Species diversity and antimicrobial susceptibility profiling of staphylococci isolated from camel milk in Algeria

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ABSTRACT

This study investigated the prevalence of staphylococcal contamination in camel milk collected from various farms in the M'sila region of Algeria and evaluated the antimicrobial susceptibility profiles of *Staphylococcus spp.* isolates. It constitutes the first study involving detailed testing of staphylococci from Algerian raw camel milk. Over a three-month period, 20 camel milk samples were collected and subjected to bacterial isolation using the spread plate technique. *Staphylococcus* species were identified through conventional methods and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Biotyper. Antimicrobial susceptibility testing was performed using disk diffusion method with various antibiotics from different classes. The results revealed a 100% prevalence of *Staphylococcus* contamination in the analysed samples. Among the 30 *Staphylococcus*

isolates, *Staphylococcus epidermidis (S. epidermidis)* (37%) and *Staphylococcus aureus (S. aureus)* (17%) were the predominant species. Antibiotic susceptibility testing revealed that only 6.66% of the isolates were sensitive to all tested antibiotics, while 93.3% exhibited resistance or intermediate susceptibility to at least one antibiotic. Notably, resistance to penicillin was highly prevalent (87%). Diverse antibiotic resistance profiles were observed, with single, double, triple, and quadruple resistance patterns. This study provides valuable insights into the prevalence of *Staphylococcus* contamination and antibiotic resistance profiles in camel milk, highlighting the need for effective strategies and measures to control and prevent the spread of antibiotic-resistant bacteria should be part of livestock management strategies to protect both animal and public health. The identification of *S. epidermidis* isolate identified as MR-MDR CNS highlights the rise of methicillin-resistant strains of CNS and the challenge they pose in maintaining the efficacy of therapeutic treatments.

Keywords: camel milk, *Staphylococcus*, prevalence, antimicrobial susceptibility

1. Introduction

Camels play a crucial role to maintain economic in pastoral communities in arid and semi-arid regions particularly in Africa and Asia (1). They are important sources of food (meat and milk) and a means of transport for the nomads (2). These animals withstand and adapt to harsh environments, making them indispensable in these areas (3). Camel milk holds significant importance as a staple food for population in arid regions and may be the sole available milk source in areas where maintaining other milking animals is challenging (4). Camel milk is considered as a rich source of proteins and fat, essential minerals like calcium, vitamins especially vitamin A and C, lactoferrin. It does not contain β-lactoglobulin (BLG) compared to cow milk (3). Recognized as the "desert white gold," camel milk has been renowned for its noteworthy nutritional and medicinal attributes (3). Its consumption is promoted due to its enhanced digestibility, lower allergenicity, and, above all, its antioxidant, immunomodulating, anti-inflammatory, anti-diabetic and anti-apoptotic properties (4).

Apart from its dietary and nutritional significance, camel milk possesses a valuable antibacterial property compared to others animals milk (4). World camel milk production was estimated at 4.11 million tons in 2022. However, the real estimate of this production may be as high as 5.4 million tons, due to undeclared traditional breeding (5). Raw camel milk is consumed without any processing, which exposes consumers to the risk of zoonotic infections such as brucellosis and tuberculosis, as well as severe infections such as *Streptococcus agalactiae* infection (6). Foodborne pathogens currently pose a significant global concern, causing disease outbreaks associated with the consumption of contaminated food, frequently caused by bacterial toxins (7). Staphylococci, particularly *Staphylococcus aureus*, are among the predominant bacteria involved in foodborne illnesses and are commonly isolated from milk in dairy herds (7). Routine mastitis diagnosis categorizes staphylococci into coagulase-negative staphylococci (CNS) and coagulase-positive staphylococci (CPS). CNS, which comprise over 15 species, are considered opportunistic pathogens causing mastitis (8). Although the transmission mode of *S. aureus* is primarily direct, the epidemiology of CNS mastitis remains unclear (8). Furthermore, various CNS species have been isolated from extramammary sites such as skin and teats, emphasizing the importance of considering these facts when promoting prudent antimicrobial use (8). These bacteria can be recovered from milk samples of dairy animals without a noticeable increase in somatic cell count (SCC) (8). Among the CNS species, *S. epidermidis, S. haemolyticus*, and *S.saprophyticus* are significant types commonly found in human infections (9). The growing emergence of antimicrobial resistance (AMR) is a serious public health concern, particularly in relation to human staphylococcal infections.

