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Raw milk as a reservoir: Exploring the drug resistance profile of *Staphylococcus aureus*

Zeenat Farukahmed Shaikh and Priyanka Rahul Patel *

Department of Research, Rapture Biotech, Ahmedabad, Gujarat, India.

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Abstract

Introduction: *Staphylococcus aureus* is a significant food-borne pathogen commonly found in raw milk and has growing public health relevance due to its increasing antibiotic resistance. Especially, MRSA is recognized as a priority pathogen globally and in India, with increasingly resistant complicated treatment efforts. This study aimed to isolate from raw milk samples using Mannitol Salt Agar (MSA) and assess their antimicrobial susceptibility to evaluate potential health risks.

Materials and Methods: Raw milk samples were collected from local dairies in Ahmedabad and processed by serial dilution, then cultured on MSA and repeatedly subcultured to obtain pure colonies. Isolates were tested against antibiotics—Amoxicillin, Ampicillin, Azithromycin, Cefexime, Cefpodoxime, Kanamycin, Streptomycin, and Vancomycin.

Results: Variable resistance patterns were observed, with specific isolates showing reduced sensitivity or complete resistance to β-lactam antibiotics such as Azithromycin and Vancomycin. At the same time, Amoxicillin and Kanamycin remained largely effective. The presence of resistant *S. aureus* in unpasteurized milk underscores the importance of adhering to stringent hygiene practices during milk production and of prudent antibiotic use in dairy farming to ensure food safety and public health.

Conclusion: The source of many multi-drug-resistant *S. aureus* strains, including MRSA, might be zoonotically transmitted to consumers. Constant monitoring and targeted strategies should be applied in the dairy sector, given the practice of raw milk consumption in many rural areas of the world. Promoting hygienic milking procedures and farming practices, as well as encouraging milk pasteurization, is necessary to reduce the risks of microbial transfer and their associated public health implications.

Keywords: *Staphylococcus Aureus*; Raw Milk; Antimicrobial; Methicillin-Resistant *Staphylococcus Aureus* (MRSA); Public Health Risk.

1. Introduction

Staphylococcus aureus is a Gram-positive spherical bacterium (0.5-1.5 μm), typically arranged in clustered form. It appears purple during Gram staining due to a thick peptidoglycan wall. It is a facultative anaerobe, non-motile, and does not form spores, and is both catalase- and coagulase-positive. [1]. *Staphylococcus aureus* is commonly found on human microbiota, the respiratory tract, armpits, and female reproductive organs. There is an easy zoonotic transmission of these microbes from animals to humans, mainly through direct skin contact. They can also spread through bodily fluids like saliva, tiny droplets released during sneezing/ coughing, and the consumption of animal products like unpasteurized milk [2]. Dairy products are recognized for their nutritional value, providing essential components such

* Corresponding author: Priyanka Rahul Patel.

as protein, calcium, and milk fat, which are beneficial for human health. Raw milk consumption is a routine practice in many rural parts of the world, where it is readily available. It is on the rise in urban areas, driven by health awareness and a preference for eating foods in their natural state. The popularity of raw milk is growing steadily. Moreover, this trend introduces health risks due to the presence of pathogenic microorganisms that can enter the dairy food chain [3]. Although often harmless, an overgrowth of *Staphylococcus aureus* can lead to serious outcomes, including major foodborne diseases and various infections such as food poisoning, skin infections, bloodstream infections, pneumonia, bronchitis, endocarditis, sepsis, and toxic shock syndrome [4].

