



## Research Article

# *Staphylococcus aureus* from Camel is Becoming Unleaded Drug-resistant Pathogen

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## Abstract

Mastitis poses a significant threat to camel milk production, compromising both quality and yield, particularly due to multidrug-resistant (MDR) *Staphylococcus aureus*. This study aimed to isolate and characterize *S. aureus* from mastitis-infected camels in the Cholistan and Suleiman ranges of Pakistan, evaluate their antibiotic susceptibility profiles, and develop potential treatment strategies. A total of 25% of isolates were MDR, with higher resistance observed in the Cholistan region. Susceptibility testing revealed alarming resistance patterns, particularly against oxacillin, ciprofloxacin, gentamicin, and trimethoprim. In vitro synergy assays indicated that combinations such as gentamicin + ampicillin and cefotaxime + ampicillin were the most effective. Field trials confirmed these findings, with the highest recovery rates observed within 3–5 days when using these combinations. These findings underscore the urgency of integrated mastitis management through targeted antibiotic use and vaccination strategies to preserve the therapeutic and commercial value of camel milk.

**Keywords:** Camel mastitis, *Staphylococcus aureus*, antimicrobial resistance (AMR), antibiotic susceptibility, drug resistance.

## 1. Introduction

Mastitis, is a serious health issue and one of the main causes of reduced productivity in dairy animals [1]. Among these, camels represent a unique dairy species due to the high nutritional and therapeutic value of their milk, which is particularly vulnerable to deterioration when mastitis occurs. Despite their smaller population relative to cattle and buffalo, camels rank third in global milk production. Camel milk is highly nutritious and especially suitable for infant feeding due to its compositional similarity to human milk [2].

Notably, camel milk has a relatively low and stable lactose content throughout lactation, making it suitable for individuals with lactose intolerance. It also contains three times more vitamin C, ten times more iron than cow milk, and comparable fat content to cattle milk, along with elevated levels of unsaturated fatty acids [3]. Moreover, it is rich in antimicrobial proteins such as lactoferrin, lysozyme, and

lactoperoxidase, which help defend against pathogens like *Lactococcus spp.*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* [4]. These properties make camel milk not a nutritional source but also a valuable nutraceutical. However, its safety and functional properties are compromised by mastitis-related bacterial contamination of the udder tissue [5]. Mastitis spreads via several routes, including environmental reservoirs, milking equipment, and microbial invasion through the teat canal, followed by colonization in the mammary gland [6]. Alarming, studies from Pakistan have shown a high prevalence of *Staphylococcus aureus* (>60%) in subclinical mastitis cases, along with increasing resistance to commonly used antibiotics [7]. This is particularly concerning given the frequent association of *S. aureus* with camel mastitis [7, 8]. Antibiotic resistance has become a major challenge to effective mastitis treatment, especially in regions where antibiotics are overused or misused [9].

The recurrent detection of multidrug-resistant (MDR) *S. aureus* strains, one of the dominant pathogens in camel mastitis, raises serious concerns about treatment failure and prolonged infection [10]. These resistant strains are capable of surviving commonly prescribed antimicrobials, thereby compromising therapeutic efficacy and posing a risk of resistance transmission through the food chain to humans [11]. Given these concerns, this study aimed to assess the prevalence of *Staphylococcus aureus* in subclinical mastitis in camels, evaluate its antibiotic resistance profiles, and investigate the therapeutic efficacy of selected drug combinations for potential treatment strategies.

## 2. Materials and methods

### 2.1. Milk sampling

Camel milk samples were obtained from the designated study area. Samples from animals with either sub-clinical or clinical mastitis were collected. The Surf Field Mastitis test was used to determine if the udder or milk was routine. The mastitis milk samples were placed in sterile tubes, kept in a container at 4°C, and then transported to the Department of Medicine at Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Panjab, Pakistan.

