



Original Article

Antimicrobial Effectiveness of Methicillin, Amoxicillin and Ampicillin against Methicillin-Resistant *Staphylococcus Aureus* (MRSA) Strains Isolated from Bovine Mastitis

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ABSTRACT

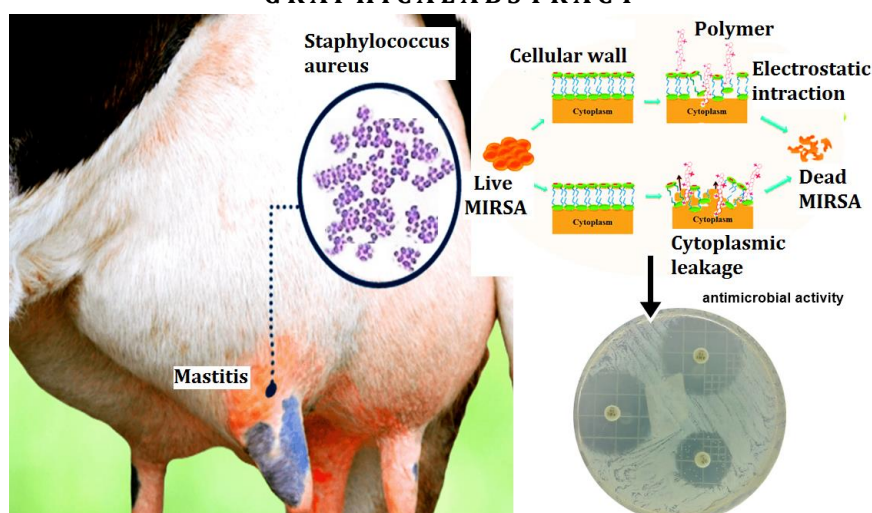
Staphylococcus aureus is a Gram-positive pathogen that is commonly associated with bovine mastitis, which is an infectious condition affecting the udder of cows. The objective of the present study is to evaluate the antimicrobial efficacy of methicillin, amoxicillin, and ampicillin for detecting methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from cases of bovine mastitis. Methodologically, it was established to carry out the antimicrobial activity using the plate disc diffusion method (Kirby-Bauer) with the use of amoxicillin and ampicillin at 2 µg and 10 µg, also following the recommendations of the Clinical and Laboratory Standards Institute. To detect resistant methicillin strains, oxacillin was used at 1 µg, and cefoxitin at 30 µg. The study was conducted against 50 strains of the bacteria under investigation, which were isolated and identified. The applied treatments demonstrated a highly significant effect ($p < 0.05$). In addition, a resistance of 50% and 60% to concentrations of 2 µg of amoxicillin and ampicillin, respectively, was observed. Furthermore, a resistance of 60% and 68% to 10 µg of amoxicillin and ampicillin, respectively, was observed, indicating that *Staphylococcus aureus* is developing mechanisms that confer antimicrobial resistance. To further investigate this, the antimicrobial activity was conducted using 1 µg oxacillin discs and 30 µg cefoxitin, revealing that 36% and 32% of the isolates were resistant to these drugs, respectively. Phenotypically, 32% ($n=16$) of the isolates were identified as methicillin-resistant *Staphylococcus aureus* (MRSA), demonstrating resistance to all beta-lactams tested.

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GRAPHICAL ABSTRACT



Introduction

Worldwide economic losses from mastitis in dairy systems represent approximately 26% of total costs. Mastitis is the most prevalent infectious disease in adult dairy cattle. Several species of bacteria, fungi, mycoplasmas, and algae have been isolated from the disease or have been shown to reproduce it experimentally [1].

The inflammation, characteristic of mastitis may be undetectable without the use of diagnostic tests applied to the milk or discharge. This condition is known as subclinical mastitis. It is important to note that mastitis is a consequence of intramammary infection caused by one or more microorganisms from various genera. These microorganisms find a nutritious substrate in the mammary gland, which promotes their growth and multiplication, contributing to the mastitis development [2].

Among these microorganisms, the bacterium *Staphylococcus aureus* stands out and can cause a wide range of diseases, ranging from relatively minor skin infections to serious and life-threatening infections within dairy herds. These include conditions such as endocarditis, omphalophlebitis in calves, pneumonia, sepsis, multi-organ abscesses, and severe clinical mastitis. In addition, its impact is reinforced by the development of resistance to antibiotics, especially methicillin-resistant *Staphylococcus aureus* (MRSA) which is resistant to practically all β -lactam antibiotics [3].

Within the dynamics of the infection, the mammary glands can be classified into one of three categories: uninfected, subclinically infected, or clinically infected, the type of pathogens also influences this classification [2].

Coliform infections tend to become clinical rapidly, whereas *Staphylococcus aureus* infections often persist as subclinical infections for weeks or months. Bacteriologic cure rates range from 90% for most coliform infections to 25% with *Staphylococcus aureus* in older cows [4].

