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Prevalence and antibiotic resistance of *Staphylococcus aureus* in raw milk produced in Northern Jordan

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ABSTRACT

This study aimed to assess contamination of raw milk with *Staphylococcus aureus* and to measure Minimum Inhibitory Concentration (MIC) of antibiotics. Furthermore, it examined effect of region, month, and milk source on Bacterial Growth Rate (BGR) and Multidrug Resistance (MDR). Results show that sample distribution is affected by month, region, and milk source. E.g., the highest infection rate was recorded in March, with majority of samples concentrated in Qasabat Irbid. Furthermore, cow's milk dominated the samples, with majority of *S. aureus* growing. Meanwhile, distribution of MDR was not significant. Furthermore, region has a significant effect on BGR and MDR. The interaction of region and response reveals that effect of region differed depending on presence of MDR. Regarding laboratory results, MIC against *S. aureus* ranges from 1.00 for the most effective Azithromycin to 4.00 µg/mL for the least effective Fosfomycin. Furthermore, there are significant differences in the WHO classifications. Accordingly, antibiotics are ranked according to their importance and priority of use. CXM-AMC may top list as the most widely used and effective first-line drug. OT, DOX, and SXT follow, which are highly important. AMP and CEP are followed by GN, ENR, CIP, FFC, and FUR, with moderate importance. Finally, CEN, AZM, SPI, TY, N, CS, and FOS are of limited use in resistant cases. Therefore, the study recommends focusing on monitoring isolates of *S. aureus*. Antibiotics should be selected based on their MIC values. Therefore, priority should be given to using highly effective antibiotics as first-line, with less-needed reserved for resistant cases.

Introduction

Raw milk is one of the most widely consumed nutritious animal products and a major source of protein and essential nutrients (Ntuli *et al.*, 2022). However, its handling and consumption without appropriate heat treatment exposes consumers to significant health risks, particularly due to contamination with pathogenic microbes (Dash *et al.*, 2022). One of the most prominent of these microbes is *S. aureus*, a major cause of food poisoning and zoonoses (Ali and Alsayeqh, 2022). Furthermore, the severity of this contamination increases when *S. aureus* isolates develop resistance to multiple antibiotics, posing a challenge to food safety, the public health sector, and veterinary medicine alike (Nunes Nascimento *et al.*, 2025). This is particularly true in agricultural settings where antibiotics are widely used to treat dairy animals (Virto *et al.*, 2022). Hence, field studies are needed to assess the prevalence of *S. aureus*, its antibiotic resistance patterns, and its impact on food safety in locally produced raw milk.

Effective control of *S. aureus* in raw milk is critical to ensuring food safety and human and animal health, especially in light of the increasing prevalence of multidrug-resistant bacterial isolates. In this context, studies in Jordan indicate that agricultural, nutritional, and technological factors directly influence the spread of this bacterium, calling for the adoption of integrated strategies that include the rational use of antibiotics (Obeidat and Al-Najjar, 2025). Accordingly, enhancing probiotics in dairy products improves food quality and health (Al-Tarawneh and Al-Najjar, 2025). Furthermore, implementing smart agriculture techniques for early detection of infections reduces their spread (Al-Lataifeh *et al.*, 2024; Dayoub *et al.*, 2024). On the other hand, socioeconomic and environmental factors, including food insecurity (Abo Znemah *et al.*, 2023; Ayasrah *et al.*, 2025), poor veterinary services (Al-Barakeh *et al.*, 2024), and regional variations in infection severity (Roukbi *et al.*, 2016), play an important role in the spread dynamics of infection. Moreover, applied innovations, such as teat cup lining (Cascos *et al.*, 2024), agricultural credit grants (Tarawneh and Al-Najjar, 2023), and agricultural extension programs (Tarawneh *et al.*, 2022; Abu harb *et al.*, 2024), contribute to improving food safety. This is achieved by supporting infrastructure and increasing health awareness, which reduces bacterial threats in raw milk production chain and limits risk of spread of multidrug-resistant bacteria.

Despite widespread use of antibiotics in Jordanian livestock production farms, data on prevalence of *S. aureus* in raw milk and its multiple resistance patterns remain limited. This lack of information hampers development of clear policies to reduce the risk of resistant strains being transmitted to consumers via food chain. Furthermore, differences in regions and milk sources may contribute to variability in bacterial contamination levels and resistance patterns, suggesting a systematic study of these variables.

This research is of great importance, as it provides updated data on prevalence of *S. aureus* and its antibiotic resistance patterns in raw milk produced in northern Jordan. Furthermore, its findings contribute to strengthening food safety and control systems, supporting national policies aimed at rational use of antibiotics in veterinary sector, and reducing health risks associated with foodborne transmission of resistant strains. Furthermore, this research is important for decision-makers and regulatory bodies to improve biosafety procedures and manage common diseases.

This study aimed to assess extent of contamination of raw milk produced in Jordan with *S. aureus*. Furthermore, it sought to identify resistance patterns of this bacterium to various antibiotics, classify it according to World Health Organization standards, and determine its mechanisms of action. In addition, it measured minimum inhibitory concentration (MIC) of antibiotics used in veterinary medicine against isolates of this bacterium. Furthermore, it sought to study influence of regional factors, month, and milk source on bacterium growth rate and its resistance pattern to multiple antibiotics.

