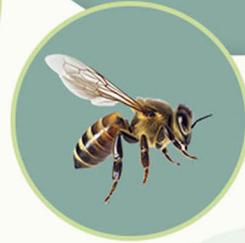
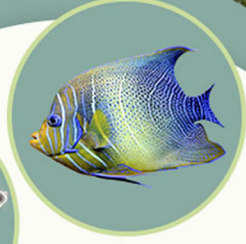




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Molecular characterization of antimicrobial resistance genes of *Staphylococcus aureus* isolated from mastitic camel milk in Egypt

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Article Info	Abstract
Article history: Received: 19 July 2023 Accepted: 02 December 2023 Available online: 15 June 2024	<p><i>Staphylococcus aureus</i> is one of the most common causes of mastitis worldwide. This study aimed to determine the prevalence and antimicrobial resistance (AMR) patterns of <i>S. aureus</i> in mastitic milk samples collected from camel farms in Matrouh Governorate, Egypt. A total of 200 mastitic camel milk samples were evaluated for <i>S. aureus</i> using both conventional culture-based and molecular-based methods. Antibiotic susceptibility testing of <i>S. aureus</i> isolates was conducted using disc diffusion and agar dilution methods, with antibiotic resistance genes identified through polymerase chain reaction with specific primers. Out of sample tested, 60 (30.00%) were positive for <i>S. aureus</i>. The isolates displayed the highest of resistance against piperacillin-tazobactam (55.00%) followed by trimethoprim-sulfamethoxazole (45.00%) and amoxicillin (40.00%). Half of the isolates were multidrug-resistant (MDR). The AMR genes included methicillin-resistant gene (<i>mecA</i>), β-lactamase gene (<i>blaZ</i>), tetracycline resistance gene (<i>tetK</i>), erythromycin resistance gene (<i>ermB</i>) and vancomycin resistant gene (<i>vanA</i>) were detected in 100%, 100%, 95.00%, 90.00% and 20.00% of the isolates, respectively. In conclusion, the presence of MDR <i>S. aureus</i> as a cause of clinical camel mastitis is a significant veterinary and public health concern. These findings highlight the importance of proper antibiotic use in Egyptian camel farms and the need for molecular techniques to fully understand the genetic profile of antimicrobial-resistant <i>S. aureus</i> isolates.</p>
Keywords: Antimicrobial resistance genes Camel Egypt Mastitis <i>Staphylococcus aureus</i>	

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Introduction

Dromedary camel milk is a crucial component in the nomadic diet because of its therapeutic benefits for various diseases and its richness in vitamins, minerals, high levels of immunoglobulins and antioxidants compared to milk from other animal species.¹ Mastitis, an inflammation of the mammary glands, is regarded as the most serious disease in the global dairy industry.² It can lead to financial loss by reducing milk output, degrading milk quality, lowering conception rates, increasing treatment costs, and spreading the infection to other animals.³ Camels, like other dairy animals, are susceptible to mastitis. Camel mastitis can be categorized as either clinical, with symptoms like redness, hotness and enlargement of the udder, or subclinical where traditional symptoms are not visible in the milk or mammary glands.⁴ The incidence of mastitis in dairy camels has been reported countries, like Somalia⁵, Kenya⁶ and various

districts in Ethiopia.⁷ Studies have shown that mastitis affects 45.66% of the camel population worldwide, with rates of 24.00% in the United Arab Emirates and 43.00% in Saudi Arabia.⁸

Mastitis is a multifactorial disease caused by various microorganisms, with bacterial pathogens being considered the primary cause of mastitis in domestic animals. Therefore, accurately identifying the specific bacterial pathogen is crucial for preventing and treating mastitis. Several research studies have reported the main causative agents of bovine mastitis.² However, there is limited knowledge about the microorganisms responsible for camel mastitis.

Staphylococcus aureus is considered one of the most recognized infectious agents of clinical and subclinical mastitis worldwide due to its extensive distribution in the environment, rapid transmission and high rate of contamination.⁹ As with all forms of contagious mastitis, *S. aureus* is spread from camel to camel during milking by

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contaminated washcloths, shared towels, the milker's hands and flies.¹⁰ The average prevalence of *S. aureus* has been observed to be 20.35% in camel udder disorders. The lowest occurrence rate (1.80%) was recorded in Saudi Arabia,⁸ while in Pakistan, *S. aureus* prevalence has been estimated to be as high as 74.50%¹¹ and 50.03%.¹²

The emergence of antimicrobial resistance (AMR) is an alarming public health concern, especially in relation to staphylococcal infections. Antibiotic resistance has developed due to the widespread usage of antimicrobial agents in food producing animals, which has decreased the effectiveness of numerous antibiotic classes for the treatment of bacterial infections in both veterinary and human medicine. This is particularly concerning for β -lactam antibiotics, which are the most frequently used antimicrobials in the treatment of infectious animal diseases including clinical mastitis.¹³ The use of these antimicrobial agents at subtherapeutic levels in developing countries, like Egypt, for growth promotion and disease prophylaxis increases the risk of emerging antibiotic-resistant bacterial pathogens.¹⁴

