Putative Organism
-	+	-	-	-	+	+	-	+	+	+	-	*Escherichia coli*
-	+	+	+	-	-	+	+	+	+	+	-	*Enterobacter spp.*
+	+	-	-	-	-	-	-	-	-	-	-	*Corynebacterium spp.*
-	+	-	-	-	-	-	-	-	-	-	-	*Pseudomonas*
-	+	-	-	+	-	-	+	+	+	-	-	*Aeromonas*
-	+	+	+	-	+	-	+	+	+	-	+	*Serratia*
-	+	+	+	-	-	-	+	+	+	+	-	*Klebsiella*
+	+	-	-	+	-	+	-	-	+	+	+	*Staphylococcus aureus*
+	+	-	-	-	-	-	-	-	+	+	+	*Staphylococcus*
+	-	-	-	-	-	-	-	-	+	-	+	*Streptococcus agalactiae*
+	-	-	-	-	-	-	-	-	+	-	+	*Streptococcus uberis*
+	-	-	-	-	-	-	-	-	+	-	+	*Streptococcus dysgalactiae*

3.2. Molecular detection of mastitis-causing bacteria

Sequencing was done on total DNA extracted from the 17 milk samples. After sequencing, several species of bacteria were identified after a BLAST search on the NCBI database. A total of 35 bacterial isolates were identified after the sequencing. Similar to biochemical testing, some of the bacteria identified after sequencing included: E. coli, Pseudomonas spp. and Enterobacter spp. Serratia, Aeromonas, Shigella, and Klebsiella. Serratia was the most predominant bacteria identified. No Gram-positive bacteria were identified.

3.3. Antibiotic susceptibility profiles

A total of 6 antibiotics were used during antibiotic susceptibility testing. The antibiotics used were: penicillin, amoxicillin, tetracycline, cloxacillin, streptomycin and chloramphenicol (Figure 4). The antibiotics were tested against 21 pure isolates that had been isolated and identified after culturing. The bacteria showed different levels of resistance towards the antibiotics as shown by the antibiotic resistance heat map (Figure 5). The levels of resistance were as follows

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

Chloramphenicol (95.2%), Penicillin (90.5%), Cloxacillin (85.7%), Amoxicillin (81%), Tetracycline (52.4%) and Streptomycin (47.6%).

Susceptibility levels towards the antibiotics were as follows Streptomycin (33.3%), Tetracycline (33.3%), Amoxicillin (19%), Penicillin (9.5%), and Cloxacillin (9.5%). No isolate was susceptible to Chloramphenicol. Intermediate resistance was present in 4 antibiotics out of the 6 used as follows Streptomycin (19%), Tetracycline (14.3%), Cloxacillin (4.8%), Chloramphenicol (4.8%) while no intermediate resistance was observed in penicillin and amoxicillin.

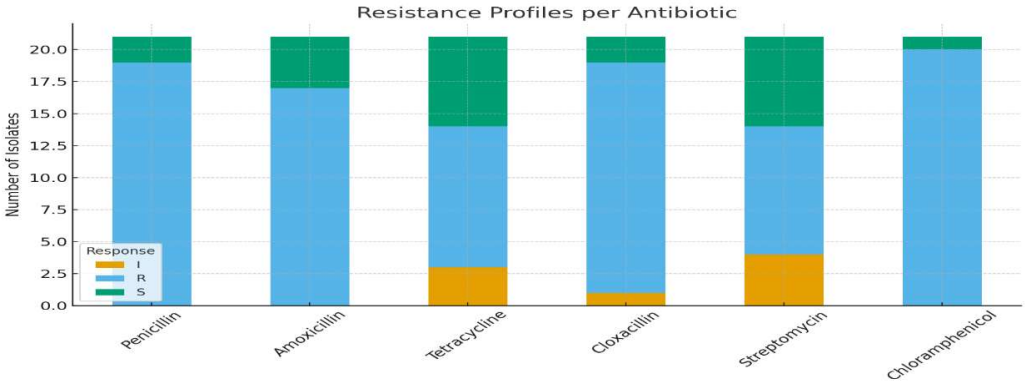

Figure 4. Resistance patterns of the 6 antibiotics used in the antibiotic susceptibility testing.

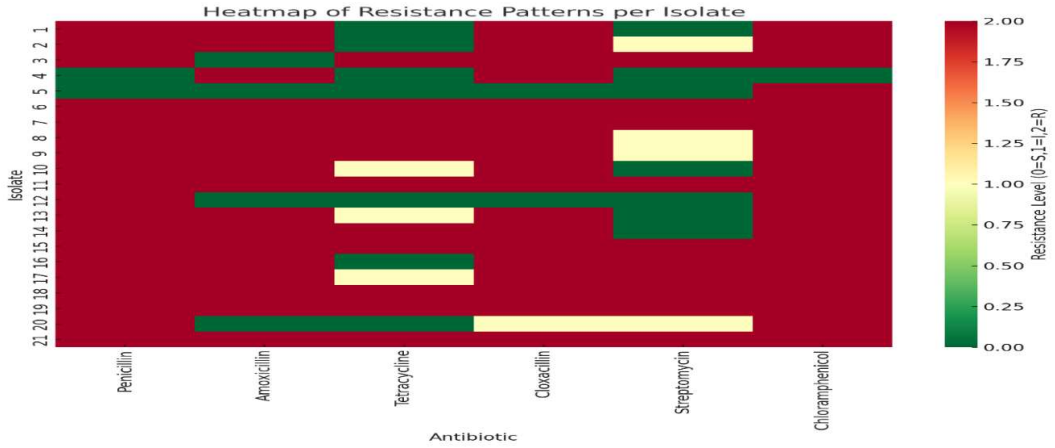

Figure 5. Isolate resistance profiles (green = susceptible, yellow = intermediate, red = resistant) and distribution showing how many antibiotics each isolate was resistant to.