It should be noted that the infected teat is a direct route of transmission, which occurs during the milking process. Most infections are subclinical and lead to an increase in cell count. Hygienic and sanitary measures are the best control measures [5].

Contagious bacteria include *Staphylococcus aureus*, coagulase-negative Staphs, *Streptococcus agalactiae*, and *Strep. Dysgalactiae*, with a herd somatic cell count greater than 200,000 mL [6].

It has been shown that it is possible to eradicate *Staphylococcus aureus* from individual cases by implementing hygienic measures during milking and by discarding chronically infected cows. However, the main limitation for achieving more effective control of *Staphylococcus aureus* remains its poor response to antibiotic therapy [7].

Experimentally, the *in vitro* demonstration of the sensitivity of *Staphylococcus aureus* to an antibiotic is not a guarantee of therapeutic success [8].

The ability of bacteria to survive within immune cells or polymorphonuclear cells, such as macrophages and epithelial cells, confers protection against the action of antibiotics. This is a starting point to believe that it can significantly contribute to their resistance to therapy. Furthermore, the pathological changes, particularly granulomas and fibrosis, induced in *Staphylococcus aureus*, render chronically infected cows essentially incurable. Even acute gangrenous infections can result in uncontrolled growth of the microorganism, producing large amounts of a toxin [9, 10].

The antibiotics most commonly used to control mastitis are those of the beta-lactam group. The mechanism of action of this group of antibiotics is the inhibition of cell wall synthesis. However, the main resistance mechanism is the beta-lactamases production by microorganisms causing clinical infections [11, 12].

Beta-lactams are partial bactericidal agents since they only act during the cell growth phase, and their efficacy is time-dependent, occurring based on the minimum inhibitory concentration. However, their rapid bactericidal capacity can cause the release of endotoxins, leading to a heightened inflammatory response. It should be noted that more than 90% of *Staphylococcus aureus* strains are resistant to penicillins [13].

Beta-lactam classification

Penicillins

Natural penicillins, such as Penicillin G, semisynthetic penicillins, penicillinase-resistant penicillins, beta-lactamase inhibitors,

cephalosporins (including the first, the second, and the fourth generation cephalosporins), monobactams, carbapenems, and methicillin are all important classes of antibiotics used in the treatment of various bacterial infections [13-17]. With everything previously described, the objective of this study was to analyze the antimicrobial effectiveness of methicillin, amoxicillin, and ampicillin against methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from bovine mastitis.

Materials and Methods

The present investigation was conducted in the General Laboratory of the Faculty of Agricultural Sciences, Natural Resources, and the Environment at the State University of Bolívar. A completely randomized design (CRD) was used for factor analysis, with the following description of the treatments (Table 1).

Obtaining the bacterial strains

The present investigation was conducted using strains of *Staphylococcus aureus* that were previously isolated from 50 cows positive for mastitis out of a total of 136 cows analyzed from the agricultural cooperative "La Colina" in San Miguel de los Bancos, Pichincha Province.

In addition, 20 cows positive for the disease out of 60 cows analyzed from the areas of La Candelaria and Releche in the Penipe, province of Chimborazo, were included. These bacterial isolates were preserved in MR-vP broth with 10% glicerol, and then recultured for the purpose of this study (Table 2).

Table 1: Treatments

Treatments	Description
T1	Staphylococcus aureus strain + Oxacillin inhibition disk 1 µg
T2	Staphylococcus aureus strain + Amoxicillin inhibition disk 2 µg
T3	Staphylococcus aureus strain + Amoxicillin inhibition disk 10 µg
T4	Staphylococcus aureus strain + Ampicillin inhibition disk 2 µg
T5	Staphylococcus aureus strain + Ampicillin inhibition disk 10 µg
T6	Staphylococcus aureus strain + NaCl Impregnated Inhibition Disc

Table 2: *Staphylococcus aureus* obtained from milk positive for bovine mastitis

Strains number	Sampling place
25	San Miguel de los bancos / Pichincha.
25	La Candelaria / Chimborazo.

Staphylococcus aureus reactivation

Fifty strains of *Staphylococcus aureus* were selected from milk samples that tested positive for bovine mastitis. For the conditioning process, the strains were removed from the freezer and allowed to rest at refrigeration temperature, followed by room temperature for 1 hour, and then 1-5 µL of each strain were taken from the cryovial and inoculated into MR-vP broth. The inoculated broth was incubated at 37 °C for 24 hours. After obtaining the bacterial strain, it was inoculated onto salt mannitol agar plate, in duplicate, to obtain a pure and homogeneous culture. The inoculation was performed by spreading the strain over the entire surface of the agar plate using a swab. The plates were then incubated for 24 hours at 37 °C.

Identification of staphylococcus aureus strains

The identification process involved macroscopic and microscopic description of the phenotypic characteristics, colony morphology, mannitol fermentation, and metabolic pathways of *Staphylococcus aureus*. As a part of the methodology, the following tests were established, and the characterization parameters are presented in Table 3.