1.2. Objectives

The main purposes of this study were, first, to differentiate *Staphylococcus* isolates from collected milk samples using the Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Biotyper (MALDI-TOF BIOTYPER). This technology has proven to be a rapid, accurate, and high-

75 throughput method for identifying bacterial species. The second objective was to assess the
76 antibiotic susceptibility of *Staphylococcus* isolates.

77

78 ## 2. Materials and Methods

79 ### 2.1. Sampling

80 Between December 2020 and February 2021, we collected 20 camel milk samples from various
81 farms in M'sila, located 200 km south of Algeria within a steppe zone. The selection criteria for the
82 farms included the absence of animals exhibiting clinical mastitis or udder inflammation, no use of
83 antibiotics, and no organoleptic changes in the initial streams of foremilk. To avoid contamination,
84 we collected raw milk samples in properly labeled screw-top bottles and transported them in a cold
85 environment using an icebox to the laboratory.

86 ### 2.2. Bacterial isolation

87 Staphylococci were isolated from raw camel milk using the spread plate technique, following the
88 EN ISO 6888-1 standard procedure (10). The milk samples were streaked onto Baird-Parker agar
89 medium supplemented with egg yolk potassium tellurite emulsion. The agar plates were then
90 incubated at 37°C for 24 to 48 hours to allow bacterial colonies to grow.

91 ### 2.3. Bacterial identification

92 #### 2.3.1. Purification and identification of *Staphylococcus* isolates

93 Presumptive staphylococcal colonies on Baird-Parker agar were confirmed using conventional
94 methods, including the assessment of colony morphology and the performance of catalase tests.

95 #### 2.3.2. Identification of Staphylococcal species by MALDI-TOF Biotyper

We identified staphylococcal species using the Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Biotyper. In triplicate, we prepared pure colonies by spotting them onto polished steel target plates. Then, we applied 1 µL of formic acid (Bruker) and 1 µL of matrix solution (α-cyano-4-hydroxycinnamic acid, Bruker) to each dried spot. The prepared plate was subsequently analyzed using the Bruker MALDI-TOF Biotyper system.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar plates, following the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020) (11). The inoculum turbidity was adjusted to match the 0.5 McFarland standard. Various antibiotics from different classes were tested, including β-lactams (Oxacillin [1µg], Penicillin [10µg]), tetracyclines (Oxytetracycline [30µg]), aminoglycosides (Gentamicin [30µg], Tobramycin [10µg]), fluoroquinolones (Ciprofloxacin [5µg]), macrolides (Erythromycin [15µg]), sulfonamides (Sulfonamide [200µg]), phosphonic acid derivatives (Fosfomycin [50 µg]), and chloramphenicol (Chloramphenicol [30µg]). For isolates identified as *S. epidermidis*, a phenotypic characterization of methicillin-resistant staphylococci (MRS) strains was performed using the disk diffusion test with 1 µg of oxacillin. Isolates demonstrating resistance to three or more different antimicrobial classes were classified as multidrug-resistant (MDR) isolates.

3. Results

3.1. Milk contamination and recovery of staphylococci isolates

Our study analyzed 20 camel milk samples, all of which tested positive for *Staphylococcus spp.*, indicating a 100% prevalence of *Staphylococcus* contamination (Table 1). While *Enterococcus* species were also present in the camel milk samples, our focus was solely on identifying *Staphylococcus* species. From these samples, we successfully retrieved a total of 30 *Staphylococcus*

isolates. Among them, 8 (27%) were coagulase-positive staphylococci (CPS), while 22 (73%) were coagulase-negative staphylococci (CNS). The most frequently isolated species from raw camel milk were *S. epidermidis*, accounting for 11 out of 30 isolates (37%), followed by *S. aureus* with 5 isolates (17%). Other coagulase-negative staphylococcal species included *S. warneri* (13%), *S. simulans* (13%), and *S. pasteuri* (10%). The CPS *S. delphini* was also identified in 3 out of 30 samples (10%).