3.4. Detection of antibiotic resistance genes

A total of 5 antibiotic resistance genes (Figure 6) were screened against bacterial DNA extracted from the 17 milk samples. The genes were, SHV, TEM, *aad*A, *tet*A and *tet*E. The *aad*A gene had the most abundance, at 29.4%. The SHV gene had an abundance of 11.8%. Similarly, *tet*A also had an abundance of 11.8%. Lastly, *tet*E had the least abundance of 5.9%. The bla_{TEM}

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

gene was not detected in any of the samples during sequencing. There was a high prevalence of phenotypic resistance to beta-lactam antibiotics among these isolates. Phenotypic resistance to tetracycline and streptomycin was moderate to high but the detected resistance genes were less frequent. The aadA gene at 29.4% frequency was notable for aminoglycoside resistance, however, there was still a significant gap between gene frequency and phenotypic resistance.

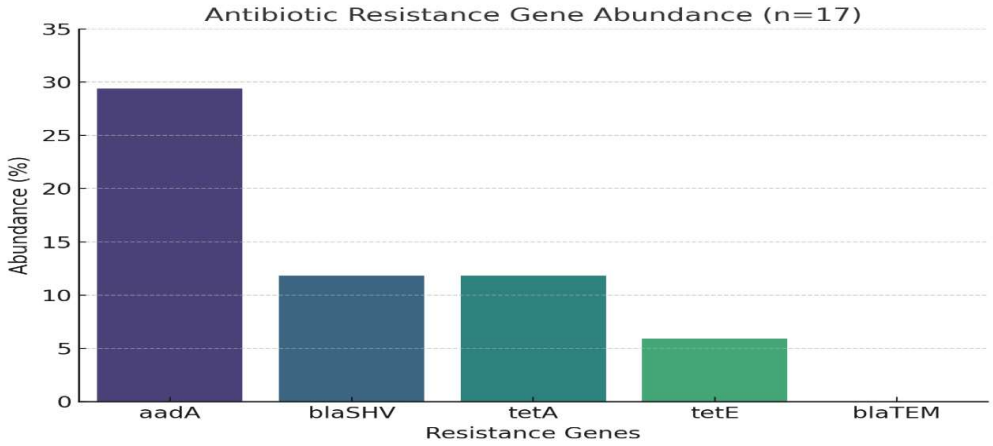

Figure 6. Abundance (%) of each resistance gene.

The dominance of aadA correlated well with the high streptomycin resistance recorded in the phenotypic tests. blaSHV presence explains the observed β-lactam resistance (penicillin, amoxicillin, cloxacillin). Tetracycline resistance (*tet*A and *tet*E) appeared at a moderate frequency, aligning with isolates that showed reduced susceptibility to tetracycline. BlaTEM was absent.

3.5. Statistical analysis

Statistical Data analysis was done using the SPSS software for windows, Version 29. One-way ANOVA (Table 4) was done to check if colony counts differ significantly between species for each medium ($p < 0.05$). In regards to resistance, the Chi-square test of independence was performed (Table 5). The test demonstrated a statistically significant difference in resistance rates across antibiotics used in the treatment and management of mastitis cases in Manyatta ($p < 0.05$).

3.6. Relationship between phenotypic and genotypic resistance patterns

The relationship between phenotypic and genotypic resistance patterns was not directly proportional. This was indicated by the varying levels of resistance observed. The phenotypic resistance levels were significantly higher than their corresponding genotypic resistance levels indicating the likely presence of other factors driving resistance patterns.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

Table 4. Anova results of colony counts across goat breeds per medium.

Medium	F-statistic	p-value	Significant?
MacConkey Agar	0.67	0.694	No
Mannitol Salt Agar	0.65	0.705	No
Sheep Blood Agar (5%)	1.2	0.398	No

For MacConkey Agar ($F = 0.67$, $p = 0.694$), Mannitol Salt Agar ($F = 0.65$, $p = 0.705$), and Sheep Blood Agar ($F = 1.20$, $p = 0.398$), the results indicated no statistically significant differences between breeds at $p < 0.05$.

Table 5: Chi-square test results for resistance vs. non-resistance across antibiotics.

Antibiotic	Resistant (%)	Susceptible/Intermediate (%)	X^2 Statistic	df	p-value
Penicillin	90.5	9.5	19.091	5	0.0018
Amoxicillin	81.0	19.0	19.091	5	0.0018
Cloxacillin	85.7	14.3	19.091	5	0.0018
Tetracycline	54.4	47.6	19.091	5	0.0018
Streptomycin	47.6	52.4	19.091	5	0.0018
Chloramphenicol	95.2	4.8	19.091	5	0.0018

Chi-square results were highly significant ($p < 0.05$), indicating that resistance levels differ significantly across the six antibiotics groups.

4.0. Discussion

This study illustrated the uniqueness of mastitis as a disease that can be caused by diverse bacteria in dairy animals such as goats (Polveiro *et al.*, 2022). These bacteria are also highly resistant (Jabbar *et al.*, 2020). Both bacteria culture and sequencing results from this study confirmed that multiple bacteria are capable of causing mastitis (Tong *et al.*, 2025). Notable bacteria identified from this study include: *Staphylococcus aureus*, *non-aureus Staphylococcus*, *Streptococcus uberis*, *Escherichia coli*, Pseudomonas spp. Klebsiella, Aeromonas and Serattia spp.(Kabui *et al.*, 2024).

Colony counts across different goat breeds involved in the study showed variation but this was not statistically significant according to ANOVA. Descriptive plot results showed some numerical variation, for instance toggenburg pure had higher mean counts on MacConkey and Mannitol Salt Agar, these differences were not statistically significant at the conventional threshold of $p < 0.05$. This indicates that the type of goat species did not strongly influence bacterial colony counts on culture media used. However, the high number of coliforms identified as causative agents in this study could be attributed to predisposing factors that would have likely contributed to the spread of these bacteria. They include poor animal management, physical wounds on the udders and poor hygiene (Abebe *et al.*, 2023).

