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Isolation and antimicrobial susceptibility of *S. Aureus* from raw camel milk

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Abstract
Camel milk is an important part of the nutrition of the pastoral population. Camel milk also includes some medicinal properties like antibacterial, antiviral, anti-tumour effects and enhancing immune defense mechanism and it also play role for enhancing immune defense mechanism. *Staphylococcus aureus* is an opportunistic infection that affects both humans and dairy animals. Illnesses transmitted by milk. Milk's microbiological quality is also influenced by the state of the water used to wash cows' udders and clean utensils, as well as by the health of the person handling the milk. The study was carried out with the aim to determine antimicrobial susceptibility pattern of raw camel milk. A total 100 raw camel milk samples collected from R and D section of Dairy Technology and Processing Unit at NRCC, Bikaner. Out of 100 raw camel milk samples 11 samples were positive for *Staphylococcus aureus*. Antibiotic susceptibility tests were performed on *S. aureus* isolates against 12 antibiotics by Kirby-Bauer disk diffusion method. Results showed that *S. aureus* isolates very sensitive to chloramphenicol (90.90%), gentamicin and oxacillin (72.72%) respectively. Intermediate resistant was recorded for oxacillin, ciprofloxacin (27.27%) and 18% for gentamicin, and azithromycin. Isolates also showed high resistant rate to penicillin (100%), ampicillin (81.82%), methicillin (72.72%) and vancomycin (36%). On the basis of this result improving the hygienic conditions of milking processes, timely treatment of infected animal and avoiding use of the antibiotics for which the pathogens had shown resistance have been recommended.

Keywords: Raw camel milk, *Staphylococcus aureus*, antibiotic sensitivity

Introduction
Food-borne infections are a major cause of concern in developing countries, resulting in numerous deaths each year as well as a significant economic impact. Foodborne illness outbreaks are prevalent in both industrialized and developing nations, and they are regarded as the most pervasive health concern in the world today, as well as a major source of reduced economic success. Foodborne disease remains a severe health risk in poor nations, accounting for a significant portion of morbidity and death (WHO, 1999) [23].

Milk is a complete food for humans and younger animals. India is top ranked position in milk production (FAOSTAT, 2022-23) [7]. Milk is rich in nutrients like carbohydrate, proteins, fats, vitamins and minerals. Milk also contains some bioactive compounds and hormones like peptides, immunoglobulins, cytokines and some food allergens like alpha-lactalbumin, beta-lactoglobulin. On the other hand, milk poses a health concern to consumers due to the presence of antimicrobial drug residues and zoonotic pathogens. In milk pathogenic organisms can be derived from the animal itself, the human hand or the environment (Bradely, 2002) [5].

Milk and milk products are especial habitat to complex microbial ecosystem, and are responsible for broad variations in taste, aroma and texture of milk and milk products. Milk and milk products are widely consumed since ancient times and demand for milk is constant across the globe. The occurrence of pathogenic bacteria in these milk and milk products can cause severe health hazards to people as they are highly susceptible to variety of microorganism because of complex chemical composition and high nutritive value (Soomro *et al*., 2003) [17].

Camel milk is an important part of the nutrition of the pastoral population, accounting for up to 30% of total annual calorie intake (Farah, 1993) [8]. Camel milk contains numerous health-promoting compounds, including bioactive peptides, lactoferrin, zinc, mono and polyunsaturated fatty acids. These compounds could help in the treatment of various essential human ailments including tuberculosis, asthma, gastrointestinal disorders, and jaundice. It has also been shown to be advantageous for the elderly, particularly in menopausal women, where calcium shortage is a high-risk factor for developing osteoporosis (Swelum *et al*., 2021) [19]. Consumption of raw milk is a frequent custom among farm households and poses a high risk leading to morbidity and mortality as it is a major mode of transmission of pathogenic microorganism (Jayarao *et al*., 2006) [11]. *Staphylococcus aureus* is an opportunistic infection that affects both humans and dairy animals. *Staphylococcus aureus* is one of the well-established sources of clinical infections in the world and it has received a lot of attention recently due to rising mortality rates (Kwon *et al*., 2006) [14]. *S. aureus* is harmful because of its entero-toxins, invasiveness, and drug resistance. *S. aureus* is a prominent cause of both nosocomial and community-acquired infections (Wenzel *et al*., 1991) [22].

Materials and Methods

Sample collection

A total 100 samples raw camel milk were collected from R and D section of Dairy Technology and Processing Unit at NRCC, Bikaner. Milk samples were aseptically collected in sterilized glass bottles and were immediately brought to laboratory under cold conditions and then processed in laboratory.

Isolation and identification of *S. aureus*

Raw camel milk samples were processed for isolation and identification of *S. aureus*. The samples were subjected to aerobic cultivation. Each sample was cultured on mannitol salt agar (MSA) plates for isolation of *S. aureus* and incubated for 24 hrs. at 37 ºC.

Antibiotic susceptibility pattern of *S. aureus* isolates

All the isolates were subjected to antibiotic sensitivity test as described by Bauer *et al*., (1966) [1]. To get rid of any extra inoculums, a sterile cotton swab was dipped in the modified suspension, spun around a few times, and then pressed hard against the tube's interior wall. After being put onto a Mueller Hinton agar plate, the swab culture was allowed to dry for three to five minutes. On the infected agar plate's surface, antibiotic discs were positioned. To make sure every disk made complete contact with the agar surface, each one was pressed down separately.

Results and Discussion

Isolation and identification of *S. aureus*

Out of 100 raw camel milk samples 11% (11/100) were positive for *S. aureus.* The isolates were identified by golden yellow colour colonies on MSA and by Gram staining techniques.