The growth morphology of *Staphylococcus aureus* is evidenced as smooth, shiny, and convex colonies. These colonies typically display an orange or porcelain white end pigment (Beta-carotenoid) and can even exhibit a golden color in the specific culture medium. *Staphylococcus aureus* is a Gram-positive bacterium that can be observed as single cells, pairs, tetrads, or larger groupings arranged in clusters resembling grapes [18]. Mannitol fermentation is a characteristic of *Staphylococcus aureus*. When grown in a medium with a high salt concentration and mannitol as the carbohydrate source, *Staphylococcus aureus* has the ability to ferment mannitol, producing acids.

This fermentation process leads to a decrease in pH, resulting in a color change of the culture medium from pink to pale yellow.

Catalase test, the catalase enzyme breaks down hydrogen peroxide into water and oxygen [16].

The Coagulase test: *Staphylococcus aureus* has two types of coagulase, one is endo-coagulase or bound coagulase (clumping factor), which causes the formation of clots or lumps in the blood plasma. It also has an exocoagulase or free coagulase that produces a fibrin clot [19].

This test was performed as follows: Initially, 10 mL of blood was obtained in Vacutainer tubes. The cellular component, leukocyte platelet, and plasma were separated by centrifugation at 3000 rpm for a period of 5 minutes. 1 mL of plasma was then transferred to Eppendorf tubes. Subsequently, a bacterial inoculum was added to the plasma. After incubation for 4 to 6 hours, clot formation can be observed.

Oxidase test

It is a colorimetric test that allows determining the presence of oxidase enzyme, which reacts in the presence of cytochrome oxidase present in the reactive strips. The test was carried out as follows: A bacterial colony was taken and placed on the test strip to observe the reaction. It is considered positive when a blue or purple coloration is observed on the test strip.

Antimicrobial activity

The analysis of antimicrobial activity (disc diffusion method) was carried out as follows: A bacterial loop was taken from a previously incubated culture, this was suspended in 5 mL of sterile distilled water. The McFarland scale was used to adjust the suspension to a turbidity equivalent to 0.5. With the help of a sterile swab, the bacterial suspension was evenly spread over the entire surface of the agar plate.

Table 3: Identification profiles used in this research

Criteria	Positive (+)	Negative (-)
Catalase	+	
Oxidase		-
Mannitol fermentation	+	
Growth in saline medium	+	
Coagulase	+	
Hemolysis Pattern	Beta hemolysis	
Gram stain	Gram-positive cocci in the form of grape clusters	
Morphology	Endopigment " <i>Staphylococcus aureus</i> " Gold color	

Finally, cellulose filter paper discs impregnated with the antibiotics under study were placed on the agar surface. The plates were then sealed and incubated at 37 °C for 24 hours.

After incubation, the diameter of the inhibition zones was measured using a vernier caliper. The measurement was adjusted by subtracting 6 mm (the diameter of the disc) from the recorded value, and the result was recorded on the data sheet for further analysis. Within the methodological process, the antimicrobial

susceptibility of ampicillin and amoxicillin at the concentration of 2 µg was established according to the EUCAST [20] recommendations.

Prevalence analysis

To estimate the occurrence of previously obtained methicillin-resistant *Staphylococcus aureus*, the resistance and susceptibility data were used to calculate the prevalence rate (PT). The following equation model was employed to determine the result as a percentage [21]:

$$\text{Prevalence} = \frac{\text{Total cases in a population and at a given time}}{\text{Total population in that place and given time}} \times 100$$

This equation allows us to calculate the prevalence rate of methicillin-resistant *Staphylococcus aureus* based on the obtained data.

Results and Discussion

The *Staphylococcus aureus* isolates were taken from the strain collection of the general laboratory of the Faculty of Agricultural Sciences and Natural Resources of the State University of Bolivar, which were previously obtained from two provinces of Ecuador (Table 4).

After performing bacterial identification tests such as mannitol fermentation, growth in a saline medium, catalase reaction (+), oxidase reaction (-), coagulation of blood plasma, and Gram staining (Gram-positive cocci arranged in clusters

resembling bunches of grapes), it was determined that 100% of the isolates were confirmed as coagulase-positive *Staphylococcus aureus*.

According to Falla *et al.* [24], in their investigation of the prevalence of *Staphylococcus aureus* that causes bovine mastitis, they obtained 129 isolates out of 150 sampled cows, resulting in a prevalence of 80%. This finding differs from the results of previous investigations from which the strains were obtained, as they reported a lower proportion of *Staphylococcus aureus* as the cause of bovine mastitis.