Similar to other previously related studies (Koskinen *et al.*, 2010; Keane *et al.*, 2013), molecular detection was able to detect a higher number of bacteria in comparison to culturing. However, the species detected by both techniques were mostly similar. In most mastitis cases, environmentally ubiquitous bacteria are normally thought to be responsible for causing

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

mastitis infections. This phenomenon was illustrated in a study by (Fredebeul-Krein *et al.*, 2022) which showed a high prevalence of coliforms isolated from clinical mastitis samples. This study recorded similar results with coliforms forming a bulk of the isolated pathogens. However, it was interesting to note that while both staphylococcus and streptococcus species which are highly associated with mastitis were isolated during culturing, both were absent from results obtained after sequencing.

The reason for the absence of the two species can be attributed to a number of factors. First, the DNA used during sequencing was extracted from overnight broth-enriched cultures rather than directly from raw milk. Nutrient broth as a culture medium is general purpose but highly permissive medium that preferentially supports the rapid proliferation of fastidious bacteria, particularly Gram-negative coliforms such as E. coli, klebsiella, and Enterobacter (Putri *et al.*, 2021). During overnight incubation, these bacteria were likely to have grown at a much faster rate compared to their Gram-positive counterparts. Gram-positive mastitis-causing bacteria like staphylococcus and streptococcus could have grown much slower, requiring more specific nutrients under similar enrichment conditions.

As a result, DNA from these Gram-positive bacteria could have been in low quantities or entirely absent in the enriched broth despite their presence in the initial milk samples and their subsequent isolation and identification through culturing techniques. Consequently, the sequencing results highlights the microbial composition of the enriched broth cultures rather than the actual bacterial strains in the milk (Jarvis *et al*., 2015). This methodological bias sheds light on the observed disparity between culture-based identification and sequencing.

However, previous studies also indicate that this could have been as a result of a shortcomings that come with 16S rRNA sequencing when dealing with mixed cultures. One of the shortcomings associated with this study is that 16S rRNA sequencing was done by targeting one variable region of the 16S rRNA gene. Targeting of multiple regions of the 16S rRNA gene to improve accuracy in detection was possible but limited by cost. Some bacteria species show high similarity in their 16S rRNA variable regions. Because of this, it is challenging to identify individual families or species without further targeting other regions of the 16S rRNA gene for more precise identification (Rizal *et al*., 2020).

Furthermore, the absence of Staphylococcus and streptococcus species after sequencing in this study could be attributed to the polymicrobial nature of the samples involved. 16S rRNA sequencing is more reliable when seeking to identify a single species organism but less reliable when sequencing polymicrobial samples. This because Sanger sequencing results are likely to yield overlapping reads that might be challenging to interpret when the samples are polymicrobial (Mularoni *et al*., 2022). This challenge can be overcome by using current Next Generation Sequencing techniques employed in metagenomics research that targets an amplified 16S rRNA gene sequence (Chacha *et al*., 2025). However, the NGS techniques remain expensive in low and middle-income countries hence the reason sanger sequencing was used during this study.

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

Polymicrobial results obtained from both sequencing and culturing highlight cases of co-infection. This was observed through detection of more than one causative agent in the same sample during sequencing. Co-infection in the causative agents was particularly a striking observation that from this study. This is because despite the fact that mastitis is caused by several bacterial species, most studies focus on the prevalence of a single bacteria species while showing little emphasis towards cases where more than one causative bacterium is detected in a sample (Rifatbegović *et al*., 2024). In this study, the detection of two or more bacteria species was consistent in both bacterial culture detection as well as in sequencing. During sampling, the duration between infection and detection was not determined. As a result, the effect of polymicrobial infection on disease progression and severity was not explored in this study.

During culturing, single samples produced mixed cultures on the different growth media further enhancing the fact that a single animal was infected by more than one type of bacteria. Over 200 species of bacteria are associated with mastitis (Song *et al*., 2024). Although almost all samples had two or more species, most of the bacteria isolated from the samples were similar in identity in most cases with serratia being the most predominant species. This can be attributed to the fact that they are coliforms normally found in animal environments (Zadoks *et al*., 2011). While serratia is not regarded as a primary mastitis pathogen, it is a known opportunistic pathogen and environmental contaminant capable of causing severe cases of both clinical and sub-clinical mastitis (Friman *et al*., 2019). The opportunistic nature of serratia coupled with nutrient broth enrichment highlights why serratia dominated among the identified bacteria. Results obtained indicated co-infection as a significant inference during this study. Due to limited information from previous studies regarding polymicrobial infection in mastitis cases, reasons for the high levels of co-infection observed, implications of co-infection towards antibiotic therapy and disease severity could not be well explained. The results however, indicated that this is a significant gap that calls for further research to probe for further understanding on the effects of co-infection cases in polymicrobial mastitis.

Occurrence of resistance in mastitis infections poses a serious challenge to dairy goat farming (Pérez *et al.*, 2020). The effects can be directly on the animals in terms of diminished animal health (Gabli *et al.*, 2019). Other effects are experienced by the farmers who suffer financially during treatment and management of the disease (Käppeli *et al*., 2019). Animals suffer from poor health due to development of antibiotic resistance which renders the antibiotics ineffective. Public health is also compromised as a result of circulation of antibiotic resistance genes in dairy products derived from goat milk (Fesseha *et al*., 2021). From this study, 4 out of the 5 resistance genes that were screened were detected to have been present.

The Chi-square test compared resistance distribution across all six antibiotics simultaneously. The results obtained were highly significant ($p = 0.0018$, $df = 5$), indicating that the different resistance levels among the antibiotics used was not random but rather due to variation in their resistance patterns. The high resistance rates (>80%) observed in penicillin (90.5%),

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

amoxicillin (81.0%), cloxacillin (85.7%), and chloramphenicol (95.2%), indicate that these antibiotics have lost efficacy against some bacterial strains that cause mastitis in Manyatta. These results are similar to a nine-year (2016-2024) study conducted in Iran that showed steady increase in resistance levels towards penicillin and amoxicillin over a 9-year period among mastitis-causing bacteria (Moradi *et al*., 2025). Moderate resistance levels were observed in tetracycline (52.4%) and streptomycin (47.6%), suggesting the two antibiotics may still retain some degree of effectiveness. In overall, the results confirmed that the resistance observed was not uniform but rather clustered more strongly in certain antibiotics, especially β-lactams and chloramphenicol.