Antibiotic susceptibility patterns of *Staphylococcus aureus* isolates

In present antibiotic sensitivity test *S. aureus* isolates show varying degree of sensitivity for different drugs such as 72.72% (8/11) for gentamycin and oxacillin, 63.64% (7/11) for tetracycline and 54.54% (6/11) for vancomycin, 0% for penicillin, 63.64% for amoxicillin, 90.90% for chloramphenicol and 27.27% sensitivity for methicillin. 18.18% (2/11) intermediate for gentamicin. Out of 11 isolates 8 isolates were showed 72.72% resistance against methicillin and 100% resistance showed for penicillin G.

Table 1: Antibiotic sensitivity test for raw camel milk

S. No.	Name of Antibiotics	Sensitive	Intermediate	Resistance
1.	Ampicillin	18.18% (2/11)	0	81.82% (9/11)
2.	Amoxillin	63.64% (7/11)	18.18% (2/11)	18.18% (2/11)
3.	Azithromycin	81.82% (9/11)	18.18% (2/11)	0
4.	Ceftriaxone	72.72% (8/11)	18.18% (2/11)	9.09% (1/11)
5.	Chloramphenicol	90.90% (10/11)	9.1% (1/11)	0
6.	Ciprofloxacin	63.64% (7/11)	27.27% (3/11)	9.1% (1/11)
7.	Gentamicin	72.72% (8/11)	18.18% (2/11)	9.1% (1/11)
8.	Methicillin	27.27% (3/11)	0	72.72% (8/11)
9.	Oxacillin	72.72% (8/11)	27.27% (3/11)	0
10.	Penicillin	0	0	100% (11/11)
11.	Tetracycline	63.64% (7/11)	27.27% (3/11)	9.1% (1/11)
12.	Vancomycin	54.54% (6/11)	9.1% (1/11)	36.36% (4/11)

Further similar results were reported by Pannu (2020) [15] examined 168 samples were collected from seven different Gauashla of Bikaner district and observed 70% susceptibility for tetracycline that is nearly similar to present study. Gebremedhin *et al*. (2022) [9] examined a total of 486 samples collected from Central Ethiopia and observed 67% susceptibility for tetracycline that is nearly similar to present study. Pannu (2020) [15] examined 168 samples sample were collected from seven different Gauashla of Bikaner district and observed 89.36% susceptibility for chloramphenicol that is similar to present study. Befekadu *et al*. (2016) [3] observed 75% susceptibility for gentamicin that is closely similar to present study. Thaker *et al*. (2012) [20] reported 90% susceptibility for gentamycin which is higher than present study. Banu and Geberemedhin (2022) [2] examined a total of 311 samples of raw cow milk and reported 82.9% susceptibility for ciprofloxacin that is higher than present finding and also showed 61% multi-drug resistance. Patel *et al*. (2018) [16] observed a total of 118 raw cattle milk samples that were collected under aseptic precautions from different places of Navsari district of south Gujarat and Showed sensitivity for Ciprofloxacin 91.66% that is higher than present study. Haque *et al*. (2018) [10] examined total 72 samples including raw cow's milk and some other dairy products sold in the local markets of Mymensingh district of Bangladesh, observed 24% intermediately resistant against vancomycin that is higher than present study. Gebremedhin *et al*. (2022) [9] observed 85% intermediate for gentamycin that

is higher than present study. Deddefo *et al*. (2022) [6] examined 38 raw milk samples collected from Ethiopia and reported 82% resistance for ampicillin that is similar to present study. Borena *et al*. (2023) [4] reported ampicillin resistance 98.48% that is higher than present study. Kumari *et al*. (2020) [13] and Jahan *et al*. (2015) [12] observed 100% antibiotic resistance for penicillin G that is similar to present study. Weldeselassie *et al*. (2020) [21] reported high levels of resistance were recorded for Penicillin G (93.8%) that is similar to present finding while resistance for vancomycin (59.4%) which is higher than present finding and also reported about 93.75% multidrug resistance. Sudhanthiramani *et al*. (2015) [18] reported 110 milk samples were collected from the local milk vendors in and around Tirupathi region of India. Antibiotic susceptibility test of positive isolates showed high resistant toward penicillin G 37/43 (86.04%) and ampicillin 32/43 (74.42%) and methicillin 6/43 (13.95%) that lower than present study. Patel *et al*. (2018) [16] A total of 118 raw cattle milk samples were collected under aseptic precautions from different places of Navsari district of south Gujarat and observed high percent resistance to Ampicillin 33.33%, Methicillin 25.00% which is much lower than present investigation.

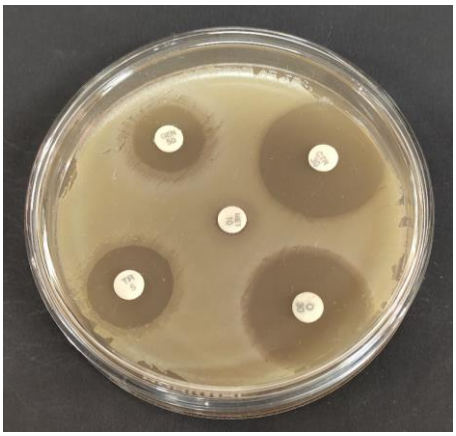

Fig 1: Microbiology lab automation

Conclusion

It can be suggested that there are shortcomings in maintaining sanitary conditions both during production and in the subsequent processing stages. It is strongly recommended that large-scale research studies regarding the quality of raw camel milk, milking protocols and sanitizing programmes should be conducted. Contamination occurs in raw milk due to unsanitary condition. The quality should be improved by complete pasteurization of raw milk.

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