Comparison of means of the treatments of 2 µg of Ampicillin and Amoxicillin versus *Staphylococcus aureus*

Table 4: Result of isolation and identification

Prevalence	<i>Staphylococcus aureus</i>
Pichincha, Mazón & Mazón [22]	29.42%
Chimborazo, Chávez (2022) [23]	68.96%

Table 5: Averages obtained from the analyzed treatments

Treatments	Mean	Tukey Grouping
2 (Amo 2 µg)	13.78	A
4 (Amp 2 µg)	13.12	B
1 (OX 1 µg)	11.82	C
6 (witness)	6.00	D

*Amo: amoxicillin, Amp: ampicillin, and Ox: oxacillin.

Table 6: Averages obtained from the analyzed treatments

Treatments	Mean	Tukey Grouping
3 (Amo 10µg)	27.30	A
5 (Amp 10µg)	27.28	A
1 (OX 1µg)	11.82	B
6 (witness)	6.00	C

*Amo: amoxicillin, Amp: ampicillin, and Ox: oxacillin.

Table 7: Normality tests of the inhibition halo obtained from concentrations of 2µg and 10µg of ampicillin against *Staphylococcus aureus*, which causes bovine mastitis

Normality tests							
Amp Concent.	Kolmogorov-Smirnov ^a			Shapiro-Wilk			
	Statistical	df	Sig.	Statistical	df	Sig.	*
2µg	0.149	50	0.007	0.944	50	0.020	NS
10 µg	0.128	50	0.040	0.960	50	0.093	NS

*Amp Concent: ampicillin concentration, df: degrees of freedom, a: Lilliefors significance correction, and NS: non-significant effect.

In the means comparison using the 5% Tukey test (Table 5), it was observed that T2 (2 µg of amoxicillin) had the highest average zone of inhibition of *Staphylococcus aureus* growth, significantly surpassing T4 (2 µg of ampicillin). There were statistic differences among the treatments according to the grouping of this comparative test. Treatment T1 (1 µg of oxacillin), which served as the positive control treatment, also showed a significant difference compared to the negative control (Disc impregnated with NaCl 0.9%), as no inhibition of bacterial growth was observed with the latter. These findings suggest that the treatment averages are statistically distinct from each other. Damasceno [25], in his research, isolated a total of 79 strains of *Staphylococcus aureus*, which accounted for 36.9% of the total isolates. The study demonstrated that among these isolates, 63.3% (n=50) were sensitive to 1 µg oxacillin, while 36.7% (n=29) showed resistance to this antibiotic. In the case of 10 µg amoxicillin, 65.8% (n=52) of the isolates were found to be sensitive, while 34.2% (n=27) exhibited resistance. Nevertheless, the EUCAST [20] recommendations

for antimicrobial susceptibility tests consider isolates sensitive to ampicillin and amoxicillin at 2 µg when they exhibit inhibition zones ≥ 15 mm in the antibiogram. Therefore, the findings of the present investigation are in disagreement with these recommendations, as the 50 strains of *Staphylococcus aureus* exhibited inhibition zones with average measurements of 13.78 mm and 13.12 mm for amoxicillin and ampicillin, respectively. However, the data distribution reveals the presence of isolates with larger inhibition zone measurements than the established averages. This suggests the existence of sensitive bacteria with inhibition zones greater than the thresholds established by the above-mentioned regulatory body.

Comparison of means of the concentration of 10 µg of amoxicillin and ampicillin versus *Staphylococcus aureus*

In the comparison of means determined by the 5% Tukey test (Table 6), it was observed that T3 (10 µg of amoxicillin) and T5 (10µg of ampicillin) did not express significant statistical differences,

They exhibited similar inhibition zones. T1 (1 µg of oxacillin), which served as the positive control treatment, showed significant differences from the negative control (disc impregnated with 0.9% NaCl), as no inhibition was observed in the latter. Khan *et al.* [26] in their investigation on antimicrobial susceptibility of beta-lactamase-producing *Staphylococcus aureus* isolated from milk positive for bovine mastitis reported that 53.3% (n=8/15) of the isolates showed phenotypic resistance to 10µg ampicillin, with halos ≤28 mm [27]. Mesquita *et al.* [28] obtained an average diameter of 18.53 mm for the inhibition zone of *Staphylococcus aureus* (ATCC 25923) against 10 µg amoxicillin. However, this finding disagrees with the cited investigations. It is important to note that the measurement of inhibition zones can vary due to various factors, including the conditions of antimicrobial handling in bovine mastitis, the underlying

factors contributing to intramammary colonization, and the potential cross-transmission of nosocomial pathogens from humans to animals, such as MRSA.

Analysis of the antimicrobial activity of ampicillin against Staphylococcus aureus, which causes bovine mastitis

According to the normality test performed on the concentrations of 2 µg and 10 µg of ampicillin on 50 strains of *Staphylococcus aureus* (Table 7), the Shapiro-Wilk test indicates that the inhibition zones follow a normal distribution. This suggests that the data is normally distributed, and there is no significant effect observed between repeated measurements.