Table 1. Diversity of staphylococci species recovered from camel milk.

Species	Coagulase test reaction	Number	Prevalence (%)
S. aureus	CPS	5	17
S. epidemidis	CNS	11	37
S. delphini	CPS	3	10
S. warneri	CNS	4	13
S. simulans	CNS	4	13
S. pasteuri	CNS	3	10
Total	-	30	100

3.2. Antibiotic susceptibility test

Our investigation included 30 *Staphylococcus* isolates to assess their susceptibility to various antibiotics. The results showed that only 2 isolates (6.66%) were sensitive to all tested antibiotics, while the vast majority (93.3%) exhibited resistance or intermediate susceptibility to at least one antibiotic. Notably, 50% of the isolates showed intermediate resistance to erythromycin, and 23% to ciprofloxacin. Further details on antibiotic sensitivity and resistance are presented in Table 2, which displays the results of the antibiotic susceptibility testing for *Staphylococcus spp*. isolates 2. The table outlines the distribution percentages for sensitivity, intermediate resistance, and full resistance to different antibiotics.

Table 2. Antibiotic susceptibility results of *Staphylococcus spp* isolates.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Tetracycline	94	3	3
Gentamicin	97	0	3
Erythromycin	43	50	7
Ciprofloxacin	67	23	10
Penicillin	13	0	87
Fosfomycin	60	0	40
Sulfonamide	77	6	17
Chloramphenicol	94	3	3
Tobramycin	97	0	3
Oxacillin*	97	0	3

*: only for *S epidermidis*

3.3. Antibiotic Resistance Profiles

Among the isolates, a diverse range of resistance profiles emerged (Figure 1). Notably, resistance to penicillin as a single antibiotic was prevalent, with a significant 26.66% (8/30) of isolates exhibiting this resistance, while 30 % (9/30) of isolates were multidrug-resistant. Resistance profiles involving two antibiotics, consistently featuring penicillin, showed varying prevalence rates, ranging from 3% to 13%. Specifically, the penicillin-fosfomycin resistance profile had a prevalence of 13%. Additionally, resistance profiles including penicillin-ciprofloxacin and penicillin-erythromycin were observed at prevalence of 3% and 7%, respectively. Profiles involving resistance to three antibiotics collectively accounted for a prevalence of 19%, with the penicillin-ciprofloxacin-sulfonamide profile accounting for 10%. Finally, profiles characterized by resistance to four antibiotics represented a prevalence of 3%.

This comprehensive analysis of antibiotic resistance profiles across various antibiotic combinations enhances our understanding of bacterial resistance mechanisms.