Materials and methods

Milk sample collection

To assess health risks associated with raw milk, 180 raw milk samples were aseptically collected from dairy animals (cows, sheep, and goats) from farms in six agricultural areas in Irbid Governorate, northern Jordan, between February and April 2024. Then the milk was tested for *S. aureus*. Additionally, the milk samples were stored at 4°C and transported to laboratory within two hours to ensure their microbiological integrity.

Isolation of Staphylococcus aureus

S. aureus bacteria were isolated from the samples by inoculating them into high-salt Trypsinized Soy Broth (TSB) with a high concentration of sodium chloride and incubating them at 37°C for 24 hours. Then they were transferred to Mannitol Salt Agar (MSA) and sheep blood agar to observe characteristic colonies. Typical colonies were collected and confirmed by Gram staining, catalase testing, and clotting tube testing. Additionally, a DNase activity test was performed to confirm the bacterial identity, as shown in Figure (1). This procedure is based on standards recommended by Clinical and Laboratory Standards Institute (CLSI) (Gaur *et al*., 2023) and ISO 6888-1:2021 for detection of *S. aureus* in food samples. Finally, the bacterial status of isolate (*S. aureus* growth or non-growth) (BGR) was determined.

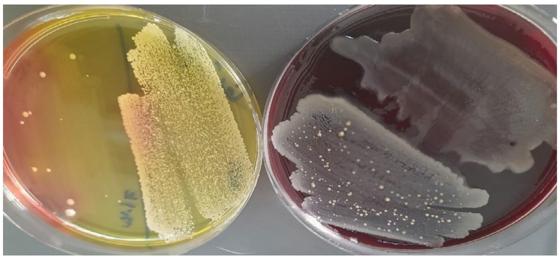

Figure 1. Shows growth of *S. aureus* on different culture media. E.g., blood agar is shown on the right, while Mannitol Salt Agar (MSA) appears yellow on the left. Thus, yellow *S. aureus* colonies are distinguished from S. epidermidis colonies on selective media.

Studied antibiotics

The study included 19 antibiotics (Cefuroxime = CXM, Amoxicillin = AMC, Gentamicin = GN, Enrofloxacin = ENR, Ciprofloxacin = CIP, Florfenicol = FFC, Colistin = CS, Ampicillin = AMP, Cephalothin = CEP, Neomycin = N, Oxytetracycline = OT, Trimethoprim = SXT, Doxycycline = DOX, Tylosin = TY, Cephalexin = CEN, Furazolidone = FUR, Azithromycin = AZM, Fosfomycin = FOS, Spiramycin = SPI). Therefore, veterinarians have used these antibiotics in field to treat animals infected with *S. aureus*.

First, laboratory classification of *S. aureus* isolates for multiple antibiotic resistance (MDR) was performed according to Papadopoulos *et al*. (2019), where an isolate was classified as multidrug resistant if it exhibited resistance to three or more antibiotics from different therapeutic classes. Next, MIC, µg/mL for each antibiotic against *S. aureus* was determined using the broth microdilution method according to Bio Laboratory Standards Institute (CLSI) recommendations. The antibiotic solution was prepared in Mueller-Hinton broth medium with a twofold dilution range (0.03–64 µg/mL). Next, a bacterial suspension prepared from fresh cultures was added and diluted to a final density of approximately 5×105 CFU/mL in each well of a 96-well microtiter plate, including growth, sterilization, and quality controls (ATCC 29213). The plates were incubated at 35.0±1.0°C for 16–20 hours, and MIC was determined as the lowest concentration at which no visible growth occurred. Furthermore, quality control results were compared to reference ranges to ensure analytical validity, according to CLSI standard (Gaur *et al*., 2023). Finally, to ensure measurement accuracy, antibiotic concentrations in the samples (µg/mL) were estimated using a standard method approved according to official protocols of the Association of Analytical Chemists (AOAC, 2019).

Antibiotic susceptibility of confirmed *S. aureus* isolates was assessed using standard disk diffusion method on Mueller-Hinton agar, according to Clinical Laboratory Standards Institute (CLSI) guidelines. Bacterial suspensions were then prepared from overnight cultures, adjusted to the McFarland 0.5 standard, and distributed onto MHA plates. Antibiotic discs were then added, and the plates were incubated at 37°C for 24 hours. Finally, the inhibition zones were measured and interpreted according to CLSI standards (Figure 2).

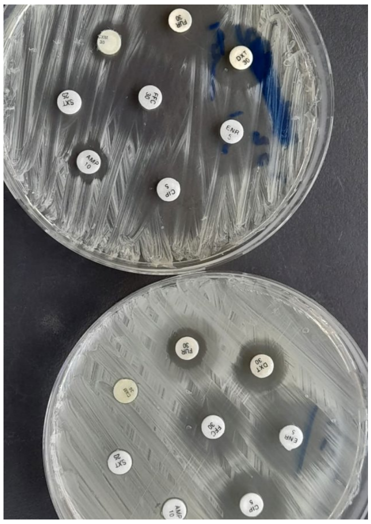

Figure 2. Shows sensitivity test of bacteria to different antibiotic discs on Mueller-Hinton medium, thus highlighting ability to distinguish between antibiotic-resistant and antibiotic-sensitive strains.