Staphylococcus aureus resistance against β -lactam antimicrobial agents is illustrated by two distinct mechanisms. First, the production of β -lactamase (penicillinase) encoded by the *blaZ* gene¹⁵ which is involved in the breakdown and attenuation of the β -lactam antibiotic. Second, the production of an altered penicillin binding protein (PBP2a) with an inaccessible active site preventing β -lactam antibiotic binding and consequently not interfering with cell wall biosynthesis allowing the bacteria to survive and grow as normally.¹⁶ The PBP2a protein is encoded by the *mec* genes (*mecA/mecC*), which are embedded within the staphylococcal cassette chromosome *mec* (*SCCmec*), a highly transmissible mobile genetic element (MGE) between bacterial populations, which could be classified into at least 14 different types.¹⁷ Furthermore, high levels of resistance in *S. aureus* have also been recorded against tetracycline, erythromycin, gentamycin, kanamycin, streptomycin, sulphonamides, phenicols and fluoroquinolones.¹⁸

The increasing occurrence of multidrug-resistant (MDR) *S. aureus* has become a global public health concern. Additionally, most MDR *S. aureus*-related foodborne illnesses have been linked to animal-derived foods, such as milk. Within MDR *S. aureus* strains, methicillin-resistant *S. aureus* (MRSA) is particularly concerning as it provides resistance to methicillin and other β -lactam antibiotics.¹⁹ The MRSA is a primary cause of widespread healthcare-associated MRSA (HA-MRSA) infections contributing to the global crisis of AMR. The MRSA is a public health concern because it can result in infections that are life-threatening and difficult to treat. Although invasive HA-MRSA infections have declined in recent years, there has been a rise in community-associated MRSA (CA-MRSA) infections among the general

population. As previously mentioned, methicillin resistance is facilitated by *SCCmec* which contains genes that encode *mecA* and its analogue *mecC*, both of which produce PBP2a. Also, HA-MRSA, livestock-associated MRSA and CA-MRSA can contaminate food consumed by humans leading to incidents of staphylococcal food poisoning.²⁰

The significance of *S. aureus* harboring multiple AMR genes in mastitic camel milk is not documented in Egypt or other developing countries. To the best of our knowledge, there is no available data concerning the occurrence of AMR genes in *S. aureus* causing clinical mastitis in camels in Egypt. Therefore, the objective of the current investigation was to determine the prevalence of *S. aureus* associated with camel clinical mastitis in Matrouh Governorate, Egypt and to assess the phenotypic and genotypic AMR profiles of some of the obtained isolates.

Materials and Methods

Ethical statement. All study procedures were carried out in compliance with the guidelines and regulations of the Research Ethics Committee of the College of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt. The ethical approval number is KFS 2017/3.

Study area, time period and population. This cross-sectional study was conducted in Matrouh Governorate, located in the northwestern part of Egypt, from January 2021 to August 2021. Two hundred milk samples were gathered from camels with clinical mastitis from various farms in desert areas of Matrouh Province. The clinical cases were characterized by a swollen, reddened and tender udder and/or detectable inflammatory abnormalities in the milk such as an alteration in the color (serum-like, blood-streaked, bloody) or consistency (clots, viscous).²¹ All specimens were collected after obtaining oral consent from the camel keepers.

Collection of milk samples. After discarding the first few squirts of milk, approximately 10.00 mL of milk was aseptically collected from each inflamed quarter into sterile, labeled falcon tubes. All samples were kept in an icebox and transferred as quickly as possible to the Bacteriology Laboratory at the Faculty of Veterinary Medicine, Kafrelsheikh University for further bacteriological examination. Each milk sample was thoroughly mixed before being tested bacteriologically for *S. aureus*.

Conventional isolation and identification procedures of *S. aureus*. The isolation of *Staphylococcus* strains was performed using a previously described method.²² Briefly, a loopful (10.00 μ L) of milk sample was enriched by inoculation in buffered peptone water and incubated aerobically for 24 hr at 37.00 °C. Then, a 0.01 mL aliquot of each dilution was aseptically streaked on the surface of 5.00% sheep blood agar (Oxoid, Basingstoke, UK) and incubated aerobically for 24 - 48 hr at 37.00 °C. The colonies grown on blood agar were sub-cultured on

mannitol salt agar (MSA; Oxoid) as selective culture media for staphylococci and incubated aerobically at 37.00 °C for 24 - 48 hr. One suspected colony was selected from MSA and purified using trypticase soya agar (Oxoid). The typical Staphylococcal isolates were identified based on colonial and morphological characteristics.²³ Next, presumptive *Staphylococcus* colonies were confirmed by biochemical assays including catalase, coagulase and sugar fermentation tests.²⁴ Purified *Staphylococcus* single colonies were finally picked up and preserved at - 20.00 °C in brain heart infusion containing 30.00% glycerol (Sigma-Aldrich, Burlington, USA) for further molecular characterization at the species level.