These findings imply treatment with β-lactams (penicillin, amoxicillin, cloxacillin) and chloramphenicol would most likely fail. Correlation tests that were performed indicated a moderate positive correlation between phenotypic resistance levels to the antibiotics tested and the availability of resistance genes. This correlation aligns with the expectation that genotypic detection partially explains phenotypic resistance but other factors and mechanisms may also contribute significantly. This was illustrated by a study comparing Phenotypic and genotypic antimicrobial resistance correlation among zoonotic isolates from human, foodstuffs and animal sources in Italy (Petrin *et al*., 2023). The correlation is not very strong, which confirms that the resistance genes detected did not fully account for the high phenotypic resistance observed. This suggests that the presence of a complex resistance pattern involving additional genetic or non-genetic mechanisms may have been responsible for the observed results (Sakagianni *et al*., 2024).

The aadA was the most abundant in this study. The *aad*A genes code for resistance towards the aminoglycoside group of antibiotics for instance streptomycin used in this study (Fazel *et al*., 2019). The *aad*A gene encodes for special enzymes known as aminoglycoside-3”-adenylyltransferases (ADD). ADDs have the ability to alter aminoglycosides when administered effectively rendering the bacteria resistant towards antibiotics such as streptomycin and kanamycin which belong to this class of antibiotics (Cameron *et al*., 2018). Some of the bacteria that carry this gene and are known for causing mastitis include; salmonella, Klebsiella and E. coli (Moradi *et al*., 2025). Resistance to streptomycin correlated with detection of the *aad*A gene (29.4%) which confers aminoglycoside resistance. The phenotypic resistance (47.6%) was higher than the gene detection frequency, indicating possible additional aminoglycoside resistance mechanisms (Petrin *et al*., 2023). In this study, the 29.4% high detection of the *aad*A gene was similar to another study conducted in northern China where the *aad*A gene had the highest prevalence (62.5%) among other genes that were screened among mastitis causing E. coli strains isolated during the study (Li *et al*., 2022). This hints at a possible trend suggesting prevalent occurrence of this gene in mastitis infections.

The bla_{SHV} which codes for resistance towards beta lactamase (Petrin *et al*., 2023) antibiotics was also detected in this study. The beta-lactamase antibiotics used during this study were penicillin, amoxicillin and cloxacillin. Beta-lactams consist of a group of broad-spectrum antibiotics that are potent against both Gram positive and Gram-negative bacteria and have a

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

beta-lactam ring in their structure (Tooke *et al*., 2019). The *bla*$_{SHV}$ gene belongs to a group of resistance genes known as Extended-spectrum beta-lactamases (ESBLs) (Husna *et al*., 2023). Bacteria carrying these genes are capable of breaking down the beta-lactam ring in the B-lactam antibiotics through the action of enzymes known as beta-lactamases (Zhang *et al*., 2024). This action renders the antibiotics used ineffective. This has been clearly illustrated in this study with the high phenotypic resistance observed towards all the β-lactams used in the management of mastitis cases in Manyatta (Penicillin (90.0%), Amoxicillin (85.7%) and Cloxacillin (81%). Some of the bacteria known to carry these genes are *Escherichia coli* and *Klebsiella pneumniae* that were present in this study (Husna *et al*., 2023).

The presence of *bla*$_{SHV}$ gene at 11.8% partially explains the phenotypic resistance to beta-lactams, however, the gene frequency was much lower than the phenotypic resistance rates observed. This was similar to the resistance patterns observed in streptomycin, suggesting that other beta-lactam resistance mechanisms may also be present as well as other beta-lactamase genes such as *bla*$_{CTX-M}$ and *bla*$_{OXA}$ genes that were not screened. The high phenotypic resistance might also have been as a result of other mechanisms such as altered penicillin-binding proteins (Sakagianni *et al*., 2024). However, *bla*$_{TEM}$ gene was not detected (0%), which was interesting because TEM beta-lactamase genes are common and often widespread in Gram-negative bacteria and hence their absence was likely due to a local resistance pattern distinct from global trends. These findings were consistent with a study in which other beta- lactamase genes were detected in mastitis cases during screening but the *bla*$_{TEM}$ was not detected (Li *et al*., 2022). However, another study conducted in different districts of Punjab detected high levels of the *bla*$_{TEM}$ gene (62. 5%) from beetal goats (Satpathy *et al*., 2023). These variations in the prevalence of the TEM gene in mastitis infections further point at the likelihood of a local resistance pattern rather than a global trend.

When it comes to tetracycline resistance, a group of genes known as the tet genes are known to be responsible for conferring this resistance in most Gram-negative bacteria. In this study *tet*A and *tet*E genes were detected to have been present. Resistance to tetracycline (52.4%) was partially highlighted by detection of *tet*A (11.8%) and *tet*E (5.9%) genes combined (17.7%), but these genes account for only a fraction of phenotypic resistance. This suggests that other tetracycline resistance genes or mechanisms are also present as observed in most of the other genes detected during this study. The tet genes are able to confer antibiotic resistance to bacteria through efflux pumping (Jahantigh *et al*., 2020). Efflux pumps are normally made up by transport proteins located in the cell membrane of the bacteria. These proteins are responsible for recognizing tetracycline antibiotics and effectively pumping them out of the cell (Kumawat *et al*., 2023). *Tet*A gene is one of the most abundant and commonly studied resistance genes when it comes to tetracycline resistance.