### 2.2. Isolation and characterization of *S. aureus*

Milk samples were first incubated in the nutrient broth at 37°C for 24 hours to encourage bacterial growth. After incubation, the cultures were centrifuged, and the sediment was streaked onto blood agar plates. These plates were then incubated for another 24 hours at 37°C. Characteristic pinpoint colonies appearing on blood agar were subcultured onto mannitol salt agar (MSA), which selectively supports the growth of *Staphylococcus* species and differentiates those capable of mannitol fermentation. Colonies that turned the MSA yellow indicated positive mannitol fermentation and were further analyzed. Gram staining revealed round, Gram-positive cocci in clusters, morphologically consistent with *Staphylococcus aureus*. To confirm the identity, a battery of biochemical tests was performed following the procedures in Bergey's Manual of Determinative Bacteriology [12]. The isolates tested positive for coagulase and catalase, confirming

their classification as *S. aureus*. They were indole-negative, methyl red-negative, and Voges-Proskauer-positive, indicating a preference for the butylene glycol fermentation pathway. Urease and citrate utilization tests were also positive. Sugar fermentation tests showed acid production without gas, confirming the organism's ability to metabolize various carbohydrates. This biochemical profile aligns with that of vaccinal *Staphylococcus aureus* strains [12, 13].

### 2.3. Antibiotic susceptibility and synergy assays

The antibiotic susceptibility of the agent was evaluated using disc diffusion and Minimum inhibitory concentration (MIC) assays against all antibiotics classes reported in previous studies. Muller Hinton agar was used for susceptibility assays against cefixime, gentamicin, amoxicillin, vancomycin, ceftaxime, ciprofloxacin, ampicillin, oxacillin, amikacin, cinoxacin, mupirocin, chloramphenicol, and trimethoprim. Briefly, fresh growth of *S. aureus* was adjusted to 0.5 McFarland, which was then swabbed on Muller-Hinton agar. Antibiotic discs were aseptically applied on Muller-Hinton agar (B3374, Oxoid, Basingstoke, UK) using a disc dispenser (Oxoid™). The plates were incubated for 24 hours at 37 °C, and inhibition zones were measured using Vernier calipers in millimeters [3]. Using standards provided by clinical laboratories and standard institutes, antibiotic inhibition zones were compared to declare the isolate as resistant, sensitive, or immediately susceptible to antibiotics. For synergy testing [14], different combinations of antibiotics based on their different target sites were prepared. The following combinations were used: a) Antibiotics are effective. b) Antibiotics are least effective with highly effective. The checkerboard method was employed to determine synergism. Each combination's fractional inhibitory concentration (FIC) index determined synergism's presence. The FIC of product A was calculated as the MIC of product A in combination with product B divided by the MIC of product A alone. Similarly, the FIC of product B was calculated as the MIC of product B in combination with product A divided by the MIC of product B alone. A fractional inhibitory concentration index  $\leq 0.5$  was considered synergistic, an FIC index  $>0.5$  and  $\leq 1.0$  was noted as additive, an FIC

index  $>1.0$  and  $\leq 4$  was considered indifferent, and an FIC index  $> 4$  was considered antagonistic.

## 2.4 Field trial

Field trials were conducted to evaluate the in vivo efficacy of selected antibiotic combinations against *Staphylococcus aureus* isolated from subclinical mastitis in camels. Camels suffering from mastitis were given treatment combinations found effective in vitro testing. The six combinations were made from drugs including oxytetracycline, ampicillin, gentamicin, and cefotaxime. The success of the drugs' efficacy was measured based on the disappearance of clinical signs of mastitis after 05 days of treatment. The response was kept on calculating until 15th days of first treatment. The milk was collected to check the disappearance of bacterial presence using microbiological techniques as described in Bergey's manual of determinative bacteriology [12].

## 2.5 Statistical analysis

The data obtained was put to univariate analysis for the antibiotic susceptibility trial. The percentage of susceptible isolates was carried out by following equation (1).

$$PCI (1)$$

Where PCI= percentage susceptible isolate

NB: Susceptible refers to either resistant, intermediate, or resistant against particular antibiotics.