Extraction of genomic DNA and molecular confirmation of *S. aureus*. All presumptive *S. aureus* isolates were further confirmed by amplification of the *S. aureus*-specific *nuc* gene (thermonuclease encoding gene) using polymerase chain reaction (PCR) as previously described.²⁵ The DNA was extracted from the identified *S. aureus* isolates using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The extracted DNA was stored at - 20.00 °C until further analysis. The primer sequence, amplicon size of the *nuc* gene and the relevant references are shown in Table 1. The reaction mixture used in this study was as follows: 25.00 µL containing 12.50 µL of Emerald Amp Max PCR master mix (Takara Bio, Kusatsu, Japan), 1.00 µL of 20.00 pmol of each primer, 5.00 µL of DNA template, and 5.50 µL of sterile nuclease-free water. The PCR cycling conditions for the *nuc* gene were as follows: initial denaturation at 94.00 °C for 5 min followed by 35 amplification cycles consisting of denaturation at 94.00 °C for 30 sec, annealing at 55.00 °C for 40 sec and extension at 72.00 °C for 40 sec followed by final elongation at 72.00 °C for 10 min (Table 2). Finally, each amplified PCR product was electrophoresed on 1.00% agarose gel (Sigma-Aldrich, Co., St. Louis, USA), prepared in 1.00 X tris borate ethylenediamine tetra-acetic acid (Sigma-Aldrich) running buffer for 45 min at 110V, and then stained with 0.50 µg mL⁻¹ ethidium bromide (Sigma-Aldrich). DNA ladder (Bio Basic, Markham, Canada) was used. The gel was then visualized and photographed under an ultraviolet transilluminator. Amplification of the 395 bp PCR products confirmed the presence of *S. aureus*.

Phenotypic assessment of antibiotic susceptibility testing. A phenotypic antibiogram profile was conducted on all molecularly confirmed *S. aureus* isolates. These isolates were tested *in vitro* for their susceptibility to a panel of antimicrobial agents from different classes of antibiotics. The testing was carried out using a Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid) according to standards and interpretive criteria set by the Clinical and Laboratory Standards Institute.²⁶ The antimicrobial discs used, along with their specified concentrations, were: norfloxacin 10.00 µg; streptomycin

10.00 µg; sulfamethoxazole/trimethoprim 23.75/1.25 µg; neomycin 30.00 µg; tobramycin 10.00 µg; erythromycin 15.00 µg; tetracycline 30.00 µg; amoxicillin 10 µg; gatifloxacin 5.00 µg; fusidic acid 10.00 µg; chloramphenicol 30.00 µg; piperacillin-tazobactam 110 µg; penicillin G 10.00 U; cefradine 30.00 µg; Rifampin 5.00 µg; Lomefloxacin 10.00 µg and Cefoxitin 30.00 µg. Moreover, all isolates were screened for their resistance against vancomycin (6.00 µg mL⁻¹) using agar dilution method by brain-heart infusion (BHI) agar to determine minimum inhibitory concentrations (MICs). The presence of more than one colony or light film of growth indicated reduced susceptibility to vancomycin and MICs breakpoints of ≤ 2.00, 4.00 - 8.00, and ≥ 16.00 µg mL⁻¹ classified the strains as susceptible, intermediate or resistant, respectively.²⁶ The selection of the antimicrobial agents was based on medications frequently used in dairy veterinary practice in Egypt. *S. aureus* ATCC 25923 was used as the quality control strain. Briefly, single colonies of each *S. aureus* isolate were inoculated into the BHI broth (Oxoid) and incubated overnight at 37.00 °C. The bacterial growth was adjusted to 0.50 McFarland units approximately equivalent to 1.50 × 10⁸ colony-forming unit using a spectrophotometric method. The suspension of bacterial growth was subsequently swabbed onto the surface of Muller Hinton agar (Oxoid) plates using cotton wool swab. Antibiotic discs were aseptically placed on the surface of dried agar plates with the aid of disc dispenser. The inoculated plates were then incubated at 35.00 - 37.00 °C for 17 hr. The inhibition zone around antibiotic discs was measured in millimeter for each isolate and the result was categorized/interpreted as susceptible, intermediate, or resistant following the Clinical Laboratory Standards Institute guidelines.²⁶ Isolates that displayed resistance to at least three or more different antimicrobial classes were categorized as MDR.

Molecular detection of *S. aureus* AMR genes using PCR assay. The molecularly identified *S. aureus* isolates were further screened for various genes that confer resistance to antibiotics using simplex PCR. The AMR genes included β-lactamase (*blaZ*), methicillin (*mecA* and *mecC*), vancomycin (*VanA*), tetracycline (*tetK*), erythromycin (*ermB*), and chloramphenicol (*fexA*) resistance determinants. The target genes, primer sequences, target fragment of PCR products and the relevant references are summarized in Table 1. All PCR assays were performed in a total volume of 25.00 µL containing 12.50 µL of Emerald Amp Max PCR master mix, 1.00 µL of 20.00 pmol of each primer, 5.00 µL of DNA template, and 5.50 µL of sterile nuclease-free water. The amplification of target genes was conducted as uniplex reactions using a thermal cycler (ABI 2720; Applied Biosystems, Waltham, USA). Several PCR protocols were used to detect the target genes of the *S. aureus* isolates. The thermal cycling conditions for each reaction are described in Table 2.

Table 1. Oligonucleotide primer sequences used in the presnet study.