Antimicrobial susceptibility of ampicillin against Staphylococcus aureus causes bovine mastitis

Table 8: Breakpoints of the zone of inhibition (mm) of penicillins against *Staphylococcus aureus* causes bovine mastitis

Standard	Inhibition halo diameter (mm)		
	R	I	S
CLSI (10 µg)	28	-	29
EUCAST (2 µg)	13	14	15

* R: resistant, I: intermediate, and S: sensitive.

Table 9: Normality test of the results obtained from antibiogram of oxacillin at 1 µg against *Staphylococcus aureus* causes bovine mastitis

Normality tests							
T1: OX1	Kolmogorov-Smirnov ^a			Shapiro-Wilk			*
	Statistical	Df	Sig.	Statistical	df	Sig.	
	,228	50	,000	,855	50	,000	**

*a: Lilliefors significance correction, OX1: oxacillin 1 µg, and **: highly significant effect.

Table 10: Antimicrobial susceptibility of oxacillin against *Staphylococcus aureus* causes bovine mastitis

Concentration	Resistance		Intermediate		Sensitive	
	Fr.	%	Fr.	%	Fr	%
1 µg	18	36	20	40	12	24

*Fr: frequency and %: percentage

According to CLSI [27], the following cutoff points are considered for all penicillins against *Staphylococcus aureus*: inhibition zones ≥ 29 mm are classified as sensitive, while inhibition zones ≤ 28 mm are classified as resistant. Similarly, EUCAST [20] recommends the use of 2 µg of ampicillin in disk diffusion method for *in vitro* studies, where inhibition zones ≤ 13 mm are

considered resistant and inhibition zones ≥ 15 mm are considered sensitive (Table 8).

Antimicrobial susceptibility of ampicillin against Staphylococcus aureus, which causes bovine mastitis

The antimicrobial susceptibility testing of *Staphylococcus aureus* with ampicillin at a concentration of 10 µg revealed that only 32% of the isolated strains (n=16) were classified as sensitive, while the remaining 68% (n=34) were classified as resistant based on the recommendations provided by CLSI [27]. Similarly, when analyzing the interaction of bacterial agent with ampicillin at a concentration of 2 µg, it was observed that 30% of the total isolates (n=15) exhibited resistance, while 60% (n=30) were classified as sensitive to this concentration. In addition, it was noted that 10% (n=5) showed intermediate resistance.

Jiménez *et al.* [9] in their investigation of resistance profiles by *Staphylococcus aureus* against 10 µg ampicillin, they obtained 81.5% sensitivity and 18.5% resistance, Sánchez *et al.* [29] of a total of 53 isolates, 60.3% (n=32) presented resistance to 10 µg ampicillin, this interaction being one of those with the highest resistance rates, Guzmán *et al.* [8] of 32 isolates observed that 86.6% presented resistance to 10 µg ampicillin, observing that when molecularly analyzing mentioned isolates, 100% presented the blaZ gene and 36.6% were positive for the mecA gene, and it is strongly linked to the phenotypic and genotypic presentation of resistance to penicillins and beta-lactams by the bacteria under study since it gives it the ability to generate beta-lactamases that inhibit the mechanism of action of beta-lactam drugs.

Analysis of the antimicrobial activity of amoxicillin against Staphylococcus aureus, which causes bovine mastitis

According to the normality test of the concentrations of 2 µg and 10 µg of amoxicillin against the strains of *Staphylococcus aureus* that cause bovine mastitis, the Shapiro-Wilk test indicates that the inhibition halos follow a normal distribution. Furthermore, the repetitions under study show no significant effect ($p>0.05$).

Antimicrobial susceptibility of amoxicillin against Staphylococcus aureus, which causes bovine mastitis

The CLSI [27] considers such cutoff points for all penicillins against *Staphylococcus aureus* as sensitive inhibition halos ≥ 29 mm and resistant inhibition halos ≤ 28 mm, as well as the EUCAST, [20] recommends the application of 2 µg of amoxicillin using the disk diffusion method for *in vitro* studies.

In this method, inhibition halos of ≤ 13 mm are considered resistant, while inhibition halos of ≥ 15 mm are considered sensitive.

Antimicrobial susceptibility of amoxicillin against Staphylococcus aureus causes bovine mastitis.

The antimicrobial susceptibility of *Staphylococcus aureus* in the interaction with amoxicillin at a concentration of 10 µg showed that only 40% of the isolated strains (n=20) were sensitive. However, the remaining 60% (n=30) were resistant according to the recommendations established by the CLSI [27].

When analyzing the interaction of bacterial agent under study with amoxicillin at a concentration of 2 µg, it was observed that, out of the total isolates analyzed, 32% (n=16) were resistant.

In addition, 50% (n=25) were manifested as resistant to this concentration. Furthermore, it was observed that 18% (n=9) of the isolates presented intermediate resistance.

These interpretative criteria were referenced to the provisions of the established guidelines. However, in the work carried out by Quispe *et al.* [30], where they isolated the bovine mastitis pathogen, 100% of the isolates were found to be sensitive to amoxicillin.