The high levels of resistance among the isolates in this study indicate the serious threat that mastitis exerts on public health. A total of seven resistant pathogens standing at 33.3%, were resistant to the select antibiotics used by local farmers. None of the screened antibiotics were effective against phenotypic antibiotic resistance shown by these bacteria (Bharadwaj *et al*.,

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

2022). The high resistance burden indicates limited treatment options and a potential spread of resistant strains (Moradi *et al*., 2025). Chloramphenicol showed the highest level of drug resistance with almost all the isolates being resistant to the antibiotic. The persistence of Chloramphenicol resistance suggests historical antibiotic pressure and horizontal gene transfer. The phenotypic resistance profile revealed very high resistance to β-lactam antibiotics, with 90.5% of isolates resistant to penicillin, 85.7% to cloxacillin, and 81% to amoxicillin. Moderate resistance was also observed to tetracycline (52.4%) and streptomycin (47.6%). In contrast, molecular screening identified relatively low prevalence of the resistance genes tested: *aad*A (29.4%), *tet*A (11.8%), *tet*E (5.9%), bla_{SHV} (11.8%), while bla_{TEM} was absent (0%).

When compared, the phenotypic resistance levels were consistently higher than the proportion of corresponding resistance genes detected. For instance, in β-lactams, the extremely high resistance rates could not be fully explained by the low detection of bla_{SHV} and absence of bla_{TEM}, suggesting the presence of other β-lactamase genes or alternative mechanisms like alterations in penicillin-binding proteins (Shivakumaraswamy *et al*., 2019). Similarly, the tetracycline resistance (52.4%) far exceeded the combined prevalence of *tet*A and *tet*E (17.7%), indicating that other tet determinants or non-genetic mechanisms such as efflux pumps may have contributed to the discrepancy. Streptomycin resistance (47.6%) showed a moderate correlation with the presence of aadA (29.4%), but the discrepancy suggests involvement of additional aminoglycoside resistance genes or possible ribosomal mutations.

All the detected genes that were screened are known to be carried by some coliforms that are zoonotic and are involved in the spread of mastitis through animals to human interactions (Song *et al*., 2024). These interactions likely result to spread of resistance genes and multi-drug resistance species capable of causing fatal infections associated with AMR in human beings. The coliforms are also capable of transferring these genes amongst themselves. These transmissions further contribute to the rising levels of AMR alongside new resistant strains. The genes are normally transmitted from one bacterium to another through the help of genetic elements that enhance transfer of genetic material such as plasmids, transposons and bacteriophages (Gundran *et al*., 2019).

The exchange of these genes can also happen directly through mechanisms such as transformation, conjugation and transduction that can happen naturally in nature (Makori *et al*., 2024). The high levels of resistance and co-infection shown in this study calls for alternative strategies in management of mastitis since single antibiotic therapy showed little efficiency. From the findings, it is imperative that ineffective β-lactam antibiotics should be shelved from use as they will only contribute to the rising levels of resistance. The high prevalence of opportunistic pathogen also necessitates improvement of farm hygiene coupled with routine CMT to ensure the spread of coliforms is contained. Broader surveillance using bias-free molecular detection techniques will significantly improve in identification of pathogenic

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

bacteria that cause mastitis and spread resistance towards antibiotics used as therapeutic options in management of mastitis infections.

5.0. Limitations of the study

The DNA for sequencing was extracted from overnight nutrient broth enrichments of the milk samples. This step selectively favoured the growth of fast-growing, Gram-negative coliforms, explaining the divergence between culture-based and sequence-based identification and the absence of Gram-positive bacteria in sequencing results. This limits the conclusions we can draw about the true relative abundance of bacterial species in the original milk samples. During sampling, the initial target was to sample at least 68 animals. However, this was not possible. This was mainly because sampling was based on consent. However, due to limited education in regards to mastitis and its effects, many farmers who were approached were skeptical about participating in the study. For this reason, only a small sample of 17 goats was used. This small sample size was a notable statistical challenge for the study despite the study being a pilot study. The, interpretation of these findings was constrained by the small sample size and the use of snowball sampling, which limited the generalizability of the results beyond the study population. The results however, show the need for further work in Manyatta Sub-County with results from this study showing how serious a problem mastitis is. The problem is not only on the animals but also on public health due to the high levels of resistance showed by causative bacteria towards antibiotics that are used in the area to control bacteria.

6.0. Conclusion

This pilot study provides a good starting point for a bigger study focusing on other alternative and effective ways of determining antibiotic resistance towards antibiotics used controlling mastitis in Manyatta. Antibiotic susceptibility testing revealed extreme phenotypic resistance to commonly used first-line antibiotics, indicating a high likelihood of clinical treatment failure if these agents are used empirically. The high β-lactam resistance is of particular concern, as it indicates limited therapeutic value of penicillin-based drugs. These findings demonstrate that routine first-line antibiotics are largely ineffective against the isolates tested, underscoring the urgent need for culture-guided therapy, revision of empirical treatment protocols, and strengthened antimicrobial stewardship to mitigate further resistance development. The variation between phenotypic and the genotypic resistance showed that the isolates carried additional resistance mechanism that were not explored. Broader molecular screening, including additional β-lactamase, tetracycline, and aminoglycoside resistance determinants, or whole-genome sequencing, would provide a more comprehensive understanding of the resistance mechanism present.

7.0 Acknowledgments

7.1 Funding

This research received no external funding.

7.2. Ethical considerations

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

The ethical approval for the study was obtained from the institutional scientific ethics review committee (ISERC), approval number: ISREC/06/2023 at the Kenya Institute of Primate Research (KIPRE). This was important in ensuring that the work was conducted in the confines of the necessary ethical guidelines and integrity.

7.3. Data availability statement

The original contributions presented in this study are included in the article.

7.4 Conflicts of interest

The authors declare that they have no competing interests.