Target genes	Primer sequence (5'-3')	Amplicon size (bp)	References
<i>mecA</i>	GTAGAAATGACTGAACGTCCGATAA	310	27
	CCAATTCCACATTGTTTCGGTCTAA		
<i>mecC</i>	GTCCTAATGCTAATGCA	304	28
	TAAGCAATAATGACTACC		
<i>blaZ</i>	TACAACGTGAATATCGGAGGG	833	29
	CATTACACTCTTGGCGGTTTC		
<i>tetK</i>	GTAGCGACAATAGGTAATAGT	360	30
	GTAGTGACAATAAACCTCCTA		
<i>vanA</i>	CATGACGTATCGGTAATAATC	885	31
	ACCGGGCAGRGTATTGAC		
<i>ermB</i>	CATTTAACGACGAAACTGGC	425	32
	GGAACATCTGTGGTATGGCG		
<i>fxaA</i>	GTACTTGTAGGTGCAATTACGGCTGA	1272	33
	CGC ATC TGA GTA GGA CAT AGC GTC		
<i>nuc</i>	ATATGTATGGCAATCGTTTCAAT	395	25
	GTAAATGCACTTGCTTCAGGAC		

Table 2. Cyclic polymerase chain reaction conditions of the different primer sets.

Target genes	Initial denaturation	Amplifications (35 cycles)			Final extension
		Denaturation	Annealing	Extension	
<i>mecA</i>	94.00 °C - 5 min	94.00 °C - 30 sec	50.00 °C - 30 sec	72.00 °C - 30 sec	72.00 °C - 7 min
<i>mecC</i>	94.00 °C - 5 min	94.00 °C - 30 sec	50.00 °C - 30 sec	72.00 °C - 30 sec	72.00 °C - 7 min
<i>blaZ</i>	94.00 °C - 5 min	94.00 °C - 30 sec	50.00 °C - 40 sec	72.00 °C - 50 sec	72.00 °C - 10 min
<i>tetK</i>	94.00 °C - 5 min	94.00 °C - 30 sec	54.00 °C - 40 sec	72.00 °C - 40 sec	72.00 °C - 7 min
<i>vanA</i>	94.00 °C - 5 min	94.00 °C - 30 sec	50.00 °C - 40 sec	72.00 °C - 50 sec	72.00 °C - 10 min
<i>ermB</i>	94.00 °C - 5 min	94.00 °C - 30 sec	51.00 °C - 40 sec	72.00 °C - 40 sec	72.00 °C - 10 min
<i>fxaA</i>	94.00 °C - 5 min	94.00 °C - 30 sec	56.00 °C - 40 sec	72.00 °C - 1 min	72.00 °C - 12 min
<i>nuc</i>	94.00 °C - 5 min	94.00 °C - 30 sec	55.00 °C - 40 sec	72.00 °C - 40 sec	72.00 °C - 10 min

Results

***Staphylococcus aureus* prevalence from the mastitic milk samples.** A total of 94 isolates were presumptively identified as *S. aureus* out of 200 investigated milk samples. Subsequently, all presumptive *S. aureus* isolates were confirmed by amplification of the *S. aureus*-specific *nuc* gene (which encodes a thermo-nuclease) using a PCR assay. Out of the 94 isolates, 60 were confirmed as *S. aureus* by species-specific PCR (Fig. 1A). Thus, the overall prevalence of *S. aureus* was 30.00%. Furthermore, 20 bacterial isolates were analyzed for their phenotypic and genotypic AMR profile.

Phenotypic antimicrobial susceptibility testing of *S. aureus* isolates. Our finding revealed that *S. aureus*

isolates were highly resistant to piperacillin-tazobactam, trimethoprim-sulfamethoxazole and amoxicillin (55.00, 45.00 and 40.00%, respectively) and moderately resistant to vancomycin (30.00%), cephadrine (20.00%) and rifampin (20.00%). However, they showed the lowest level of resistance against streptomycin, penicillin G, erythromycin, norfloxacin, tetracycline, cefoxitin and chloramphenicol (15.00, 15.00, 10.00, 10.00, 5.00, 5.00 and 5.00%), respectively. In contrast, all the isolates did not exhibit resistance to neomycin, tobramycin, gatifloxacin and lomefloxacin. Interestingly, multiple drug resistance phenotypes (resistance to at least one anti-microbial agent in three or more antimicrobial categories) were found in 50.00% of the tested *S. aureus* isolates.

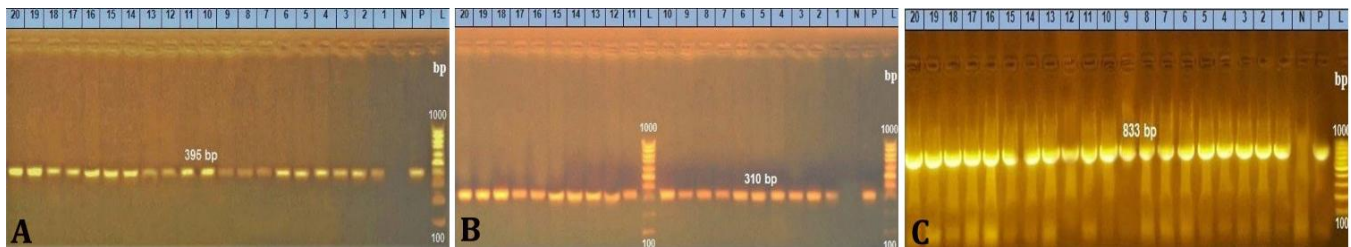


Fig. 1. Agarose gel electrophoresis of the polymerase chain reaction products for amplification of **A)** *nuc* gene, **B)** *mecA* gene and **C)** *blaZ* gene of representative *Staphylococcus aureus* isolates. Lane L: 100-bp DNA marker (DNA ladder, Bio Basic, Markham, Canada); Lane P: positive control; Lane N: negative control; Lanes 1 to 20: test samples.