Previous studies have shown that raw milk and dairy products derived from it significantly contribute to human disease outbreaks. Contamination, typically caused by pathogenic bacteria, often occurs during the processing and handling of cows with mastitis, particularly in unhygienic environments [5]. *S. aureus* is widely known as one of the leading causes of subclinical mastitis in dairy animals. Globally, the prevalence of *S.* aureus in mastitis cases varies widely, ranging from as low as 5.6% in Korea to as high as 70% in Hungary, with notable rates reported in China and the United States at 36% and 47-62%, respectively. This widespread presence is concerning not only because of the infection itself but also because of the frequent detection of antibiotic resistance genes in these bacteria [6]. Additionally, fluctuating temperatures from 37°C to 42°C can lead to the production of heat-resistant staphylococcal enterotoxins, which again are harmful to public health [7]. Virulence factors have been associated with clinical symptoms and severity of infections caused by *S. aureus* and represent major concerns for the food processing industry. Key factors include hemolysins (alpha, beta, gamma, and delta), staphylococcal enterotoxins, leukocidin, and toxic shock syndrome toxin-1 [8].

Methicillin was first used in 1959 to treat infections caused by Penicillin-resistant *Staphylococcus aureus*. Nevertheless, in 1961, the first isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) were identified in the United Kingdom and then globally. The Methicillin acquired (mecA) gene is a gene that codes for Penicillin-binding protein 2a in MRSA. These strains represent a significant public health threat since they are frequently multidrug-resistant and cause both nosocomial and community-onset infections [9]. Antimicrobial resistance is a primary global public health concern. The emergence of resistance in both human and animal bacterial pathogens has been closely linked to antimicrobials or their use as animal growth promoters [10]. In particular, MRSA represents a growing public health challenge for both hospitals and the community environment, primarily driven by the extensive use of antibiotics, in agriculture, veterinary practice, and human medicine [11],[12]. *MRSA* is a type of bacteria that has become resistant to certain antibiotics, specifically beta-lactam antibiotics like Methicillin [13].

MRSA has been identified as a priority pathogen by the World Health Organization's 2024 Bacterial Priority Pathogens List and the Department of Biotechnology, India's 2021 Priority Pathogen List. As rising trends in Antimicrobial Resistance (AMR) threaten to bring a post-antibiotic era along, the Gujarat State Biotechnology Mission (GSBTM), under the Department of Science and Technology, initiated 'Network Program on AMR Superbugs-one health policy tandem with the state action plan', which was launched as well. This initiative aims to monitor the increasing antimicrobial resistance across Gujarat [14]. According to the national average detection rate in 2023, MRSA reached 29.1%, up slightly from 2022's 0.2%. Also, *S. aureus* commonly uses antibiotics, which are gradually declining, with penicillin G resistance reported at 92%. Global Antimicrobial Resistance Surveillance System (Glass), 2022: Staphylococcus aureus infections have increased across 82 countries and regions, making it the third most common bacterial pathogen globally. It is also the second leading cause of bloodstream infection among bacteria and exhibits high levels of drug resistance, with 135,631 reported cases [15]. This study aims to isolate *S. aureus* from raw milk and evaluate its antimicrobial susceptibility.

2. Material and methods

2.1. Sample collection and processing

The raw milk samples were collected from a local dairy unit, using pre-sterile collection tubes, and stored with 4-degree gel packs during transit. Primary screening was carried out by spreading the samples on a Mannitol Salt Agar (MSA) plate and incubating at 37°C for 24 to 48 hours. The colony-forming units (CFU) were counted for each positive sample according to the standard protocol. The isolated colonies were sub-cultured thrice under the same conditions to obtain pure isolated colonies for antibiotic profiling.

2.2. Antimicrobial susceptibility profiling

The isolated microbial strains were subjected to an antibiotic well diffusion assay for antimicrobial susceptibility profiling against Amoxicillin, Ampicillin, Azithromycin, Cefixime, Cefpodoxime, Kanamycin, Streptomycin, and Vancomycin. The average zone of inhibition was measured as per the standard protocol.

3. Results and discussion

A total of ten raw milk samples were collected from various local dairy units for bacteriological analysis. Of these, three samples exhibited characteristic growth on MSA, indicating presumptive positivity for *S. aureus*, while the remaining seven samples showed no growth on MSA. From the three MSA-positive samples, a total of nine *S. aureus* isolates were successfully recovered for further characterization.