Analysis of the antimicrobial activity of oxacillin against Staphylococcus aureus causes bovine mastitis

According to the Shapiro-Wilk test, performed on 50 strains of *Staphylococcus aureus* (degrees of freedom) against 1 µg of oxacillin (Table 9 and 10), the distribution of the inhibition halo measurements was found to be non-normal. The test revealed a highly significant effect ($p<0.05$), indicating that the experimental units did not exhibit similar behavior in response to the inhibitory effect of the studied drug.

Antimicrobial susceptibility of oxacillin against *Staphylococcus aureus* causes bovine mastitis

The results obtained from the diameter of the inhibition halo were compared with the cutoff points used in the research by Farzaneh *et al.* [31] for *Staphylococcus aureus*, where the cut-off points for *S. aureus* is Sensitive 13 mm in halo diameter, intermediate 11 to 12 mm, and resistant 10 mm.

Table 11 indicates the susceptibility of *Staphylococcus aureus* to 1 µg of oxacillin, revealing that over 35% (n=18) of the isolates exhibited resistance to the drug. According to Gómez *et al.* [32], the emergence of oxacillin-resistant *Staphylococcus aureus* was observed in 1960. However, resistance is conferred by a modified penicillin-binding protein (PBP2a). This particular strain is recognized as a nosocomial pathogen primarily found in hospital settings and predominantly affecting humans. Hryniewicz and Garbaz [33] further explained that within oxacillin resistance, both homogeneous and heterogeneous phenotypic resistance can be observed. This resistance may be expressed by the entire bacterial population or only a portion, leading to the classification of *Staphylococcus aureus* as Bordeline (BROSA).

Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA)

For this analysis, the plate-disc diffusion method was applied with 1 µg oxacillin discs and 30 µg cefoxitin. The distribution of inhibition halos was then examined for the 50 strains studied in relation to these drugs.

According to the Shapiro-Wilk test, it was found that the distribution of inhibition halo

measurements with the use of 1 µg oxacillin was heterogeneous among the 50 strains studied. This indicates that the isolates varied in their response to the inhibitory effect of the drug.

On the other hand, the distribution of inhibition halo measurements with the use of 30µg cefoxitin discs was observed to be homogeneous, indicating that the 50 isolates exhibited a similar response to the inhibitory effect of this drug. The cutoff points established for cefoxitin (30µg) against *Staphylococcus aureus* are: 22 mm of susceptible halo and 21 are considered resistant. When analyzing the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) using the phenotypic analysis of resistance to 1 µg oxacillin and 30 µg cefoxitin, the CLSI parameters were used as a reference.

The *mecA* gene, which is associated with resistance to the mechanism of action of beta-lactam antibiotics, was considered in this analysis.

In a total of 50 strains of *Staphylococcus aureus*, it was found that 36% of the isolates were resistant to 1 µg oxacillin, 40% showed intermediate resistance, and the remaining 24% was sensitive to the drug.

In the case of cefoxitin with a concentration of 30 µg, 32% of the isolates were resistant, while 68% were sensitive to the drug.

Tesfaye *et al.* [34] conducted a study where they identified *Staphylococcus aureus* in 37 out of 121 milk samples with mastitis, accounting for 30.6% of the total isolates. Among these isolates, 12 (32.4%) were identified as methicillin-resistant *Staphylococcus aureus* (MRSA) strains. From this finding, they further observed that 5 strains exhibited resistance to two or more beta-lactam antibiotics.

Table 11: Normality tests for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) causing bovine mastitis

	Kolmogorov-Smirnov ^a			Shapiro-Wilk			*
	Statistical	df	Sig.	Statistical	df	Sig.	
Oxa1	0.228	50	0.000	0.855	50	0.000	**
Cefox30	0.167	50	0.001	0.965	50	0.139	NS

^aa: Lilliefors significance correction.

Similarly, Jiménez *et al.* [9] conducted a study on resistance profiles of *Staphylococcus aureus*, where they isolated 36 strains. They found that 92.3% of the isolates were sensitive and 7.7% were resistant to 1 µg oxacillin.

In addition, they observed that 95.4% of the isolates were sensitive to cefoxitin, while 4.6% exhibited resistance. These findings differ from the cited publications, as the present study reported a prevalence of 32% of methicillin-resistant *Staphylococcus aureus*.