Genotypic AMR pattern of *S. aureus* isolates. The genotypic AMR pattern revealed that all of the examined *S. aureus* strains harbored the *mecA* gene and were categorized as MRSA. The detected AMR genes included: *mecA* 20/20 (100%), *blaZ* 20/20 (100%), *ermB* 19/20 (95.00%), *tetK* 18/20 (90.00%) and *vanA* 4/20 (20.00%), (Figs. 1 and 2). Other antibiotic-resistant genes, such as *mecC* and *fexA* were not identified. All isolates harbored three and more AMR genes. The combined existence of antibiotic-resistant genes includes: *mecA* + *blaZ* 20 (100%), *mecA* + *blaZ* + *tetK* + *ermB* 14 (70.00%), *mecA*+*blaZ*+ *tetK*+*vanA*+*ermB* 3 (15.00%), *mecA* + *blaZ* + *vanA* + *ermB* 1 (5.00%), *mecA* + *blaZ* + *ermB* 1 (5.00%) and *mecA* + *blaZ* + *tetK* 1 (5.00%).

Discussion

Camel mastitis is an extremely serious disease that not only results in milk loss but also adversely affects the health of the mammary gland.³⁴ Research studies conducted before the 20th century listed camel mastitis as one of the diseased conditions with a lower prevalence, due to its high content of antimicrobial factors, which rendered camel mastitis a negligible priority for epidemiological investigations. However, recent studies have revealed that 45.66% of the camel population is suffering from mastitis with the lowest prevalence rate of 16.00% in Somalia³⁵ and the highest incidence rate of 90.50% in Pakistan.³⁶

The current investigation focused on *S. aureus*, one of the most common infectious agents of mastitis worldwide, from clinical camel mastitis in Matrouh Governorate, Egypt. The overall *S. aureus* prevalence (30.00%) in this study was in line with Ahmad *et al.* who detected *S. aureus* isolates in 33.33% (4/12) of clinically affected camels in Pakistan.³⁷ However, it was higher than that previously estimated by Abo Hashem *et al.*³⁸ who stated that the total prevalence of *S. aureus* mastitis was 9.10% in camels in South Sinai, Egypt and Husein *et al.*,³⁹ who reported a prevalence rate of 4.16% (6/144) in Jijiga town, Ethiopia. It was lower than Al-Juboori *et al.*,⁴⁰ who found that 13 out of 29 (44.82%) milk samples were contaminated with *S. aureus* in the United Arab Emirates and Mohamud *et al.*, who reported that the prevalence of *S. aureus* mastitis in camels was 46.15% (12/26) in Benadir region, Somalia.³⁵

The variation in prevalence rates among the different studies could be attributed to several factors. These include the sample size of tested camels, seasonal alterations, different breeds, diverse rearing systems, environmental conditions based on geographical location, preventive measures and sanitation practices, milk samples preservation methods, various sources of contamination in each study location, genetic profile and diversity of *S. aureus* strains and laboratory diagnostic techniques used for identifying of *S. aureus* isolates. Furthermore, irregular excretion of bacteria in milk and low inoculum sampling may lead to false-negative identification results.⁸

The presence of tick vectors, along with the thorny desert plants and the use of anti-suckling apparatus appear to be potential risk factors for the development of *S. aureus* mastitis in camels in the study region.⁴¹ These issues can result in teat injuries and udder skin lesions which subsequently provide an optimal environment for *S. aureus* entry and pathogenicity. Moreover, the unsanitary milking practices and insufficient management strategies in the study district may have potentially contributed to the occurrence of clinical *S. aureus* mastitis in the camel populations tested during the current study.

The detection of *S. aureus* in the camel milk samples suggests the need for strict hygienic precautions during the production and handling of camel milk to minimize the public health risks. Additionally, educating camel breeders about the importance of sanitary milking procedures would help reduce the negative impact of mastitis on both the quantity and quality of camel milk.⁴⁰ In addition, preventing udder lesions, early identifying subclinically affected udders, culling chronically infected she-camels and immediate treatment of all clinical cases with appropriate antibiotics should be standard practices.

Also, the tested *S. aureus* isolates were highly resistant to piperacillin-tazobactam, sulfonamides and amoxicillin because these injectable antimicrobial agents are widely and inappropriately used in the veterinary field in Egypt.⁴² They also exhibited moderate resistance against penicillin G and cephadrine β -lactam antibiotics, as they possessed the *blaZ* and *mecA* genes responsible for resistance to penicillin and other β -lactam antimicrobial agents.⁴³ Additionally, the unusual display of vancomycin resistance may indicate that camels have become a secondary