Table 1 Results of raw milk samples on Mannitol Salt Agar for Staphylococcus aureus screening

Sample	Result on MSA	CFU/mL
1	Negative	-
2	Positive	14.56
3	Negative	-
4	Positive	14.3
5	Positive	1.13
6	Negative	-
7	Negative	-
8	Negative	-
9	Negative	-
10	Negative	-

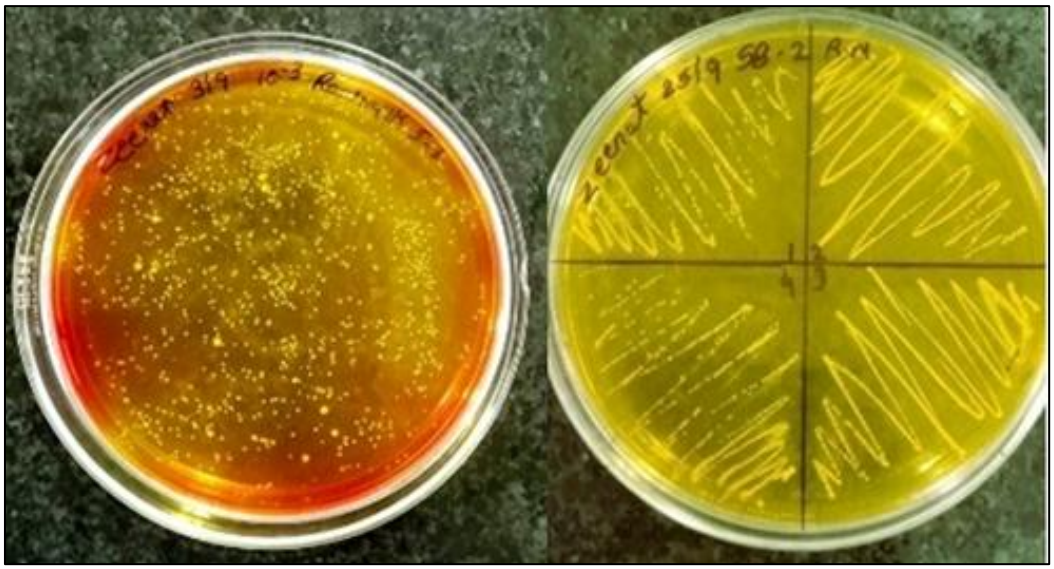

Figure 1 1(a) - Sample on MSA Plate, 1(b) - Subculturing of the sample isolate

3.1. Antimicrobial Susceptibility Test

Antibiotic susceptibility testing of the isolates against eight antibiotics showed inhibition zone diameters ranging from 2 to 36.2 mm, suggesting inconsistent mechanisms of action. Higher susceptibility was observed against Amoxicillin (7/9; 77.8%) and Kanamycin (6/9; 66.7%), followed by moderate susceptibility to Ampicillin and Cefpodoxime (5/9; 55.6%). Cefexime and Streptomycin showed intermediate activity (4/9; 44%), whereas Vancomycin exhibited limited susceptibility (3/9; 33.3%), along with decreased susceptibility or resistance to Azithromycin (1/9; 11.1%). These findings reflect the presence of sensitive and resistant *S. aureus* in a small number of samples; hence, they reinforce the importance of routine monitoring of antibiotic resistance in dairy products.

Table 2 Antimicrobial susceptibility profile of *Staphylococcus aureus* isolates recovered from raw milk samples against selected antibacterial agents, showing the distribution of susceptible, intermediate, and resistant phenotypes

	Average Zone of inhibition (in mm)								
Antibiotics	**S1C1**	**S1C2**	**S1C3**	**S1C4**	**S1C1**	**S1C2**	**S1C3**	**S1C4**	**S1C1**
Amoxicillin	21	33	26.5	26.5	35	29	27	12.5	35
Ampicillin	22.5	25.5	24	12.5	14.5	11	26.5	28	13.5
Azithromycin	14	18.5	5	12	21	16.5	6.5	2	6
Cefixime	20.5	20.5	18.5	15	26	11	27.5	19	33
Cefpodoxime	23.5	11	20	18	27.5	13	32	23.5	28
Kanamycin	5	20.5	19	19	26	28	28	29	36.2
Streptomycin	16.5	20.5	21	21	3	21.5	18.5	20	18.5
Vancomycin	17.5	18.5	18.5	19.5	10.5	21	25	20.5	13.5