Furthermore, the antibiotic susceptibility of *S. aureus* isolates was classified using a binary system (BSS) based on MIC value according to CLSI standards (2023). Therefore, an isolate is classified as "susceptible" when (MIC) is ≤ 2 µg/mL, meaning it is sensitive to antibiotic at usual therapeutic concentrations. In contrast, it is classified as "non-susceptible" when the MIC is greater than 2 µg/mL, meaning it is resistant or requires concentrations higher than therapeutic limit to achieve inhibition.

Antibiotics were classified according to their mechanism of action (MMA) against *S. aureus*, based on their properties (Gaur *et al*., 2023). These antibiotics act by different mechanisms, including: (1) targeting bacterial cell wall, (2) inhibiting protein synthesis, and (3) interfering with DNA synthesis, leading to inhibition of bacterial cell growth. Additionally, the studied antibiotics (WCU) were classified into three categories according to World Health Organization (WHO, 2023) recommendations: (1) commonly used (Access), routinely used to treat *S. aureus* as a first-line treatment option; (2) intermediate use (Watch), used when resistance is present in first-line treatment; and (3) non-preferred use (Reserve), limited to multi-resistant cases. Finally, to measure rate of field antibiotic use (FCU), they were classified into three main categories: high (≥7%), intermediate (3%), and low (<3%).

Statistical analyses

A statistical analysis was conducted using SAS (2012) to estimate differences in relative distributions of various categories of variables studied. These included sample collection months, regions, milk source, *S. aureus* growth rate, and multiple antibiotic resistance (MDR) pattern. The chi-square test and P values were calculated to determine significance at each level. Furthermore, the Maximum Likelihood method was applied to estimate influence of main sources of variance (month, region, milk source, response interaction, and two-way interactions). In addition, MIC, µg/mL of antibiotics were estimated according to *S. aureus* susceptibility, mechanism of action, WHO classification of antibiotics, and local usage levels. Then means and standard errors were estimated for each one. Analysis of variance was used to test for differences between means, with Duncan (1955) post hoc test applied to determine significance. In addition, Antibiotics were classified based on their importance and priority of use, according to WHO guidelines and local usage standards. Finally, they were classified based on their therapeutic use (first-line, second-line, or reserve) and field use rate (high, intermediate, or low). These were based on a review of scientific literature, local veterinary regulations, and analysis of sensitivity data and field use rates included in the study.

Results

Distribution of demographic and biological variables

Table 1 shows distribution of variables in study sample. Significant differences were found in distribution of samples by month, with the highest percentage recorded in March, and by region, with a high concentration in Qasabat Irbid, and milk samples, where cow's milk predominated. Furthermore, concerning bacterial growth rate (BGR), majority of samples showed growth of *S. aureus*, confirming importance of these variables in shaping samples. In contrast, multidrug resistance (MDR) variable, despite its high percentage, did not show significance, meaning that sample distribution was not affected by it.

Factors affecting BGR and MDR in raw milk

Table 2 shows that regions had a significant effect on both BGR and MDR, indicating importance of location in determining bacterial characteristics. Furthermore, relationship between animals from which milk samples were taken and BGR was significant, indicating that milk sample source significantly determined the bacteria's ability to grow, while this source did not affect MDR. Furthermore, interaction between region and response (MDR) was significant, indicating that effect of region varied depending on presence or absence of bacterial MDR. In contrast, month or milk source variables alone had no significant effect on bacterial characteristics, nor did most other interactions between variables.

Minimum inhibitory concentration (MIC) of antibiotics against S. aureus

Figure 3 illustrates variation in MIC µg/mL for a range of tested antibiotics. Specifically, the bars reflect relative strength of each antibiotic, with lower MIC values indicating greater efficacy. MIC values range from

Table 1. Distribution of demographic and biological variables of the sample.

Variables	Categories	Percent	Chi-Square	Pr > ChiSq
Months	February	30	8.4	0.02
	March	43.33		
	April	26.67		
Regions	Qasabat Irbid	55	197.86	0.01
	Bani Kinanah	14.44		
	Ar-Ramtha	2.78		
	Al-Wasatiyah	8.89		
	Al-Mazar Al-Shamali	9.44		
	Al-Kourah	9.44		
Milk source	Cow	65.56	93.73	0.01
	Goat	7.78		
	Sheep	26.67		
Bacterial growth rate	No *S. aureus*	26.11	41.08	0.01
	S. aureus	73.89		
Multidrug resistance	MDR	54.89	1.27	0.25
	Non-MDR	45.11		

Table 2. Maximum Likelihood Analysis for factors influencing BGR and MDR

Maximum Likelihood Analysis of Variance				
Traits	Bacterial Growth Rate (BGR)		Multidrug Resistance (MDR)	
Source of Variance	Chi-Square	Pr > ChiSq	Chi-Square	Pr > ChiSq
Months	1.52	0.46	2.66	0.26
Regions	16.57	0.01	12.13	0.03
Milk source (Animals)	1.24	0.53	3.61	0.16
Response (BGR / MDR)	0.02	0.95	3.87	0.06
Month × Res	2.4	0.3	3.53	0.17
Region × Res	7.47	0.11	8.33	0.03
Milk source × Res	16.52	0.01	0.11	0.94
Month × Region	9.13	0.24	11.59	0.07
Month × Milk source	4.01	0.4	0.51	0.91
Region × Milk source	3.23	0.66	1.72	0.63
Likelihood Ratio	6.32	0.27	1.34	0.51

Table 3. Means and standard error of antibiotic concentrations (MIC, µg/mL) by sensitivity, mechanism of action, and classifications of use.