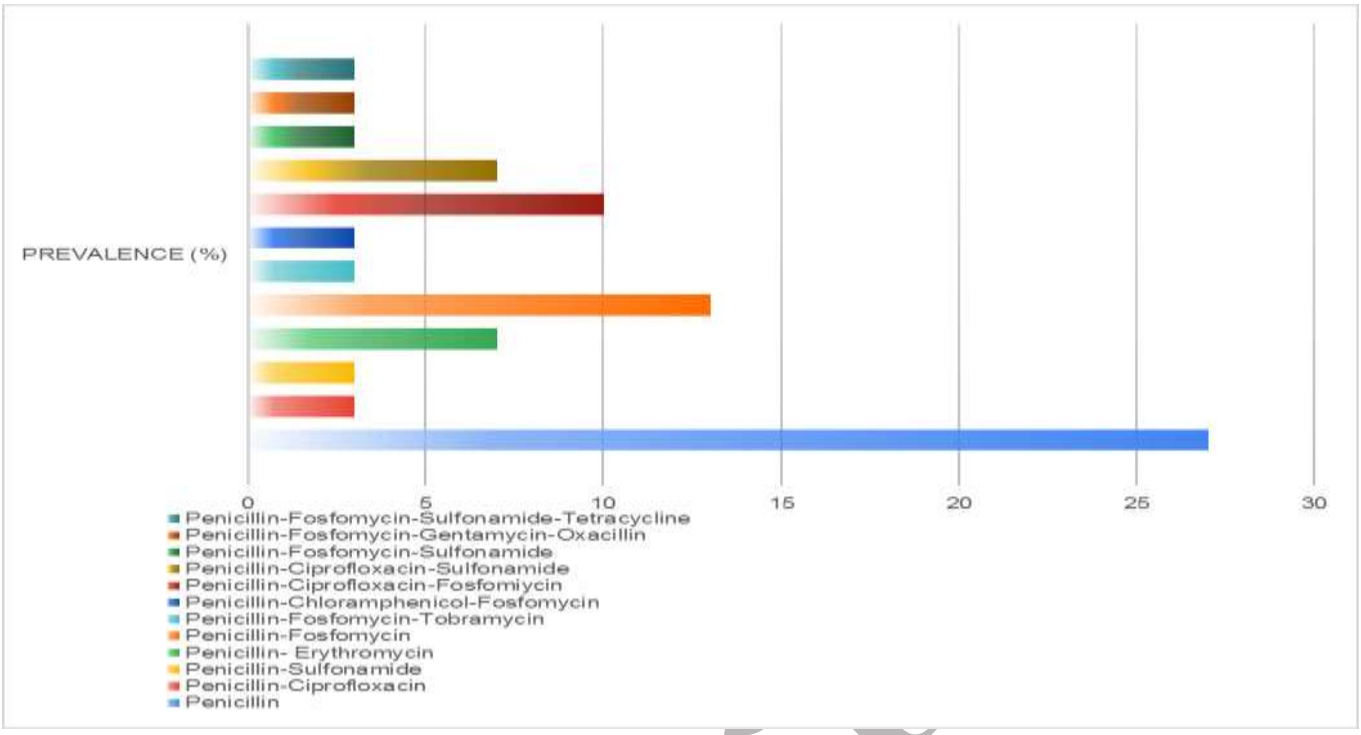

Figure 1. Antibiotic resistance profiles of different *Staphylococcus* species.

4. Discussion

Our investigation into camel milk samples yielded compelling results concerning *Staphylococcus* contamination, especially when compared to previous studies. While *Staphylococcus* is a common constituent of skin flora in both humans and animals, our study's standout finding was the 100% prevalence of *Staphylococcus spp.* contamination detected in the samples. Several previous studies on bacterial contamination of camel milk have reported varying prevalence of *Staphylococcus spp.*, ranging from 46.7% to 89.8% (12, 13). These results highlight the dynamic nature of microbial populations and their potential shifts over time (14). The significant prevalence observed underscores the need for a nuanced understanding of the microbial landscape in food products, particularly since certain *Staphylococcus* strains can produce toxins that pose health risks (15). Therefore, while the presence of *Staphylococcus* in milk is expected, rigorous monitoring and risk assessment are crucial to ensuring food safety and public health. Comparing the distribution of isolated staphylococcal species with earlier studies reveals both consistencies and disparities. MALDI-TOF confirmation of isolates identified 27% coagulase-positive staphylococci (CPS) and 73% coagulase-negative staphylococci (CNS). The dominance of CNS in raw milk is a pattern already reported by Elhosseny et al. (16), who found 61% CNS vs. 39% CPS, and by Njage et al. (12), who reported 55% CNS vs. 45% CPS. In contrast, Kirwa et al. (17) reported a higher prevalence of CPS compared to CNS (83.6% vs. 16.4%). This variance in CPS prevalence