Statistical software SPSS version 22 was used for this project to analyze data, while a 5% probability was set for carrying out the research trial [15].

## 3. Results

### 3.1. Prevalence and antibiotic susceptibility profile of

### *Staphylococcus aureus*

The study found 25% of MDR *Staphylococcus aureus* isolates from subclinical camel mastitis from both areas (Table 1). The prevalence of pathogenic *S. aureus* was non-significant between the Cholistan and Suleiman ranges. However, the prevalence of MDR bacteria was lower in the Suleiman Range compared to the Cholistan Range.

There were variable responses against antibiotics as shown by *S. aureus* isolated from milk of camels located in Cholistan area of Punjab and Suleiman range of Baluchistan (Table 2,3 Figure A, B) . There, 40% of *S. aureus* isolated from camel milk were resistant to oxacillin, cefoxitin, trimethoprim, tetracycline, gentamicin, oxytetracycline, and otrepomicin. In this case, higher percentages of intermediate susceptible strains of *S. aureus* present an alarming situation toward a rise in antimicrobial resistance in the future. However, there were still isolates sensitive to most antibiotics in that 50, 40, 50, 40, 50, 50, 50, and 40% of *S. aureus* were sensitive to oxacillin, cefoxitin, ciprofloxacin, gentamicin, oxytetracycline, streptomycin, and amikacin, respectively (Table 2). It is thus pertinent to note that drug resistance is higher in camel milk-based *S. aureus* than was considered because of lactoferrin. *S. aureus* isolates from the Sulaiman range responded differently to those of the Cholistan area regarding antibiotic responses (Table 3). However, the general trend was more or less similar to others. The highest resistance was found against oxacillin (40%), trimethoprim (30%), ciprofloxacin (40%), gentamicin (40%), streptomycin (40%), and amikacin (30%). Again, intermediate susceptible isolates were considerable in this case, too, as 10-30% were among intermediate susceptible isolates.

**Table 1.** Prevalence of Subclinical Mastitis, *Staphylococcus aureus*, MDR *S. aureus*.

Category	n	Prevalence
Milk Samples Collected	200	-
Subclinical Mastitis	85	$\frac{85}{200} \times 100 = 42.25\%$
<i>S. aureus</i>	40	$\frac{40}{85} \times 100 = 47.06\%$
MDR <i>S. aureus</i>	10	$\frac{10}{40} \times 100 = 25\%$