Variables	Categories	Mean	SE	Pr > F
Overall mean		2.13	0.14	
S. aureus sensitivity	Sensitive	1.65[a]	0.12	0.02
	Insensitive	2.49[b]	0.17	
Mechanism of antibiotic action	Targeting the cell wall	2.36[a]	0.31	0.75
	Inhibiting protein synthesis	1.95[a]	0.12	
	Interfering with DNA formation	2.17[a]	0.46	
WHO classification of antibiotics	Commonly used	2.00[b]	0.14	0.03
	Intermediate use	2.02[b]	0.17	
	Reserve	3.13[a]	0.87	
Local use of antibiotics	High	1.82[a]	0.15	0.42
	Medium	1.982[a]	0.09	
	Low	2.402[a]	0.26	

Table 4. Classification of antibiotics according to level of importance and priority of use.

Antibiotics	Importance level	Priority of use
CXM – AMC	Highest	First line/commonly used
OT – DOX – SXT	High	First line but partial resistance
GN – ENR – CIP – FFC – FUR	Intermediate	Second-line/ medium rate use
AMP – CEP	Less	low usage
CEN – AZM – SPI – TY – N – CS – FOS	Low	Reserve + Low rate

1.00 µg/mL for Azithromycin, the most, to 4.00 µg/mL for Fosfomycin, the weakest effective of the antibiotics studied. In general, the most effective antibiotics, such as Enrofloxacin and Cefuroxime, require lower concentrations, while the least effective antibiotics, such as Neomycin and Cephalexin, require much higher concentrations to inhibit microbial growth.

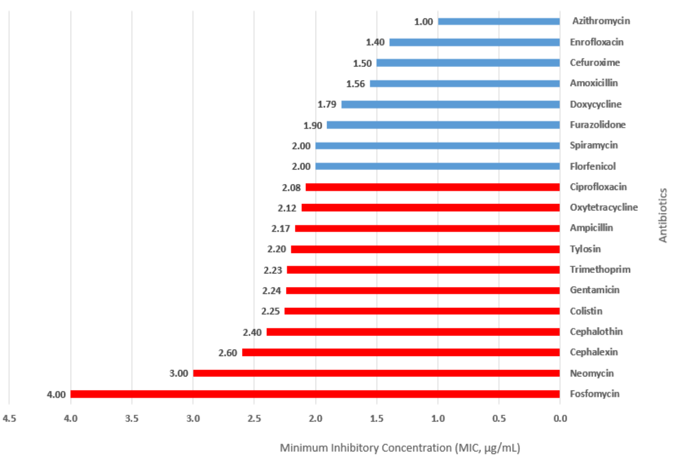

Figure 3. Mean inhibitory concentrations (µg/mL) of the antibiotics studied against bacterial isolates (*S. aureus*).

Effect of bacterial sensitivity and antibiotic classification

Table 3 shows significant differences in average (MIC) of antibiotics based on *S. aureus* susceptibility. These concentrations are significantly higher for non-susceptible antibiotics than for susceptible ones. Furthermore, the WHO classification of antibiotics shows significant differences. The MICs were significantly higher for reserve antibiotics than for commonly used or intermediate antibiotics. In contrast, comparisons based on the mechanism of action of antibiotics (cell wall targeting, protein inhibition, or interference with DNA synthesis), and local use classifications both show no significant differences in MICs.

Classification of antibiotics in veterinary field

Table 4 classifies antibiotics based on their level of importance and priority for veterinary use. First, this classification is topped by a group of the highest importance, such as CXM-AMC, which are commonly used first-line drugs with a high rate of usage. In addition, they are effective against common infections such as staphylococci, highly sensitive, and widely available.

Next, a group of high importance includes OT, DOX, and SXT. Despite their widespread use, these drugs are of low importance due to partial resistance or moderate use. A group of moderate importance, including GN, ENR, CIP, FFC, and FUR, is placed at the third level. These are considered second-line antibiotics or alternatives commonly used in cases of first-line failure or in special therapeutic situations.

Less important drugs, such as AMP and CEP, although classified as commonly used, their low local use reduces their field priority. Finally, there is a group of less important drugs (CEN, AZM, SPI, TY, N, CS, and FOS), which include drugs with less topical use, and in addition, some of them are reserved and usually reserved for the treatment of complex or resistant cases.