underscores the potential influence of factors such as geographical location, farming practices, and the methods of isolation and identification used in previous studies. Phenotypic characterization by MALDI-TOF identified six species within the *Staphylococcus* genus. The CPS were represented by *S. aureus* (17% prevalence) and *S. delphini* (10%). Numerous studies have reported the presence of *S. aureus* in raw camel milk at prevalence ranging from 10% to 62% (12, 16). A previous study on raw livestock milk from northern Kenya reported 0% contamination by *S. aureus* in camel milk (18). This variation in contamination rates is primarily attributed to differences in the methods used to isolate and identify *Staphylococcus* species. Many studies rely on the presumptive appearance of colonies on agar and biochemical identification using catalase and coagulase tests to confirm *S. aureus*, whereas our study showed that among the 8 coagulase-positive isolates, 3 were identified as *S. delphini*. This species has recently been implicated in human infections with the first documented case described by Magleby et al. (19). This highlights the need of using advanced molecular tools to ensure accurate identification of *Staphylococcus* species. *S. delphini* has been isolated from various sources, including retail food, poultry meat, bulk and goat milk, and some dairy products (20). Seligsohn et al. (21) have also reported it in camel milk. Among the isolated coagulase-negative staphylococci (CNS), the dominant species was *S. epidermidis*, with a prevalence of 37%. These results are consistent with the results of Njage et al. (12). The other CNS observed were *S. pasteuri*, *S. simulans*, and *S. warneri*, all of which have already been documented in camel milk, with their distributions varying between studies (12). CNS are generally considered as having a low pathogenic potential. However, the longstanding focus on coagulase-positive staphylococci, which were traditionally seen as the strains of primary importance, has likely led to underestimations of the prevalence of enterotoxin- producing CNS (12). Antibiotic sensitivity testing of the staphylococcal isolates obtained in this study confirmed antibiotic resistance in nearly all isolates (Table 1). Only two isolates were classified as sensitive to all the antibiotics from the various classes tested. Additionally, multi-resistance was demonstrated, with resistance profiles consistently involving penicillin and fosfomycin (Figure1).The search for methicillin resistance, where only *S. epidermidis* was tested, resulted in the identification of an isolate classified as methicillin-resistant *Staphylococcus* (MRS), as well as being multi-drug resistant (MDR) categorizing it as an MR-MDR CNS. Kirwa et al. (17) in Kenya reported that among *Staphylococcus spp*. isolated from camel milk, the lowest resistance was observed to chloramphenicol and tetracycline (1.6% and 3%), which corroborates the results of this study. However, they also reported high resistance rate to cephalexin (81.9%) and streptomycin (72.1%), whereas our study highlighted high resistance to penicillin (87%) and fosfomycin (40%). Numerous studies have focused on the antibiotic susceptibility of *Staphylococcus* species, particularly *Staphylococcus aureus* from camels. Methicillin resistance has

often been the primary focus in research investigating methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical isolates from nasal swabs and infectious cases. However, data on the antimicrobial resistance of *Staphylococcus* species in camel milk remain scarce. This study represents the first report case of MR-MDR CNS in camel milk. Multidrug resistance (MDR), often described as the "silent pandemic," was observed in 30 % of isolates, with resistance to a β-lactam antibiotic (penicillin) consistently associated. This multidrug resistance was observed in both coagulase-positive *staphylococci* (CPS) and coagulase-negative staphylococci (CNS). The increasing resistance to antibiotics could be attributed to their misuse by herders who self-medicate their camels, as well as the easy access to antibiotics. This study involved a thorough analysis of *Staphylococcus* contamination and antibiotic susceptibility profiles in camel milk samples collected from various farms in M'sila, Algeria. The findings showed a 100% prevalence of *Staphylococcus* contamination, with *S. epidermidis* and *S. aureus* identified as the predominant species. A high prevalence of resistance to Penicillin and multidrug-resistant isolates was observed, indicating a need for enhanced management practices in camel farming to reduce the risk of antibiotic resistance. The variety of antibiotic resistance profiles ranging from single-agent resistance to complex multidrug resistance, illustrates the intricate nature of bacterial resistance mechanisms. These results offer valuable insights into antimicrobial resistance in camel milk production systems and stress the importance of ongoing surveillance and responsible antibiotic use practices. Implementing measures to control and prevent the spread of antibiotic-resistant bacteria should be part of livestock management strategies to protect both animal and public health. The high level of MDR-CNS, including methicillin-resistant (MR-CNS) strains, poses a direct risk to public health by expanding the resistance gene pool from which pathogenic bacteria can acquire resistance traits.

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Authors' Contribution

Study concept and design: F. M and C.B

Acquisition of data: F.M, C.B and L.B

Analysis and interpretation of data: F.M, C.B and L.B

Drafting of the manuscript: F.M and C.B

Administrative, technical, and material support: F.M, S.L.A, N.L and L.B

Study supervision: F.M and L.B

Ethics

We confirm that we have followed and respected the instructions to the authors. The ethics in publishing policy, and conflicts of interest disclosure on behalf of all authors.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

The authors affirm that all the results are given in this article.

Artificial Intelligence (AI)

This study was conducted without using AI.

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