**Table 2.** Drug response of *Staphylococcus aureus* isolated from the milk of camels located in Cholistan, Punjab.

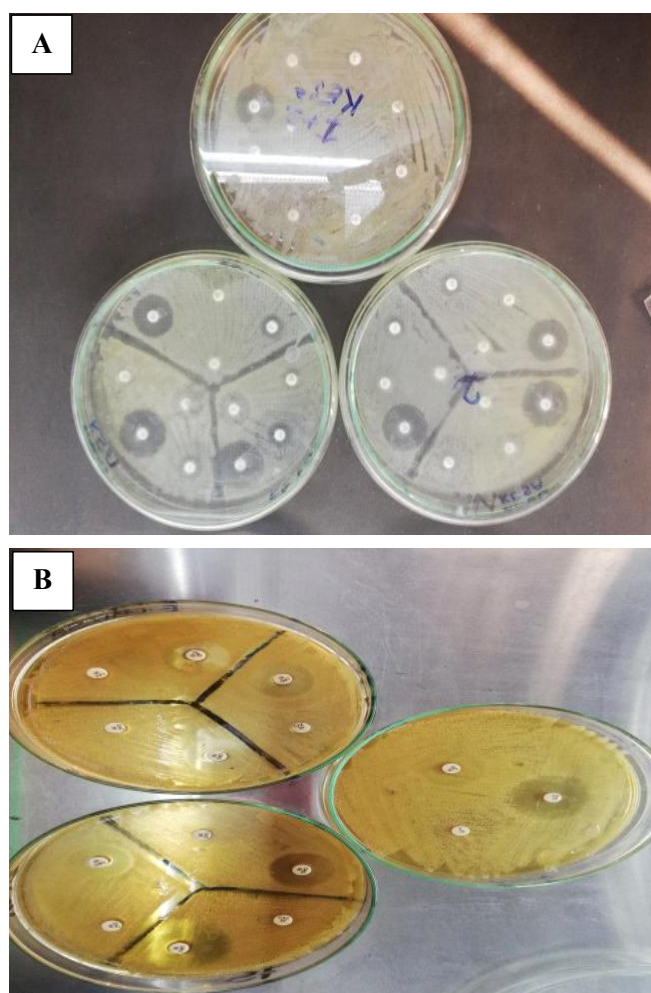
Antibiotic	Potency	S (%)	I (%)	R (%)
Oxacillin	10µg	50	10	40
Cefoxitin	30 µg	40	20	40
Ampicillin	10µg	30	40	30
Trimethoprim	25µg	50	10	40
Ciprofloxacin	5µg	40	30	30
Gentamicin	10µg	50	10	40
Vancomycin	30µg	30	40	30
Oxytetracycline	30µg	70	10	20
Oxytetracycline	30µg	50	10	40
Streptomycin	10µg	50	10	40
Amikacin	30µg	40	40	20

Note: S=Sensitive, I=Intermediate, R=Resistant

Sensitive strains of *S. aureus* against various antibiotics were higher than those isolated from camels in Cholistan. *S. aureus* showed the highest sensitivity against ampicillin, followed by cefoxitin, vancomycin, oxytetracycline, streptomycin, amikacin, ciprofloxacin, and gentamicin, presenting 70, 60, 60, 60, 60, 60, 60, 50, and 40%, respectively. It is interesting to note that *S. aureus* isolated from mountain ranges presented comparatively lower resistance than *S. aureus* isolated from camels residing in Cholistan.

### 3.2. Drug interaction in an *in vitro* trial

The checkerboard approach was used to evaluate the medications' synergy to determine how much each drug interacted with the others and whether or not they could be used together in the field (Table 4). None of the combinations was antagonistic, while another cadre had variable percentages. Gentamicin and ampicillin were among the most effective drugs, presenting 70% of isolates synergistically and the rest in partial synergism. Oxytetracycline and Gentamicin presented 30% synergistic, 30% partially synergistic, 10% additive, and 30% in different responses. Oxytetracycline and Cefotaxime showed 50% synergism, 40% partial synergism, and 10% additive response. Oxytetracycline with ampicillin presented 30% synergism, 50% partial synergism, and 30% were among the in different categories. In combination with cefotaxime, gentamicin showed 60% synergism, 20% partial synergism, 10% additive, and 10% indifferent responses.



**Figure 4** Susceptibility of *S. aureus* against different antibiotics. Susceptibility assay on (A) Nutrient agar, and (B) Mueller Hinton against antibiotics (oxacillin, ceftriaxone, ampicillin, trimethoprim, ciprofloxacin, gentamicin, vancomycin, oxytetracycline, and amikacin).

### 3.3. Field trials of drug combinations

The combinations tested through *in vitro* trials were further tested on camels suffering from mastitis. Camels are located in different areas of Cholistan (Punjab) and the Sulaiman range (Balochistan). The combinations of oxytetracycline, gentamicin, cefotaxime, and ampicillin were used. The trial showed diversified responses of drugs in curing camels suffering from *S. aureus* infection. Ampicillin in combination with gentamicin and ampicillin in combination with cefotaxime showed highest response, in that 40% of cases recovered in 3-5 days' duration while least response was

noted in case of oxytetracycline in combination with ampicillin (Table 5). The latter found 40% of camels did not respond to the treatment even after 10<sup>th</sup> day. Percentages of recovered cases during 6-7<sup>th</sup> day post start of treatment showed a 20-30% range of success rate, while 8-10 days duration presented 10-40% success range. Except oxytetracycline in combination with cefotaxim, all other combinations showed 20-40% of cases did not respond to treatment combinations after 10<sup>th</sup> day of treatment. Such a scenario presents concerns over the use of preventive measures in practice to avoid loss of available treatment regimens.