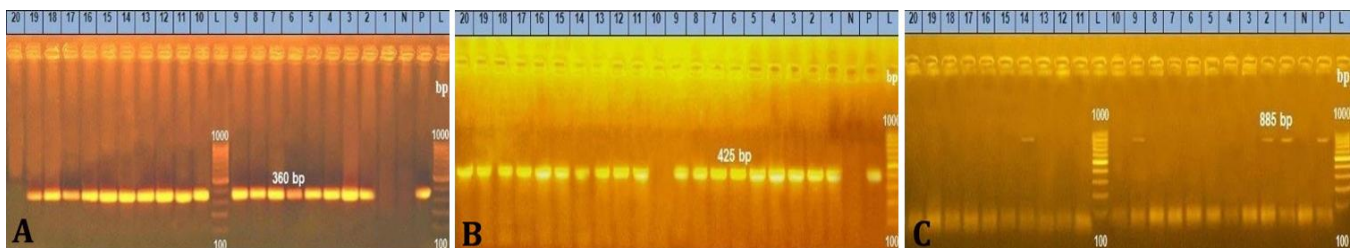


Fig. 2. Agarose gel electrophoresis of the polymerase chain reaction products for amplification of **A)** *tetK* gene, **B)** *ermB* gene and **C)** *vanA* gene of representative *Staphylococcus aureus* isolates. Lane L: 100-bp DNA marker (DNA ladder, Bio Basic, Markham, Canada); Lane P: positive control; Lane N: negative control; Lanes 1 to 20: test samples.

reservoir of vancomycin-resistant *S. aureus* after being exposed to the primary human reservoir of such vancomycin resistant staphylococci.⁴⁴ This interpretation is supported by the fact that vancomycin is a glycopeptide antibiotic medication used as a last resort to treat serious bacterial infections in human medicine and has never been used in the treatment of infectious animal diseases. Similarly, all *S. aureus* strains did not display any resistance against neomycin, tobramycin (aminoglycosides), gatifloxacin and lomefloxacin (fluoroquinolones). This finding could be associated with the lack of use of these antibiotic medications in veterinary practice in Egypt. Additionally, this suggests that these antimicrobial agents could potentially be used for the efficient treatment of clinical mastitis cases in camels in Egypt.

Similar patterns of susceptibility of the *S. aureus* isolates in the current investigation to some of the aforementioned antimicrobial agents have been reported.^{38,40} The diversity in phenotypic AMR patterns observed among *S. aureus* isolates in different studies could be related to geographical variations, environmental sanitation and regulations governing antimicrobial use in the study location.

The increased multi-drug resistance phenotypes in the current study could be attributed to the fact that camel owners usually keep dogs with their camels. It is known that such dogs shed a huge number of multi-drug resistant *S. aureus*, creating a link between dogs, humans and the environment.⁴⁵ Additionally, all the tested *S. aureus* strains were genotypically identified as MRSA, and the vast majority of MRSA strains are MDR pathogens.⁴⁶ This multi-drug resistance of MRSA strains may be due to their enhanced affinity for obtaining MGE such as plasmids and transposons which include AMR genes.⁴⁷ Subsequently, the high incidence of multiple antibiotic resistant *S. aureus* and methicillin resistant *S. aureus* could pose both clinical and public health issues.

Remarkably, our study revealed that some *S. aureus* strains carrying *blaZ*, *ermB* and *tetK* resistance genes did not exhibit penicillin, erythromycin and tetracycline resistance phenotypes, respectively. These phenotypic and genotypic variations were in accordance with Silva *et al.*⁴⁸ and this may be attributed to the lack of expression of the corresponding *blaZ*, *ermB* and *tetK* AMR genes. On the contrary, the *fexA* and *vanA* genes were not detected in one chloramphenicol-resistant isolate and two vancomycin-resistant isolates, respectively, suggesting the potential presence of other AMR genes or a modification in the phenotypic resistance mechanism, such as mutations in the bacterial cell wall receptors.⁴⁹ The scarcity of comparable results to our findings may be due to a lack of previous reports elucidating the molecular identification of AMR genes in *S. aureus* strains isolated from clinically affected camels. This shortage of data underscores the need for further molecular diagnostic technologies such as

DNA microarray and complete genome sequencing, which enable real-time identification of several molecular targets including AMR determinants and virulence markers.

The present study sheds light on the prevalence and antibiogram profile of *S. aureus* from clinical camel mastitis in Egypt. The findings revealed an alarming number of MRSA strains and multidrug resistant *S. aureus* in mastitic camel milk specimens. This situation limits the therapeutic options for such mastitis cases and poses a potential public health threat. Therefore, the prohibition of non-prescription antibiotic usage in Egyptian camel farms is strongly advised and limiting the unauthorized use of antimicrobial agents is an essential preventive strategy in all camel breeding sites in Egypt. Additionally, good hygiene practices should be continuously implemented in camel farms to reduce the incidence of *S. aureus* mastitis in camels. Lastly, further research should be conducted to gain a better understanding of the genetic background of the multidrug-resistant *S. aureus* isolated from mastitic camel milk in Egypt.

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Conflict of interest

None of the authors have no conflict of interest to declare.