Discussion

Data presented in Table 1, the results reveal clear patterns in distribution of demographic and biological variables that reflect influence of temporal and regional factors and milk source on prevalence of the studied bacteria (Acharya *et al*., 2021). Furthermore, the highest percentage of sample collection occurred during March (43.3%) compared to February (30.0%) and April (26.7%) (p=0.02), reflecting seasonal influence on milk production and availability, as well as environmental conditions favorable to bacterial growth, such as temperature and humidity (Bokharaeian *et al*., 2022). In addition, the highest concentration of samples was found in Qasabat Irbid (55.0%) compared to other regions (p=0.01), which may indicate a concentration of livestock production activities or a larger farm size in this area. This is consistent with studies that emphasize importance of regional distribution in determining risk of microbial contamination

(Karanth *et al*., 2023).

In addition, dominance of cow's milk (65.6%) compared to sheep's milk (26.7%) and goat's milk (7.8%), with p=0.01, is indicative of prevailing pattern of animal production and reflects potential for exposure to wider sources of contamination due to larger supply chains (Nyokabi *et al*., 2020). Regarding microbial results, the vast majority of samples showed *S. aureus* growth (73.9%) compared to no growth (26.1%) (p=0.01), reinforcing the importance of this microbe as an indicator of health risks and thus underscoring need to intensify veterinary and health control programs for raw dairy products (Ntuli *et al*., 2022).

As for MDR, despite its high rate (54.9% vs. 45.1%), it did not show significant differences (p=0.25). This may reflect the homogeneity of this phenomenon between sites or its association with other factors not included in current study, such as differences in husbandry methods or treatment practices (Caneschi *et al*., 2023). Therefore, this calls for more in-depth future studies to understand determinants and mechanisms of spread of MDR in context of animal production and local ecosystems, considering environmental, social, and economic variables that may influence this pattern (Pepi and Focardi, 2020).

Results in Table 2, indicate that regional factors play a pivotal role in determining bacterial characteristics, with effect of region clearly evident on both BGR and MDR, with p=0.01 and p=0.03, respectively. This supports previous studies indicating importance of spatial distribution in explaining microbial variations and drug resistance patterns (Yudhanto and Varga, 2023). Furthermore, relationship between milk source (animal species) and BGR was found to be significant (p=0.01), indicating that source of the milk sample contributes to determining growth capacity of bacteria. However, the source of milk did not show a significant effect on MDR (p=0.94). This may reflect different patterns of antibiotic use or differences in veterinary practices across animal species (Caneschi *et al*., 2023).

In addition, interaction between region and response revealed significance for MDR, p=0.03, indicating that effect of region factors on multiple resistance may vary depending on presence or absence of MDR. This reflects potential presence of region foci with distinct resistance characteristics (Pereyre and Tardy, 2021). In contrast, results show that months had no significant effect on bacterial characteristics of BGR (p=0.46) or MDR (p=0.26), and that animal species (milk source) had no significant effects on BGR (p=0.53) or MDR (p=0.16). This suggests that temporal effect is less important compared to spatial or qualitative factors (Su and Li, 2025). Finally, most other interactions between variables did not achieve significance, reinforcing that region factors and animal species are the more influential factors in bacterial behavior and spread of drug resistance than time (Caneschi *et al*., 2023).

Figure 3 indicates a significant difference in MIC between different antibiotics against *S. aureus* isolates, thus reflecting variability in their effectiveness against the studied bacteria (FeBler *et al*., 2023). In other words, the lower MIC value, the more effective antibiotic inhibiting microbial growth. For example, values range from 1.00 µg/ml for Azithromycin, which is the most effective, to 4.00 µg/ml for Fosfomycin, which is the least effective. Furthermore, this is consistent with previous reports on variability in ability of antibiotics to penetrate bacterial cells, inhibit protein synthesis, or interfere with DNA (Tungare *et al*., 2025).

In addition, results show that the most effective antibiotics, such as Enrofloxacin and Cefuroxime, require relatively low concentrations (1.20–1.40 µg/ml) to achieve inhibition, while less effective antibiotics, such as Neomycin and Cephalexin, require higher concentrations, ranging from 3.20 to 3.80 µg/ml. This underscores importance of variation in bacterial susceptibility patterns and dose required to achieve therapeutic efficacy (Kim *et al*., 2024).

This pattern therefore promotes selection of appropriate antibiotics based on actual MIC values rather than traditional classification or prevalence rates, as some reserved or less frequently used antibiotics may require much higher concentrations to inhibit growth. Finally, this calls for a re-evaluation of field use protocols and development of more precise strategies to rationalize antibiotics and improve their efficacy against *S. aureus* isolates.

Data from Table 3, showed that MIC of antibiotics varies significantly according to *S. aureus* susceptibility and WHO classifications. This highlights importance of these factors in determining bacterial resistance levels. Overall mean was 2.13±0.14 µg/ml for all samples (n=19), with higher concentrations recorded for non-susceptible samples (2.49±0.17 µg/ml) compared to susceptible samples (1.65±0.12 µg/ml), with significance (p=0.02). Furthermore, this is consistent with studies indicating that resistant bacteria require concentrations higher than MIC to achieve inhibition (FeBler *et al*., 2023).

Moreover, WHO classification shows significant differences (p=0.03), with reserve antibiotics recording the highest mean MIC of 3.13±0.87 µg/ml, compared to 2.00±0.14 µg/ml for commonly used antibiotics, or 2.02±0.17 µg/ml for moderate use. This reflects the increasing need for higher concentrations when treating more conservative antibiotics or those intended for resistant cases (Ruzante *et al*., 2022).