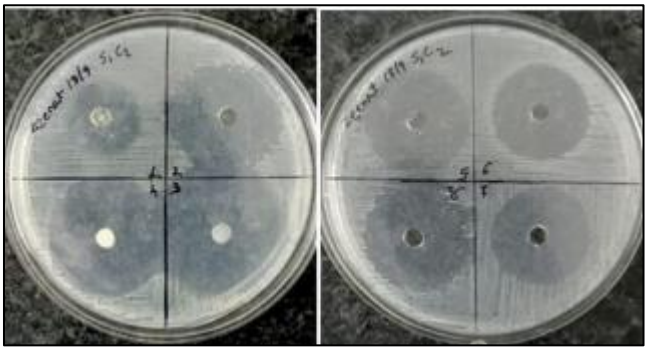

Figure 2 Antimicrobial Susceptibility of different Antibiotics

Dairy products have long been recognized as important sources of *S. aureus*, together with conceptual premises of the multiple contamination resources stating that an infectious udder is prone to contaminate milk, unsanitary milking procedures offer one more opportunity for contamination, and poor practices during processing and storage may lead to a failure in quality control [1], [11]. Infected cows with mastitis are a common source of infection, as the bacteria can enter milk directly and persist in many favourable environmental conditions [7].

In the present study, antimicrobial susceptibility testing of nine *S. aureus* showed resistance to several β-lactam antibiotics, especially amoxicillin and Ampicillin, which was in line with previous studies conducted in Mansoura, Egypt [10], [8]. Resistance to β-lactams has public health implications because these agents are widely used in veterinary and human medicine. The *Staphylococcus aureus* isolates in this study exhibited higher susceptibility towards Vancomycin and Kanamycin than to the other antibiotics used. These observations align with previous studies, in which Vancomycin and Kanamycin remained active against food-borne *Staphylococci* isolates present in milk [12]. The epidemiological trend of MRSA has increasingly reported in both hospitals and community settings over the recent decade. The findings of this study report that more than 50% of *S. aureus* isolates were methicillin-resistant, representing a marked increase from previous years. These MRSA isolates exhibited high resistance to beta-lactam antibiotics, posing significant treatment challenges. This example illustrates the need to continue surveillance for MRSA and to rely on newer antibiotics, such as Vancomycin, to manage these complicated infections effectively [16].

Meanwhile, examples of multidrug-resistant (MDR) and Methicillin-resistant *S. aureus* (MRSA) strains in dairy products have been widely reported [12], [13]. The presence of *S. aureus* in raw milk underscores the zoonotic risk of consuming unpasteurized dairy products. In addition to causing direct infection, *S. aureus* produces heat-stable enterotoxins that may survive incomplete pasteurization and precipitate food poisoning outbreaks [17]. The heterogeneity in resistance profiles across studies can be attributed to variations in antibiotic use, farm management, and geographic conditions. This highlights the importance of antimicrobial resistance surveillance in dairy herds and of regulating antibiotic use in

food animals. Good milking hygiene, pasteurizing milk before sale, and preventing the misuse of antibiotics should be implemented - these are measures to protect public health.

4. Conclusion

This investigation demonstrates that raw milk serves as a reservoir for Staphylococcus aureus strains with diverse antimicrobial resistance profiles, including β-lactam-resistant isolates. The high rate of isolation for Methicillin-resistant *S. aureus* (MRSA) raised a considerable concern regarding the emerging resistance that can cause problems in both therapy and community control. The diversity of resistance patterns among isolates underscores the importance of regularly monitoring antimicrobial profiles in the dairy industry. Encouraging good milking hygiene, deterring abuse of antibiotics on farms, and advocating for pasteurization are essential interventions to reduce the risk of transmission and protect consumer health. Continued monitoring and targeted strategies are critical to effectively managing this emerging threat.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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