Conclusion

In susceptibility tests of *Staphylococcus aureus*, it is recommended to use amoxicillin and ampicillin at a concentration of 10 µg, as the cutoff points for these drugs are calibrated at this concentration. In the present study, it was found that over 50% of the isolated *Staphylococcus aureus* strains exhibited resistance to this concentration. Similarly, when these two antibiotics were tested at a concentration of 2 µg, resistance was observed in 50% of the isolates included in the study. The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) was estimated to be 32% based on the antimicrobial activity analysis.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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References

- [1]. Filor V., Seeger B., De Buhr N., Von Köckritz-Blickwede M., Kietzmann M., Oltmanns H., Meibner J., Investigation of the pathophysiology of bacterial mastitis using precision-cut bovine udder slices, *Journal Dairy Science*, 2022, **105**:7705 [Crossref], [Google Scholar], [Publisher]
- [2]. Aguilar F., Álvarez C., Mastitis Bovina; Fisiología y Fisiopatología de la glandula mamaria; factores determinantes; agentes infecciosos, *Universidad Técnica De Machala*, 2019, 152 [Publisher]
- [3]. Vargas Hoyos K., Vidal Arboleda J., Olivera-Angel M., Validación de método cualitativo para detección de *Staphylococcus aureus* y *Streptococcus agalactiae* en muestras de leche para diagnóstico de mastitis bovina, *Revista U.D.C.A Actualidad & Divulgación Científica*, 2018, **21**:271 [Crossref], [Google Scholar], [Publisher]
- [4]. Pedroza A., Manejo técnico de mastitis y calidad de leche, *Sitio Argentino De Producción Animal*, 2018, 7 [Google Scholar], [Publisher]
- [5]. Gusmann M., Steeneveld W., Kirkeby C., Hogeveen H., Nielen M., Farre M., Halasa T., Economic and epidemiological impact of different intervention strategies for clinical contagious mastitis, *Journal of Dairy Science*, 2018, **102**:1483 [Crossref], [Google Scholar], [Publisher]
- [6]. Rojas Rodríguez C., Factores asociados con la mastitis subclínica bovina en fincas lecheras de

- Zipaquirá, Cundinamarca, *Tesis de grados, Universidad de Rosario*, 2017, 119 [[Crossref](#)], [[Publisher](#)]
- [7]. Lee A.S., De Lencastre H., Garau J., Kluytmans J., Malhotra-Kumar S., Peschel A., Harbarth S., Methicillin-Resistant *Staphylococcus Aureus*, *Nature Reviews Disease Primers*, 2018, 4 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)].
- [8]. Guzmán-Rodríguez J.J., Salinas-Pérez E., León-Galván F., Barboza-Corona J.E., Valencia-Posadas M., Ávila-Ramos F., Hernández-Marín J.A., Ramírez-Sáenz D., Gutiérrez-Chávez A.J., Relationship between antibiotic resistance and biofilm production of *Staphylococcus Aureus* isolates from bovine mastitis, *Revista Mexicana de Ciencias Pecuarias*, 2021, 12:1117 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [9]. Jiménez Velásquez S.D.C., Torres Higuera L.D., Parra Arango J.L., Rodríguez Bautista J.L., García Castro F.E., Patiño Burbano R.E., Perfil de resistencia antimicrobiana n asilamientos de *Staphylococcus* Spp obtenidos de leche bovina en Colombia, *Revista Argentina de Microbiología*, 2020, 52:121 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [10]. Yadav R., Kumar A., Singh V.K., Yadav S.K., Prevalence and antibiotyping of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in domestic animals in India, India: *Journal of Global Antimicrobial Resistance*, 2018, 15:222 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [11]. Duse A., Persson K., Pedersen K., Microbial Aetiology, antibiotic susceptibility and pathogen-specific risk factors for udder pathogens from clinical mastitis in dairy cows, (1er Ed.) *Animals*, 2021, 11:2113 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [12]. Boireau C., Cazeau G., Jarrige N., Calavas D., Madec J.Y., Leblond A., Haenni M., Gay É., Antimicrobial resistance in bacteria isolated from mastitis in dairy cattle in France, 2006–2016, *Journal of Dairy Science*, 2018, 101:9451 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [13]. Guo Y., Song G., Sun M., Wang J., Wang Y., Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus aureus*, *Frontiers in Cellular and Infection Microbiology*, 2020, 10:107 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [14]. Bui T., Preuss C.V., Cephalosporins, In: *StatPearls [Internet], Treasure Island (FL): StatPearls*, 2023 [[Google Scholar](#)], [[Publisher](#)]
- [15]. Rojas G., Vásquez Y., Rodríguez M., García P., Rojas T., Mecanismos de resistencia a antibióticos betalámicos en enterobacteriales aislados en hemocultivos, Maracay, Estado de Aragua, Venezuela, *Kasmera*, 2021, 49, 1 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [16]. Tomazi T., Veiga Dos Santos M., Antimicrobial use for treatment of clinical mastitis in dairy herds from Brazil and its association With Herd - Level Descriptors. *Preventive veterinary medicine*, 2020, 176:104937 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [17]. Espínola S., Piraino P., Alcaraz P., Mora D., Guía para el diagnóstico y tratamiento de pacientes que refieren alergia a betalactámicos, (F. P. Chemistry, Ed.) *Archivos de alergia e inmunología clínica*, 2020, 51:95 [[Google Scholar](#)], [[Publisher](#)]
- [18]. Taylor T.A., Unakal C.G., *Staphylococcus aureus* Infection, In: *StatPearls [Internet], Treasure Island (FL)*, 2022 [[Google Scholar](#)], [[Publisher](#)]
- [19]. Montoya García N., Acosta J.P., Bedolla C., Castañeda H., Cal L., Velázquez V., Mecanismos de defensa e inmunidad de la glándula mamaria en la vaca lechera, *Uaem – Fmvz*, 2019, 30 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [20]. EUCAST, Eucast European Committee on Antimicrobial Susceptibility Testing, *Obtenido De European Society of Clinical Microbiology and Infectious Diseases*, 2022 [[Publisher](#)]
- [21]. Cuenca-Condoy M., García-Bracho D., Reinoso-García L., González-Rojas J., Torracchi-Carrasco J., Detección de mastitis subclínica bovina y factores asociados en fincas lecheras de la provincia del Cañar-Biblián, Ecuador. *Review Científica Fcv-Luz*, 2021, 31:93 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [22]. Mazón J., Mazón D., Análisis de Resistencia y Susceptibilidad Antimicrobiana en bacterias aisladas de mastitis bovina en la Cooperativa Agropecuaria “La Colina”, Provincia de Pichincha (Ecuador), Guaranda, Bolívar, Ecuador, *Tesis de grado, Universidad Estatal de Bolívar*, 2022, 260 [[Publisher](#)]