**Table 3.** Drug response of *Staphylococcus aureus* isolated from camel milk located in Suleman range, Balochistan

Antibiotic	Potency	S (%)	I (%)	R (%)
Oxacillin	10µg	50	10	40
Cefoxitin	30µg	60	20	20
Ampicillin	10µg	70	20	10
Trimethoprim	25µg	40	30	30
Ciprofloxacin	5µg	50	10	40
Gentamicin	10µg	40	20	40
Vancomycin	30µg	60	20	20
Oxytetracycline	30µg	60	20	20
Oxytetracycline	30µg	60	20	20
Streptomycin	10µg	50	10	40
Amikacin	30µg	60	10	30

Note: S=Sensitive, I=Intermediate, R=Resistant

**Table 4.** Percentages of synergy combinations against MDR *S. aureus* based on FIC in dices

Antibiotic combinations	Synergistic	Partial Synergistic	Additive	Indifferent	Antagonistic
Oxytetracycline + Gentamicin	30	30	10	30	-
Oxytetracycline + Cefotaxime	50	40	10	-	-
Oxytetracycline + Ampicillin	30	40	-	30	-
Gentamicin + Ampicillin	70	30	-	-	-
Gentamicin + Cefotaxime	60	20	10	10	-
Ampicillin + Cefotaxime	-	30	-	70	-

**Table 5.** Percentages of recovered cases of mastitis with novel drug combinations at different time intervals

Antibiotic combinations	3-5 days	6-7 days	8-10 days	>10 days or no recovery until 15 days
oxytetracycline+Gentamicin	30%	30%	20%	20%
oxytetracycline+Cefotaxime	20%	30%	40%	10%
oxytetracycline+Ampicillin	10%	20%	30%	40%
Gentamicin + Ampicillin	40%	20%	20%	20%
Gentamicin + Cefotaxime	30%	20%	30%	20%
Ampicillin + Cefotaxime	40%	30%	10%	20%

Note: Antibiotic therapy was carried out for 05 days, while response was noted until 15 days.

## 4. Discussion

### 4.1. Prevalence of *Staphylococcus aureus* in camel milk

The present study recorded a 25% prevalence of multidrug-resistant (MDR) *Staphylococcus aureus* in cases of subclinical camel mastitis across both the Cholistan and Suleiman ranges. Although the difference in prevalence between the two regions was statistically nonsignificant, there was a notably higher proportion of MDR isolates in Cholistan, suggesting regional variation in antibiotic use, hygiene practices, or environmental pressures.

This prevalence aligns with previous studies, which have consistently reported *S. aureus* as a major pathogen of the camel udder [15]. Notably, *S. aureus* is known for its ability to colonize the teat canal and survive in the udder environment, making detection difficult without repeated sampling and pre-enrichment steps [16,17]. This characteristic intermittent shedding might explain underreporting in some regions and also highlights the importance of using sensitive diagnostic approaches, including pre-incubation of milk samples, for reliable detection [18]. Molecular studies have also shown diverse virulence gene profiles in camel mastitis isolates, such as *clfA*, *nuc*, and *tsst-1*, emphasizing the pathogen's adaptability and variation by region [19]. The current finding of a 25% MDR prevalence should raise concern, particularly given the zoonotic potential of *S. aureus* and the increasing interface between camels, humans, and other animals in pastoral communities.