8.0 References

Abebe, E., Gugsa, G., Ahmed, M., Awol, N., Tefera, Y., Abegaz, S., & Sisay, T. (2023). Occurrence and antimicrobial resistance pattern of E. coli O157:H7 isolated from foods of Bovine origin in Dessie and Kombolcha towns, Ethiopia. *PLOS Neglected Tropical Diseases*, *17*(1), e0010706. https://doi.org/10.1371/journal.pntd.0010706

Bharadwaj, A., Rastogi, A., Pandey, S., Gupta, S., & Sohal, J. S. (2022). Multidrug-Resistant Bacteria: Their Mechanism of Action and Prophylaxis. *BioMed Research International*, *2022*, 5419874. https://doi.org/10.1155/2022/5419874

Cameron, A., Klima, C. L., Ha, R., Gruninger, R. J., Zaheer, R., & McAllister, T. A. (2018). A Novel aadA Aminoglycoside Resistance Gene in Bovine and Porcine Pathogens. *mSphere*, *3*(1), e00568-17. https://doi.org/10.1128/mSphere.00568-17

Chacha, R. R., Were, S., Neondo, J., Issa, M., Khayeli, J., & Omar, F. (2025). Metagenomic analysis of bacterial diversity in potable water and ice pop samples from Juja: Implications for public health. *JOURNAL OF AGRICULTURE, SCIENCE AND TECHNOLOGY*, *24*(2), 73–98. https://doi.org/10.4314/jagst.v24i2.6

Cheng, W. N., & Han, S. G. (2020). Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments — A review. *Asian-Australasian Journal of Animal Sciences*, *33*(11), 1699–1713. https://doi.org/10.5713/ajas.20.0156

Fazel, F., Jamshidi, A., & Khoramian, B. (2019). Phenotypic and genotypic study on antimicrobial resistance patterns of E. coli isolates from bovine mastitis. *Microbial Pathogenesis*, *132*, 355–361. https://doi.org/10.1016/j.micpath.2019.05.018

Fesseha, H., Mathewos, M., Aliye, S., & Wolde, A. (2021). Study on Prevalence of Bovine Mastitis and Associated Risk Factors in Dairy Farms of Modjo Town and Suburbs, Central Oromia, Ethiopia. *Veterinary Medicine : Research and Reports*, *12*, 271–283. https://doi.org/10.2147/VMRR.S323460

Fredebeul-Krein, F., Schmenger, A., Wente, N., Zhang, Y., & Krömker, V. (2022). Factors Associated with the Severity of Clinical Mastitis. *Pathogens*, *11*(10), Article 10. https://doi.org/10.3390/pathogens11101089

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

Friman, M. J., Eklund, M. H., Pitkälä, A. H., Rajala-Schultz, P. J., & Rantala, M. H. J. (2019). Description of two Serratia marcescens associated mastitis outbreaks in Finnish dairy farms and a review of literature. *Acta Veterinaria Scandinavica*, *61*, 54. https://doi.org/10.1186/s13028-019-0488-7

Gabli, Z., Djerrou, Z., Gabli, A. E., & Bensalem, M. (2019). Prevalence of mastitis in dairy goat farms in Eastern Algeria. *Veterinary World*, *12*(10), 1563–1572. https://doi.org/10.14202/vetworld.2019.1563-1572

Giuliano, C., Patel, C. R., & Kale-Pradhan, P. B. (2019). A Guide to Bacterial Culture Identification And Results Interpretation. *Pharmacy and Therapeutics*, *44*(4), 192–200.

Gundran, R. S., Cardenio, P. A., Villanueva, M. A., Sison, F. B., Benigno, C. C., Kreausukon, K., Pichpol, D., & Punyapornwithaya, V. (2019). Prevalence and distribution of blaCTX-M, blaSHV, blaTEM genes in extended- spectrum β- lactamase- producing E. coli isolates from broiler farms in the Philippines. *BMC Veterinary Research*, *15*, 227. https://doi.org/10.1186/s12917-019-1975-9

Huang, C.-H., & Kusaba, N. (2022). Association between differential somatic cell count and California Mastitis Test results in Holstein cattle. *JDS Communications*, *3*(6), 441–445. https://doi.org/10.3168/jdsc.2022-0249

Husna, A., Rahman, Md. M., Badruzzaman, A. T. M., Sikder, M. H., Islam, M. R., Rahman, Md. T., Alam, J., & Ashour, H. M. (2023). Extended-Spectrum β-Lactamases (ESBL): Challenges and Opportunities. *Biomedicines*, *11*(11), 2937. https://doi.org/10.3390/biomedicines11112937

Jabbar, A., Saleem, M. H., Iqbal, M. Z., Qasim, M., Ashraf, M., Tolba, M. M., Nasser, H. A., Sajjad, H., Hassan, A., Imran, M., & Ahmad, I. (2020). Epidemiology and antibiogram of common mastitis-causing bacteria in Beetal goats. *Veterinary World*, *13*(12), 2596–2607. https://doi.org/10.14202/vetworld.2020.2596-2607

Jahantigh, M., Samadi, K., Dizaji, R. E., & Salari, S. (2020). Antimicrobial resistance and prevalence of tetracycline resistance genes in Escherichia coli isolated from lesions of colibacillosis in broiler chickens in Sistan, Iran. *BMC Veterinary Research*, *16*, 267. https://doi.org/10.1186/s12917-020-02488-z

Jarvis, K. G., White, J. R., Grim, C. J., Ewing, L., Ottesen, A. R., Beaubrun, J. J.-G., Pettengill, J. B., Brown, E., & Hanes, D. E. (2015). Cilantro microbiome before and after nonselective pre-enrichment for Salmonella using 16S rRNA and metagenomic sequencing. *BMC Microbiology*, *15*, 160. https://doi.org/10.1186/s12866-015-0497-2

Kabui, S., Kimani, J., Ngugi, C., & Kagira, J. (2024). Prevalence and antimicrobial resistance profiles of mastitis causing bacteria isolated from dairy goats in Mukurweini Sub-County, Nyeri County, Kenya. *Veterinary Medicine and Science*, *10*(3), e1420. https://doi.org/10.1002/vms3.1420