- [23]. Chávez-Morales M., Evaluación in vitro de la actividad antimicrobiana del aceite esencial de tomillo rojo (*Thymus Zygis*) mediante la técnica de Kirby-Bauer en *Staphylococcus aureus* aislada de mastitis bovina, *Guaranda, Bolívar, Ecuador. Tesis de grado, Universidad Estatal de Bolívar*, 2022, 95 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [24]. Tapias S.F., Prevalencia de *staphylococcus aureus* en mastitis bovina. Municipio de Campoalegre – Huila, *Revista Agropecuaria Y Agroindutrial La Angostura*, 2020, 7:1 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [25]. Souza G.A.A.D., Carvalho C.M.C., Xavier E.D., Borges L.F.F., Gonçalves S.F., de Almeida A.C., *Staphylococcus Aureus* Resistente a metilicina e meropenem en leite de vacas con mastitis subclínica, *Brazilian Journal of Development*, 2020, 6:98067 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [26]. Khan A., Firyal S., Khan I., Awan A.R., Tayyab M., Wasim M., Shabbir M.Z., Ali M.M., Zohaib A., Phenotypic and genotypic characterization of beta-lactams resistant *Staphylococcus Aureus* isolates from bovine mastitis and its zoonotic implications, *Pakistan Veterinary Journal*, 2020, 40: 523 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [27]. CLSI Clinical & Laboratory Standards Institute, *Standars Staphylococcus Aureus*, 2019 [[Publisher](#)]
- [28]. Mesquita S., Araujo B., Dos Santos M., Lisboa K.A., De Albuquerque F., Carvalho T., Bossi A., Resistência de *Staphylococcus aureus* e *Escherichia coli* à Antibióticos, *Research, Society and Development*, 2022, 11:1 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [29]. Sánchez M., Arceo T., Sandoval G., Alva P.N., Jiménez R., Loeza P.D., Uso de nisina y quitosano para la inhibicion de *Staphylococcus aureus* resistente a antibióticos y asociado a mastitis bovina, *Revista Mexicana de Ciencias Pecuarias*, 2018, 9:792 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [30]. Quispe R., Peña G., Andía V., Resistencia antimicrobiana de *Síaphylococcus aureus* y *Sírepíococcus agalacíae* aislados de leche de vacas con mastitis, *Revista veterinaria*, 2021, 32:79 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [31]. Farzaneh F., Razavi M., Khamar T., Comparison of Antimicrobial Effect of *Pistacia atlantica* with Antibiotics in Urinary Tract Infection Zahedan Journal of Research in Medical Science, 2018 20:e63589 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [32]. Gómez L., Nuñez D., Perozo M., Bermúdez J., Marín M., *Staphylococcus Aureus* con resistencia múltiple a los antibióticos (Mdr) en un hospital de Maracaibo- Venezuela, *Kasmera*, 2016, 44:53 [[Google Scholar](#)], [[Publisher](#)]
- [33]. Hryniewicz, M., & Garbacz, K. (2017). *Staphylococcus Aureus* Resistente a la oxacilina límite (borsa): ¿un problema más común de lo esperado? *Journal of Medical Microbiology*, 66:1367 [[Crossref](#)], [[Publisher](#)]
- [34]. Tesfaye K., Gizaw Z., Feleke A., Prevalence of mastitis and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* in lactating dairy cows of selected dairy farms in and around Adama Town, Central Ethiopia. *Environmental Health Insights*, 2021, 15: 11786302211021297 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

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