### 4.2. Antibiotic resistance and therapy

Antimicrobial susceptibility profiling in this study revealed a worrying trend: high resistance rates to commonly used antibiotics, including oxacillin, cefoxitin, ciprofloxacin, gentamicin, and trimethoprim. In the Cholistan isolates, resistance to oxacillin, cefoxitin, and streptomycin was as high as 40%, with intermediate susceptibility seen in up to 30% of isolates. This suggests a potential shift toward full resistance, a hallmark of emerging antimicrobial resistance (AMR). Such resistance to penicillin and sulfonamide groups has also been reported in earlier studies [20, 21]. Furthermore, both ecological zones showed similar resistance patterns, indicating region-wide misuse or overuse of antibiotics, particularly those in the beta-lactam group [10]. This underscores the urgent need for strategic interventions, including antibiotic stewardship and farmer education on drug withdrawal periods.

To address AMR, drug combination therapy was evaluated using a checkerboard method. Combinations like gentamicin + ampicillin and gentamicin + cefotaxime showed strong synergistic effects (70% and 60% synergy, respectively), suggesting that combining drugs with different mechanisms, e.g., cell wall synthesis inhibition and ribosomal targeting, can overcome individual drug resistance. The synergy observed between two beta-lactam antibiotics, such as ampicillin and cephalosporins, likely stems from their different protein binding targets [22, 23], while cephalosporins also possess some beta-lactamase inhibitory potential [24, 25].

Field trials mirrored these lab findings: the most effective combinations *in vivo* were gentamicin + ampicillin and

ampicillin + cefotaxime, which resulted in a 40% recovery within 3–5 days [26, 27, 28]. On the other hand, combinations like oxytetracycline + ampicillin had poor outcomes, with 40% of animals showing no recovery even after 10 days. These results validate the importance of evidence-based drug pairing and demonstrate how certain combinations can fail in practice despite theoretical promise [29, 30, 31].

This dual approach, lab-based synergy testing combined with field validation, offers a practical model for mastitis management. However, the fact that even the most effective combinations left 20–30% of animals untreated highlights the limits of antibiotic therapy in advanced or resistant infections. If prevalence continues to rise, so will the treatment failure rate, leading to economic losses and increased risk of resistant zoonotic transmission [32].

## 5. Conclusion

This study highlights a concerning prevalence of multidrug-resistant *Staphylococcus aureus* (MDR *S. aureus*) in subclinical camel mastitis from both the Cholistan and Sulaiman ranges. Although the overall prevalence of pathogenic *S. aureus* was not significantly different between the two regions, the Cholistan area exhibited a higher frequency of MDR strains. Antimicrobial susceptibility testing revealed varied resistance patterns, with a considerable proportion of isolates showing resistance to oxacillin, trimethoprim, ciprofloxacin, gentamicin, and other commonly used antibiotics. Alarming, a substantial number of isolates exhibited intermediate susceptibility, suggesting an impending threat of escalating resistance. In vitro drug interaction assays demonstrated that combinations like gentamicin + ampicillin and gentamicin + cefotaxime yielded the most promising synergistic effects. Field trials supported these findings, with higher recovery rates observed in camels treated with these combinations. However, some regimens, particularly oxytetracycline-based combinations, showed poor therapeutic outcomes, underscoring the urgent need for rationalized antibiotic use and continuous surveillance. The results advocate for region-specific therapeutic strategies and strengthen the case for preventive interventions, including

vaccine development, to mitigate the growing threat of antimicrobial resistance in camel mastitis pathogens.

## Author Contribution Statements

Zeeshan Arif: Methodology, Writing – original draft; Mariam Anjum: Writing – review & editing; Amjad Islam Aqib: Project administration, Conceptualization, Supervision, Writing – original draft; Mahreen Fatima: Methodology, Writing – review & editing; Mohsin Khan: Data curation, Formal analysis; Noor-e-Ali: Investigation, Project administration, Visualization, Validation.

## Ethical approval

Not Applicable

## Conflicts of Interest

The authors report no conflicts of interest.

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## Data Availability statement

The data presented in this study are available on request from the corresponding author.

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