Käppeli, N., Morach, M., Zurfluh, K., Corti, S., Nüesch-Inderbinen, M., & Stephan, R. (2019). Sequence Types and Antimicrobial Resistance Profiles of Streptococcus uberis Isolated

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

From Bovine Mastitis. *Frontiers in Veterinary Science*, *6*. https://doi.org/10.3389/fvets.2019.00234

Keane, O. M., Budd, K. E., Flynn, J., & McCoy, F. (2013). Pathogen profile of clinical mastitis in Irish milk-recording herds reveals a complex aetiology. *Veterinary Record*, *173*(1), 17–17. https://doi.org/10.1136/vr.101308

Koskinen, M. T., Wellenberg, G. J., Sampimon, O. C., Holopainen, J., Rothkamp, A., Salmikivi, L., Haeringen, W. A. van, Lam, T. J. G. M., & Pyörälä, S. (2010). Field comparison of real-time polymerase chain reaction and bacterial culture for identification of bovine mastitis bacteria. *Journal of Dairy Science*, *93*(12), 5707–5715. https://doi.org/10.3168/jds.2010-3167

Kumawat, M., Nabi, B., Daswani, M., Viquar, I., Pal, N., Sharma, P., Tiwari, S., Sarma, D. K., Shubham, S., & Kumar, M. (2023). Role of bacterial efflux pump proteins in antibiotic resistance across microbial species. *Microbial Pathogenesis*, *181*, 106182. https://doi.org/10.1016/j.micpath.2023.106182

Li, K., Hou, M., Zhang, L., Tian, M., Yang, M., Jia, L., Liang, Y., Zou, D., Liu, R., & Ma, Y. (2022). Analysis of Antimicrobial Resistance and Genetic Correlations of Escherichia Coli in Dairy Cow Mastitis. *Journal of Veterinary Research*, *66*(4), 571–579. https://doi.org/10.2478/jvetres-2022-0055

Lianou, D. T., & Fthenakis, G. C. (2022). Use of Antibiotics against Bacterial Infections on Dairy Sheep and Goat Farms: Patterns of Usage and Associations with Health Management and Human Resources. *Antibiotics*, *11*(6), 753. https://doi.org/10.3390/antibiotics11060753

Makori, A., Neondo, J., Mwangi, I., & Mweu, C. (2024). Genetic diversity of pathogenic bacteria and incidence of selected antibiotic resistant genes in water used by eateries and open markets in Juja, Kenya. *JOURNAL OF AGRICULTURE, SCIENCE AND TECHNOLOGY*, *23*(1(1)), 125–147. https://doi.org/10.4314/jagst.v27i1.8

Matuozzo, M., Spagnuolo, M. S., Hussein, H. A., Gomaa, A. M., Scaloni, A., & D'Ambrosio, C. (2020). Novel Biomarkers of Mastitis in Goat Milk Revealed by MALDI-TOF-MS-Based Peptide Profiling. *Biology*, *9*(8), 193. https://doi.org/10.3390/biology9080193

Michira, L., Kagira, J., Maina, N., Waititu, K., Kiboi, D., Ongera, E., & Ngotho, M. (2023). Prevalence of subclinical mastitis, associated risk factors and antimicrobial susceptibility pattern of bacteria isolated from milk of dairy cattle in Kajiado Central sub-county, Kenya. *Veterinary Medicine and Science*, *9*(6), 2885–2892. https://doi.org/10.1002/vms3.1291

Moradi, A., Bidarian, B., Mohammadian, F., Akbarian, F., Kalateh Rahmani, H., Tashakkori, N., & Khoramian, B. (2025). Trends in Antimicrobial Resistance of Major Mastitis-Causing Pathogens: A Nine-Year Study. *Veterinary Medicine and Science*, *11*(4), e70417. https://doi.org/10.1002/vms3.70417

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

Muhamad Rizal, N. S., Neoh, H., Ramli, R., A/L K Periyasamy, P. @ R., Hanafiah, A., Abdul Samat, M. N., Tan, T. L., Wong, K. K., Nathan, S., Chieng, S., Saw, S. H., & Khor, B. Y. (2020). Advantages and Limitations of 16S rRNA Next-Generation Sequencing for Pathogen Identification in the Diagnostic Microbiology Laboratory: Perspectives from a Middle-Income Country. *Diagnostics*, *10*(10), 816. https://doi.org/10.3390/diagnostics10100816

Mularoni, A., Mikulska, M., Barbera, F., Graziano, E., Medaglia, A. A., Di Carlo, D., Monaco, F., Bellavia, D., Cascio, A., Raffa, G., Sciacca, S., Luca, A., Pilato, M., & Conaldi, P. G. (2022). Molecular Analysis With 16S rRNA PCR/Sanger Sequencing and Molecular Antibiogram Performed on DNA Extracted From Valve Improve Diagnosis and Targeted Therapy of Infective Endocarditis: A Prospective Study. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *76*(3), e1484–e1491. https://doi.org/10.1093/cid/ciac452

Novac, C. S., & Andrei, S. (2020). The Impact of Mastitis on the Biochemical Parameters, Oxidative and Nitrosative Stress Markers in Goat's Milk: A Review. *Pathogens*, *9*(11), 882. https://doi.org/10.3390/pathogens9110882

Pérez, V. K. C., Costa, G. M. da, Guimarães, A. S., Heinemann, M. B., Lage, A. P., & Dorneles, E. M. S. (2020). Relationship between virulence factors and antimicrobial resistance in *Staphylococcus aureus* from bovine mastitis. *Journal of Global Antimicrobial Resistance*, *22*, 792–802. https://doi.org/10.1016/j.jgar.2020.06.010