In contrast, comparisons based on mechanism of action of antibiotics do not show significant differences (p=0.75), with similar mean MICs for cell wall inhibitors (2.36±0.31 µg/ml), protein synthesis inhibitors (1.95±0.12 µg/ml), and DNA synthesis inhibitors (2.17±0.46 µg/ml). This suggests that mechanism of action may have a lesser impact on MICs than susceptibility factors or global classification (Caneschi *et al*., 2023).

Furthermore, local use classifications showed no differences (p=0.42), with means ranging from 1.82±0.15 µg/ml for high use, 1.98±0.09 µg/ml for medium use, and 2.40±0.26 µg/ml for low use. This suggests that bacterial susceptibility and global antibiotic classification are the more influential factors in determining MICs than mechanism of action or pattern of topical use.

Data in Table 4 highlighted a clear classification of antibiotics according to their importance and priority of use, reflecting field practices and regulatory considerations for antibiotic rationalization. Group 1, with the greatest importance (CXM-AMC), is classified as the most frequently used first-line drug, representing more than 60% of all veterinary prescriptions (Tarillion *et al*., 2025). Consequently, its high efficacy against staphylococci, high sensitivity, and widespread availability explain why veterinarians continue to rely on it as a primary treatment option.

Furthermore, high-risk group (OT-DOX-SXT) follows, representing commonly used drugs with a partial resistance rate of 25–30% and an average usage rate of approximately 40% of total field use (Kim *et al*., 2024). Similarly, this reflects emergence of resistance pressures in this group due to frequent use.

At the third level is the intermediate-risk group (GN-ENR-CIP-FFC-FUR), which is considered second-line drugs or common therapeutic alternatives, with a usage rate of no more than 20-25% of all cases and is often reserved for cases that do not respond to first-line treatment (Tarillion *et al*., 2025). This, in turn, reflects a gradual strategy in veterinary antibiotic prescribing to limit the spread of resistance.

As for AMP-CEP drugs, they are considered less important due to their relatively low field use (less than 15%), which reduces their priority in treatment programs (Craven *et al*., 2022). This reflects changing microbial patterns or a shift to the more effective alternatives.

Finally, the low-risk group (CEN-AZM-SPI-TY-N-CS-FOS) stands out, with a usage rate of no more than 5-10%. In this context, many of these drugs are classified as reserve drugs, reserved for complicated or resistant cases (Morris *et al*., 2017). This underscores importance of carefully managing this group to maintain its long-term effectiveness and prevent spread of antimicrobial resistance.

Overall, this classification pattern reflects diversity of veterinary practices in Jordan and highlights need to adopt clear policies for rational use, in line with recommendations of World Health Organization and regulatory bodies, to ensure sustainable effectiveness of antibiotics in veterinary field.

Results indicate that prevalence of *S. aureus* in raw milk is primarily influenced by regional factors and milk source rather than temporal factors, with potential spatial foci of resistance. Furthermore, they revealed significant differences in minimum inhibitory limit (MIC) values among antibiotics, with bacterial susceptibility and WHO classifications playing a pivotal role in determining their effectiveness. This highlights importance of relying on actual measurements and re-evaluating field use protocols. Consequently, these data confirm that antibiotic prioritization is a fundamental tool for rationalizing veterinary practice, requiring strict regulatory policies to ensure rational use and maintain their effectiveness in face of increasing resistance.

Conclusion

Effective control of microbial risks and antibiotic resistance in raw milk requires an integrated approach that combines an understanding of regional, seasonal, and milk source factors. Relying on actual MIC measurements, bacterial susceptibility, and WHO classifications when selecting antibiotics is essential. Therefore, strict regulatory policies must be implemented to rationalize field use and direct veterinary interventions to the most important foci and sources to ensure sustainable treatment effectiveness and limit spread of resistance.

Conflict of interest

The authors have no conflict of interest to declare.

References

Abo Znemah, S., Tarawneh, R., Shnaigat, S., Al-Najjar, K., 2023. Estimating Some Indicators of Food Security for Rural Households. Asian Journal of Agriculture and Food Sciences. Volume 11 – Issue 1. https://doi.org/10.24203/ajafs.v11i2.7171

Abu harb, S., Dayoub, M., Al-Najjar, K., 2024. The impact of agricultural extension program effectiveness on sustainable farming: A survey article. International Journal of Agricultural Technology 20,477-492. http://www.ijat-aatsea.com/

Acharya, K.R., Brankston, G., Slavic, D., Greer, A.L., 2021. Spatio-Temporal Variation in the Prevalence of Major Mastitis Pathogens Isolated From Bovine Milk Samples Between 2008 and 2017 in Ontario, Canada. Frontiers in Veterinary Science 8, 742696. https://doi.org/10.3389/fvets.2021.742696

Al-Barakeh, F., Khashroum, A.O., Tarawneh, R.A., Al-Lataifeh, F.A., Al-Yacoub, A.N., Dayoub, M., Al-Najjar, K., 2024. Sustainable Sheep and Goat Farming in Arid Regions of Jordan. Ruminants 4, 241-255. https://doi.org/10.3390/ruminants4020017