References

1. Selva Muthukumar M, Mudgil P, Baba WN, et al. A comprehensive review on health benefits, nutritional composition and processed products of camel milk. *Food Rev Int* 2023; 39(6): 3080-3116.
2. Oikonomou G, Bicalho ML, Meira E, et al. Microbiota of cow's milk; distinguishing healthy, sub-clinically and clinically diseased quarters. *PLoS One* 2014; 9(1): e85904. doi: 10.1371/journal.pone.0085904.
3. Klaas IC, Zadoks RN. An update on environmental mastitis: Challenging perceptions. *Transbound Emerg Dis* 2018; 65(Suppl1): 166-185.
4. El Tigani-Asil ETA, Abdelwahab GE, Veedu JTVP, et al. Gangrenous mastitis in dromedary camels in UAE caused by *Streptococcus agalactiae*. *BMC Vet Res* 2020; 16(1): 174. doi: 10.1186/s12917-020-02382-8.
5. Abdurahman OA, Agab H, Abbas B, et al. Relations between udder infection and somatic cells in camel (*Camelus dromedarius*) milk. *Acta Vet Scand* 1995; 36(4): 423-431.
6. Younan M, Ali Z, Bornstein S, et al. Application of the California mastitis test in intramammary *Streptococcus agalactiae* and *Staphylococcus aureus* infections of camels (*Camelus dromedarius*) in Kenya. *Prev Vet Med*

- 2001; 51(3): 307-316.
7. Abera M, Abdi O, Abunna F, et al. Udder health problems and major bacterial causes of camel mastitis in Jijiga, Eastern Ethiopia: implication for impacting food security. *Trop Anim Health Prod* 2010; 42(3): 341-347.
 8. Aqib AI, Muzammil I, Naseer MA, et al. Pathological insights into camel mastitis. *Acta Trop* 2022; 231:106415. doi: 10.1016/j.actatropica.2022.106415.
 9. Khan A, Hussain R, Javed MT, et al. Molecular analysis of virulent genes (COA and SPA) of *Staphylococcus aureus* involved in natural cases of bovine mastitis. *Pak J Agric Sci* 2013; 50(4): 739-743.
 10. Cheng WN, Han SG. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. *Asian-Australas J Anim Sci* 2020; 33(11):1699-1713.
 11. Aqib AI, Ijaz M, Durrani AZ, et al. Prevalence and antibiogram of *Staphylococcus aureus*, a camel mastitogen from Pakistan. *Pakistan J Zool* 2017; 49(3): 861-867.
 12. Ali M, Avais M, Ijaz M, et al. Epidemiology of subclinical mastitis in Dromedary camels (*Camelus dromedarius*) of two distinct agro-ecological zones of Pakistan. *Pakistan J Zool* 2019; 51(2): 527-532.
 13. Ocloo R, Nyasinga J, Munshi Z, et al. Epidemiology and antimicrobial resistance of staphylococci other than *Staphylococcus aureus* from domestic animals and livestock in Africa: a systematic review. *Fron Vet Sci* 2022; 9: 1059054. doi: 10.3389/fvets.2022.1059054.
 14. Grema HA, Geidam YA, Gadzama GB, et al. Methicillin resistant *Staphylococcus aureus* (MRSA): a review. *Adv Anim Vet Sci* 2015; 3(2): 79-98.
 15. Zhang HZ, Hackbarth CJ, Chansky KM, et al. A proteolytic transmembrane signaling pathway and resistance to β -lactams in staphylococci. *Science* 2001; 291(5510): 1962-1965.
 16. Rey Pérez J, Zálama Rosa L, García Sánchez A, et al. Multiple antimicrobial resistance in methicillin-resistant *staphylococcus sciuri* group isolates from wild ungulates in Spain. *Antibiotics* 2021; 10(8): 920. doi: 10.3390/antibiotics10080920.
 17. Urushibara N, Aung MS, Kawaguchiya M, et al. Novel staphylococcal cassette chromosome mec (*SCCmec*) type XIV (5A) and a truncated *SCCmec* element in SCC composite islands carrying *speG* in ST5 MRSA in Japan. *J Antimicrob Chemother* 2020; 75(1): 46-50.
 18. Jamali H, Paydar M, Radmehr B, et al. Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control* 2015; 54: 383-388.
 19. Chatterjee SS, Otto M. Improved understanding of factors driving methicillin-resistant *Staphylococcus aureus* epidemic waves. *Clin Epidemiol* 2013; 5: 205-217.
 20. Le HH, Dalsgaard A, Andersen PS, et al. Large-scale *Staphylococcus aureus* foodborne disease poisoning outbreak among primary school children. *Microbiol Res* 2021; 12(1): 43-52.
 21. Radostits O, Gay C, Hinchcliff KW, et al. *Veterinary medicine: A text book of the diseases of cattle, sheep, pigs, goats and horses*. 10th ed. Philadelphia, USA: Saunder Ltd 2010; 603-700.
 22. Quinn PJ, Markey BK, Carter M, et al. *Veterinary microbiology and microbial disease*. 1st ed. Ames, USA: Wiley 2002; 536-539.
 23. Forbes BA, Sahm DF, Weissfeld AS. *Bailey & Scott's diagnostic microbiology: 12th ed*. Michigan, USA: Elsevier Mosby 2007; 842-855.
 24. Wakabayashi Y, Umeda K, Yonogi S, et al. Staphylococcal food poisoning caused by *Staphylococcus argenteus* harboring staphylococcal enterotoxin genes. *Int J Food Microbiol* 2018; 265: 23-29.
 25. Gao J, Ferreri M, Liu XQ, et al. Development of multiplex polymerase chain reaction assay for rapid detection of *Staphylococcus aureus* and selected antibiotic resistance genes in bovine mastitic milk samples. *J Vet Diagn Invest* 2011; 23(5): 894-901.
 26. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 28th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
 27. McClure JA, Conly JM, Lau V, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. *J Clin Microbiol* 2006; 44(3): 1141-1144.
 28. Cuny C, Layer F, Strommenger B, et al. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans in Germany. *PLoS One* 2011; 6(9): e24360. doi: 10.1371/journal.pone.0024360.
 29. Bagcigil AF, Taponen S, Koort J, et al. Genetic basis of penicillin resistance of *S. aureus* isolated in bovine mastitis. *Acta Vet Scand* 2012; 54(1): 69. doi: 10.1186/1751-0147-54-69.
 30. Duran N, Ozer B, Duran GG, et al. Antibiotic resistance genes & susceptibility patterns in staphylococci. *Indian J Med Res* 2012; 135(3): 389-396.
 31. Patel R, Uhl JR, Kohner P, et al. Multiplex PCR detection of *vanA*, *vanB*, *vanC-1*, and *vanC-2/3* genes in enterococci. *J Clin Microbiol* 1997; 35(3): 703-707.
 32. Schlegelova J, Vlkova H, Babak V, et al. Resistance to erythromycin of *Staphylococcus* spp. isolates from the food chain. *Veterinarni Medicina* 2008; 53(6): 307-314.
 33. Kehrenberg C, Schwarz S. Distribution of florfenicol resistance genes *fexA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. *Antimicrob Agents Chemother* 2006; 50(4): 1156-1163.
 34. Al-Otaibi M, El-Demerdash H. Nutritive value and