Petrin, S., Orsini, M., Massaro, A., Olsen, J. E., Barco, L., & Losasso, C. (2023). Phenotypic and genotypic antimicrobial resistance correlation and plasmid characterization in Salmonella spp. Isolates from Italy reveal high heterogeneity among serovars. *Frontiers in Public Health*, *11*, 1221351. https://doi.org/10.3389/fpubh.2023.1221351

Polveiro, R. C., Vidigal, P. M. P., de Oliveira Mendes, T. A., Yamatogi, R. S., da Silva, L. S., Fujikura, J. M., Da Costa, M. M., & Moreira, M. A. S. (2022). Distinguishing the milk microbiota of healthy goats and goats diagnosed with subclinical mastitis, clinical mastitis, and gangrenous mastitis. *Frontiers in Microbiology*, *13*, 918706. https://doi.org/10.3389/fmicb.2022.918706

Putri, N., Ramadhani, R., & Wasito, E. (2021). Gram Negative Bacteria (Escherichia coli) Win Against Gram Positive Bacteria (Staphylococcus aureus) in The Same Media. *Biomolecular and Health Science Journal*, *4*, 113. https://doi.org/10.20473/bhsj.v4i2.30177

Rifatbegović, M., Nicholas, R. A. J., Mutevelić, T., Hadžiomerović, M., & Maksimović, Z. (2024). Pathogens Associated with Bovine Mastitis: The Experience of Bosnia and Herzegovina. *Veterinary Sciences*, *11*(2), 63. https://doi.org/10.3390/vetsci11020063

Sahoo, S., Behera, M. R., Mishra, B., Sahoo, P., & Kar, S. (2023). Antibiotic-resistant bacteria in bovine milk in India. *Journal of Advanced Veterinary and Animal Research*, *10*(1), 21–29. https://doi.org/10.5455/javar.2023.j648

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

Sakagianni, A., Koufopoulou, C., Koufopoulos, P., Kalantzi, S., Theodorakis, N., Nikolaou, M., Paxinou, E., Kalles, D., Verykios, V. S., Myrianthefs, P., & Feretzakis, G. (2024). Data-Driven Approaches in Antimicrobial Resistance: Machine Learning Solutions. *Antibiotics*, *13*(11), 1052. https://doi.org/10.3390/antibiotics13111052

Satpathy, M. M., Sharma, N. S., Kaur, P., & Arora, A. K. (2023). Detection of antimicrobial resistance genes in extended spectrum beta-lactamase-producing Escherichia coli from milk of indigenous Beetal goats of Punjab. *Iranian Journal of Veterinary Research*, *24*(1), 37–41. https://doi.org/10.22099/IJVR.2023.43480.6365

Schadt, I. (2023). Health concerns about possible long-term effects of legally marketed milk and dairy from animals with intramammary infections. *Frontiers in Public Health*, *11*. https://doi.org/10.3389/fpubh.2023.1200924

Shivakumaraswamy, S. K., Deekshit, V. K., Vittal, R., Akhila, D. S., Mundanda, D. M., Mohan Raj, J. R., Chakraborty, A., & Karunasagar, I. (2019). Phenotypic & genotypic study of antimicrobial profile of bacteria isolates from environmental samples. *The Indian Journal of Medical Research*, *149*(2), 232–239. https://doi.org/10.4103/ijmr.IJMR_2097_17

Song, X., Wang, Y., Bai, R., Pei, X., Xu, H., Zhu, K., & Wu, C. (2024). Antimicrobial resistance profiles of common mastitis pathogens on large Chinese dairy farms. *JDS Communications*, *5*(3), 185–189. https://doi.org/10.3168/jdsc.2023-0413

Stergiadis, S., Nørskov, N. P., Purup, S., Givens, I., & Lee, M. R. F. (2019). Comparative Nutrient Profiling of Retail Goat and Cow Milk. *Nutrients*, *11*(10), 2282. https://doi.org/10.3390/nu11102282

Tong, X., Barkema, H. W., Nobrega, D. B., Xu, C., Han, B., Zhang, C., Yang, J., Li, X., Gao, J., Tong, X., Barkema, H. W., Nobrega, D. B., Xu, C., Han, B., Zhang, C., Yang, J., Li, X., & Gao, J. (2025). Virulence of Bacteria Causing Mastitis in Dairy Cows: A Literature Review. *Microorganisms*, *13*(1). https://doi.org/10.3390/microorganisms13010167

Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H. A., Takebayashi, Y., & Spencer, J. (2019). β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *Journal of Molecular Biology*, *431*(18), 3472–3500. https://doi.org/10.1016/j.jmb.2019.04.002

Yamagishi, J., Sato, Y., Shinozaki, N., Ye, B., Tsuboi, A., Nagasaki, M., & Yamashita, R. (2016). Comparison of Boiling and Robotics Automation Method in DNA Extraction for Metagenomic Sequencing of Human Oral Microbes. *PLoS ONE*, *11*(4), e0154389. https://doi.org/10.1371/journal.pone.0154389

Yang, W.-T., Ke, C.-Y., Wu, W.-T., Lee, R.-P., & Tseng, Y.-H. (2019). Effective Treatment of Bovine Mastitis with Intramammary Infusion of Angelica dahurica and Rheum officinale Extracts. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2019*, 7242705. https://doi.org/10.1155/2019/7242705

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8

Zadoks, R. N., Middleton, J. R., McDougall, S., Katholm, J., & Schukken, Y. H. (2011). Molecular Epidemiology of Mastitis Pathogens of Dairy Cattle and Comparative Relevance to Humans. *Journal of Mammary Gland Biology and Neoplasia*, *16*(4), 357–372. https://doi.org/10.1007/s10911-011-9236-y

Zhang, S., Liao, X., Ding, T., & Ahn, J. (2024). Role of β-Lactamase Inhibitors as Potentiators in Antimicrobial Chemotherapy Targeting Gram-Negative Bacteria. *Antibiotics*, *13*(3), 260. https://doi.org/10.3390/antibiotics13030260

ISSN 1561-7645 (online)
doi: 10.4314/jagst.v25i2.8