Ali, S., Alsayeqh, A.F., 2022. Review of major meat-borne zoonotic bacterial pathogens. Frontiers in Public Health 10, 1045599. https://doi.org/10.3389/fpubh.2022.1045599

Al-Lataifeh, F.A., Tarawneh, R., Al-taha'at, E.S., Dayoub, M., Sutinen, E., Al-Najjar, K., 2024. Smart Technologies for Livestock Sustainability and Overcoming Challenges: A Review. Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/ Journal of Xi'an Shiyou University, Natural Sciences Edition. (67, 09, 196-204). Zenodo. https://doi.org/10.5281/zenodo.13744058

Al-Tarawneh, N.H., Al-Najjar, K., 2025. Enhancing the potential of probiotic bacteria to improve labneh's nutritional value. Journal of Tianjin University Science and Technology 58, 12-28. https://doi.org/10.5281/zenodo.14630684

AOAC, 2019. Association of Official Analytical Chemists. Official methods of analysis of AOAC International (21st Ed.). AOAC International.

Ayasrah, A.I.I., Shnaigat, S.F., Obeidat, H.M., Al-Najjar, K., 2025. Demographic, Health, and Socioeconomic Determinants of Food Insecurity and its Association with Osteoporosis. Journal of Tianjin University Science and Technology 58. https://doi.org/10.5281/zenodo.15123550

Bokharaeian, M., Toghdory, A., Ghoorchi, T., Ghassemi Nejad, J., Esfahani, I.J., 2022. Quantitative Associations between Season, Month, and Temperature-Humidity Index with Milk Yield, Composition, Somatic Cell Counts, and Microbial Load: A Comprehensive Study across Ten Dairy Farms over an Annual Cycle. Animals 13, 3205. https://doi.org/10.3390/ani13203205

Caneschi, A., Bardhi, A., Barbarossa, A., Zaghini, A., 2023. The Use of Antibiotics and Antimicrobial Resistance in Veterinary Medicine, a Complex Phenomenon: A Narrative Review. Antibiotics 12, 487. https://doi.org/10.3390/antibiotics12030487

Craven, A.J., Pegram, C.A., Packer, R.M., Jarvis, S., McGreevy, P.D., Warnes, C., Church, D.B., Brodbelt, D.C., 2022. Veterinary drug therapies used for undesirable behaviours in UK dogs under primary veterinary care. PLOS ONE 17, e0261139. https://doi.org/10.1371/journal.pone.0261139

Dash, K.K., Fayaz, U., Dar, A.H., Shams, R., Manzoor, S., Sundarsingh, A., Deka, P., Khan, S.A., 2022. A comprehensive review on heat treatments and related impact on the quality and microbial safety of milk and milk-based products. Food Chemistry Advances 1, 100041. https://doi.org/10.1016/j. focha.2022.100041

Dayoub, M., Shnaigat, S., Tarawneh, R.A., Al-Yacoub, A.N., Al-Barakeh, F., Al-Najjar, K., 2024. Enhancing Animal Production through Smart Agriculture: Possibilities, Hurdles, Resolutions, and Advantages. Ruminants 4, 22-46. https://doi.org/10.3390/ruminants 4010003

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11, 1-42. http://176.9.41.242/doc/statistics/1955-duncan.pdf

FeBler, A.T., Wang, Y., Burbick, C.R., *et al.*, 2023. Antimicrobial susceptibility testing in veterinary medicine: performance, interpretation of results, best practices and pitfalls. One Health Adv. 1, 26. https://doi.org/10.1186/s44280-023-00024-w

Gaur, P., Hada, V., Rath, R.S., *et al.*, 2023 Interpretation of Antimicrobial Susceptibility Testing Using European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) Breakpoints: Analysis of Agreement. Cureus 15, e36977. https://doi.org/10.7759/cureus.36977

Karanth, S., Feng, S., Patra, D., Pradhan, A.K., 2023. Linking microbial contamination to food spoilage and food waste: The role of smart packaging, spoilage risk assessments, and date labeling. Frontiers in Microbiology 14, 1198124. https://doi.org/10.3389/ fmicb.2023.1198124

Kim, S., Kim, H., Kim, J., Min, K., 2024. Assessment of Antimicrobial Use for Companion Animals in South Korea: Developing Defined Daily Doses and Investigating Veterinarians' Perception of AMR. Animals 15, 260. https://doi.org/10.3390/ani15020260

Morris, D.O., Loeffler, A., Davis, M.F., Guardabassi, L., Weese, J.S., 2017. Recommendations for approaches to meticillin-resistant staphylococcal infections of small animals: Diagnosis, therapeutic considerations and preventative measures. Veterinary Dermatology 28, 304-e69. https://doi.org/10.1111/vde.12444

Ntuli, V., Sibanda, T., Elegbeleye, J.A., Mugadza, D.T., Seifu, E., Buys, E.M., 2022. Dairy production: Microbial safety of raw milk and processed milk products. Present Knowledge in Food Safety. pp. 439-454. https://doi.org/10.1016/B978-0-12-819470-6.00076-7