- characterization properties of fermented camel milk fortified with some date palm products chemical, bacteriological and sensory properties. *Int J Nutr Food Sci* 2013; 2(4):174-180.
35. Mohamud AI, Mohamed YA, Jama OS, et al. Prevalence and major pathogens associated with clinical and subclinical mastitis in dairy camel (*Camelus dromedarius*) in Benadir Region of Somalia. *Vet Sci Res Rev* 2020; 6(2):132-137.
36. Qamar MF, Chaudhary HR, Ahmd W, et al. Prevalence of sub-clinical mastitis in one humped camel of Lesser Cholistan, Pakistan. International Workshop on Dairy Science Park, Peshawar, Pakistan 2011; <https://en.engormix.com/dairy-cattle/articles/prevalence-sub-clinical-mastitis-t35158.htm>. Accessed March 31, 2024.
37. Ahmad S, Yaqoob M, Bilal MQ, et al. Risk factors associated with prevalence and major bacterial causes of mastitis in dromedary camels (*Camelus dromedarius*) under different production systems. *Trop Anim Health Prod* 2012; 44(1):107-112.
38. Abo Hashem M, Ibrahim S, Goda AS, et al. Diversity of microorganisms associated to She camels' subclinical and clinical mastitis in South Sinai, Egypt. *Suez Canal Vet Medi J (SCVMJ)* 2020; 25(2): 307-319.
39. Husein A, Haftu B, Hunde A, et al. Prevalence of camel (*Camelus dromedaries*) mastitis in Jijiga Town, Ethiopia. *Afr J Agric Res* 2013; 8(24): 3113-3120.
40. Al-Juboori AA, Kamat NK, Sindhu JI. Prevalence of some mastitis causes in dromedary camels in Abu Dhabi, United Arab Emirates. *Iraq J Vet Sci* 2013; 27(1): 9-14.
41. Abdelgadir AE. Mastitis in camels (*Camelus dromedarius*): Past and recent research in pastoral production system of both East Africa and Middle East. *J Vet Med Anim Health*. 2014; 6(8): 208-216.
42. Tarabees R. Molecular characterization of *Staphylococcus* species isolated from clinical and subclinical mastitic buffaloes in El-Behera governorate, Egypt. *Alex J Vet Sci* 2021; 69(1): 20-32.
43. Seedy FRE, Samy AA, Salam HSH, et al. Polymerase chain reaction detection of genes responsible for multiple antibiotic resistance *Staphylococcus aureus* isolated from food of animal origin in Egypt. *Vet World* 2017; 10(10): 1205-1211.
44. Bhat AH. Bacterial zoonoses transmitted by household pets and as reservoirs of antimicrobial resistant bacteria. *Microb Pathog* 2021; 155:104891. doi: 10.1016/j.micpath.2021.104891.
45. Shoaib M, Rahman SU, Aqib AI, et al. Diversified epidemiological pattern and antibiogram of *mecA* gene in *Staphylococcus aureus* isolates of pets, pet owners and environment. *Pak Vet J* 2020; 40: 331-336.
46. Pinchuk IV, Beswick EJ, Reyes VE. Staphylococcal enterotoxins. *Toxins* 2010; 2(8): 2177-2197.
47. Zayda MG, Masuda Y, Hammad AM, et al. Molecular characterisation of methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylo-coccus aureus* isolated from bovine subclinical mastitis and Egyptian raw milk cheese. *Int Dairy J* 2020; 104: 104646. doi: 10.1016/j.idairyj.2020.104646
48. Silva ATF, da Silva JG, Aragão BB, et al. Occurrence of β -lactam-resistant *Staphylococcus aureus* in milk from primiparous dairy cows in the northeastern region of Brazil. *Trop Anim Health Prod* 2020; 52(5): 2303-2307.
49. Spanu V, Spanu C, Viridis S, et al. Virulence factors and genetic variability of *Staphylococcus aureus* strains isolated from raw sheep's milk cheese. *Int J Food Microbiol* 2012; 153(1-2): 53-57.