Nunes Nascimento, J.C., Alvarenga, V.O., Paulino, B.N., de Cerqueira E., Silva, A.B., Matos, J.R., Rosário, I.L. dos S., Costa, M., 2025. *Staphylococcus aureus* in food safety: antimicrobial resistance, detection technologies, and future perspectives. Critical Reviews in Food Science and Nutrition 1–24. https://doi.org/10.1080/10408398.2025.2528165

Nyokabi, S.N., De Boer, I.J., Luning, P.A., Korir, L., Lindahl, J., Bett, B., Oosting, S.J., 2020. Milk quality along dairy farming systems and associated value chains in Kenya: An analysis of composition, contamination and adulteration. Food Control 119, 107482. https://doi.org/10.1016/j.foodcont.2020.107482

Obeidat, J., Al-Najjar, K., 2025. Integrating Biomedical and Sustainable Strategies for Effective Parasite Control in Livestock: A Review. Hunan Daxue Xuebao/Journal of Hunan University Natural Sciences 62, 98-105. https://doi.org/10.5281/zenodo.15259217

Papadopoulos, P., Papadopoulos, T., Angelidis, A.S., Kotzamanidis, C., Zdragas, A., Papa, A., Filioussis, G., Sergelidis, D., 2019. Prevalence, antimicrobial susceptibility and characterization of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* isolated from dairy industries in north-central and north-eastern Greece. International Journal of Food Microbiology 291, 35-41. https://doi.org/10.1016/j.ijfoodmicro.2018.11.007

Pepi, M., Focardi, S., 2020. Antibiotic-Resistant Bacteria in Aquaculture and Climate Change: A Challenge for Health in the Mediterranean Area. International Journal of Environmental Research and Public Health 18, 5723. https://doi.org/10.3390/ijerph18115723

Pereyre, S., Tardy, F., 2021. Integrating the Human and Animal Sides of Mycoplasmas Resistance to Antimicrobials. Antibiotics 10, 1216. https://doi.org/10.3390/ antibiotics10101216

Roukbi, M., Al-Omar, A.N., Al-Najjar, K., Salam, Z., Al-Suleiman, H., Mourii, M., Gourie, S., 2016. Seroprevalence of antibodies to Chlamydophila abortus in Small Ruminants in Some Provinces in Syria. Net Journal of Agriculture Science 4, 29-34. https://www.netjournals.org/pdf/NJAS/2016/2/16-019.pdf

Ruzante, J.M., Harris, B., Plummer, P., Raineri, R.R., Loy, J.D., Jacob, M., Sahin, O., Kreuder, A.J., 2022. Surveillance of antimicrobial resistance in veterinary medicine in the United States: Current efforts, challenges, and opportunities. Frontiers in Veterinary Science 9, 1068406. https://doi.org/10.3389/fvets.2022.1068406

SAS, 2012. Institute Inc.: SAS/STAT User's Guide: Version 9.1, SAS Institute Inc., and Cary, NC, USA. https://www.sas.com/en_us/software/stat.html

Su, T., Li, C., 2025. Spatial-temporal characteristics and influence factors of high-quality development of animal husbandry industry in China. PLOS ONE 20, e0313906. https://doi.org/10.1371/journal.pone.0313906

Tarawneh, R.A., Al-Najjar, K., 2023. Assessing the Impacts of Agricultural Loans on Agricultural Sustainability in Jordan. IOSR Journal of Economics and Finance (IOSR-JEF) 14, 49-56. https://doi.org/10.9790/5933-1406014956

Tarawneh, R.A., Tarawneh, M.S., Al-Najjar, K., 2022. Agricultural Policies among Advisory and Cooperative Indicators in Jordan. International Journal of Research-GRANTHAALAYAH 10, 10–17. https://doi.org/ 10.29121/ granthaalayah. v10.i2.2022.4489

Tarillion, M., Hertzsch, R., Richter, A., 2025. Off-Label Use of Antibiotics in Small Animal Medicine—A Germany-Wide Anonymous Online Survey. Antibiotics 14, 419. https://doi.org/10.3390/antibiotics14040419

Tungare, K., Gurav, P., De, A., Kadam, A., Bhori, M., Garse, S., 2025. Combination of Antimicrobial Drugs with Nanocarrier Drug Delivery System for Veterinary Therapeutics. In: Lahiri, D., Nag, M., Bhattacharya, D., Ray, R.R. (eds) Biofilm Associated Livestock Diseases and their Management. Livestock Diseases and Management. Springer, Singapore. https://doi.org/10.1007/978-981-96-1885-9_11

Virto, M., Amores, G., Hernández, I., 2022. Antibiotics in Dairy Production: Where Is the Problem? Dairy, 3(3), 541-564. https://doi.org/10.3390/dairy3030039

WHO, 2023. Paediatric drug optimization for antibiotics: meeting report, 30 November, 5-7 December 2022. World Health Organization. https://books.google.co.uk/books

Yudhanto, S., Varga, C., 2023. Knowledge and Attitudes of Small Animal Veterinarians on Antimicrobial Use Practices Impacting the Selection of Antimicrobial Resistance in Dogs and Cats in Illinois, United States: A Spatial Epidemiological Approach. Antibiotics 12, 542. https://doi.org/10.3390/antibiotics12030542