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"Prevalence And Study Of Antibiogram Pattern Of *S. Aureus* Isolated From Various Milk Samples"

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Abstract

The current study was carried out with the aim to isolate *Staphylococcus aureus* from various milk samples collected from different area of Akola and Khamgoan and determine the antibiogram pattern of *S. aureus* isolates. in this study, the hygiene of milk product containers was significantly associated with the occurrence of *S. aureus* due to the poor-quality milk product containers used (30.77%). The prolonged use of poor-quality plastic materials for handling milk products was common in milk collection centers and among farmers presenting milk products to the markets. Utensils used for milking and storage determine the safety of milk and milk products. The current study was carried out with the total 20 milk samples collected from home, daries of different areas of Khamgoan and Akola.The identification of *Staphylococcus aureus* in the given sample is based on their morphological characteristics. *Staphylococcus aureus* was frequently encountered from milk samples.

Keywords: Milk, Microbial contamination, Spoilage Public, health risks, Pathogens, *Salmonella*, *Brucella*, *Staphylococcus aureus (S. aureus*), Antibiotic resistance.

Introduction

Milk serves as a highly nutritious food but is extremely susceptible to microbial contamination, leading to spoilage and significant public health risks (Nicklas et al., 2003; Soomro et al., 2002). Pathogens such as *Salmonella*, *Brucella*, *Staphylococcus aureus*, *Listeria*, *E. coli*, and coliforms frequently contaminate milk due to unhygienic production, handling, and storage practices (Nanu et al., 2007). Notably, *S. aureus*, including methicillin-resistant strains (MRSA), poses a major threat due to its association with foodborne illnesses, mastitis in dairy animals, and increasing antibiotic resistance (Fitzgerald, 2012; Paton & Le Loir, 2014). Contamination often occurs during milking, processing, and distribution, particularly in developing countries where pasteurization standards may be inadequate (Genigeorgis, 1976). Despite pasteurization, improper handling can reintroduce pathogens, and *S. aureus* biofilms and heat-stable enterotoxins further challenge food safety (Asao et al., 2003; Rill et al., 2008).

Raw milk consumption in regions like Ethiopia, Egypt, and China exacerbates risks, emphasizing the need for improved hygiene, antimicrobial resistance monitoring, and public awareness (Wu et al., 2018a; Rahman et al., 2018). Effective control measures, including proper pasteurization and hygienic dairy practices, are essential to mitigate outbreaks and ensure milk safety (Ruegg, 2003; Bianchi et al., 2014).

Milk serves as an excellent high-quality food that provides essential nutritional requirements for humans, yet it remains highly susceptible to microbial spoilage (Nicklas et al., 2003). Unhygienic production practices and improper storage contribute to bacterial contamination, which not only deteriorates milk's quality but also poses significant health risks to consumers. *Staphylococcus aureus* is a particularly concerning pathogen in milk and dairy products, capable of causing food poisoning through its enterotoxins. Studies in China have detected persistent *S. aureus* strains in powdered infant formula and pasteurized milk, highlighting concerns about contamination levels, antibiotic resistance profiles, and virulence factors (Wang et al., 2012; Jamali et al., 2015) In the dairy industry, *S. aureus* remains a persistent contaminant, with β-lactamase production further complicating treatment options (CIS et al., 2018). These findings emphasize the need for stringent hygiene practices, antimicrobial resistance monitoring, and improved safety measures across the milk production chain to mitigate risks to public health (K. McMillan et al., 2016).

Staphylococcus aureus, particularly methicillin-resistant strains (MRSA), poses significant public health risks through milk contamination, exhibiting multidrug resistance mediated by βlactamases and PBP2a expression (Woodford et al., 2009; Ache et al., 2020). Studies reveal high prevalence in dairy products, with 75% of isolates resistant to ≥3 antibiotic classes and 100% capable of biofilm formation, enhancing persistence (R.M. Bundi et al., 2014; Pekana et al., 2017). Virulence genes (e.g., enterotoxins) and zoonotic transmission further complicate control (Fitzgerald, 2012). Indiscriminate antibiotic use in livestock drives resistance, underscoring the need for surveillance and prudent antimicrobial practices (Hudzicki, 2009; CLSI, 2019).

Material and method

Milk and dairy products are not common causes of *staphylococcal* food poisoning outbreaks in developed countries, possibly because of an effective quality control program during production, processing and distribution. In developing countries, however, where milk pasteurization is not common and sanitary standards in dairies are not well practiced, staphylococci may play a more important role in food-borne disease.

Collection of samples

1. Milk samples were collected from different areas in khamgoan and Akola.
2. Milk samples swabs were prepared by using sterile cotton tipped.
3. Apply an even rotate the swab .
4. The plates were then incubated at 37° c for 24 hours.

Isolation and identification of *S.aureus*

Isolation was done by using various selective media such as N.agar, Mannitol salt agar . Krimi.G *et.al*. identification was done on the basis of cultural, morphological and biochemical characteristics and was confirmed on the basis of Bergey's Manual Determinative Bacteriology.

Methylene Blue Reduction Test

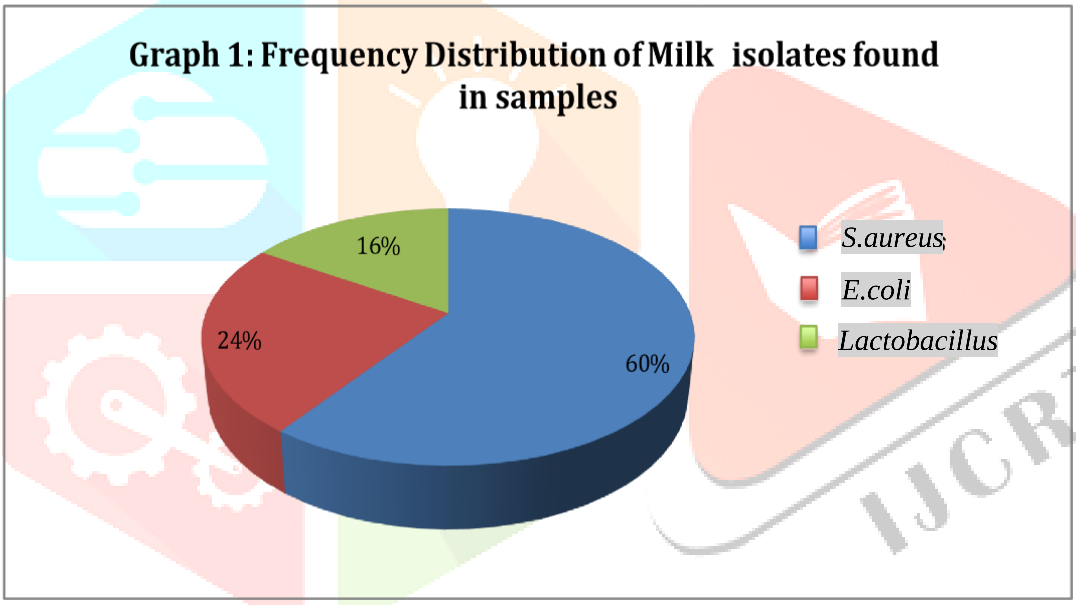
The Methylene Blue Reduction Test (MBRT) is a simple and widely used method for assessing the microbial quality of milk, specifically to determine the presence of bacteria that may reduce the oxygen content in milk, typically due to contamination with microorganisms Methylene blue is a dye that is blue in color when oxidized. When bacteria are present in milk they use oxygen in the milk, leading to a reduction of the dye. The time it takes for the dye to lose its color (i.e., get reduced) is proportional to the number of microorganisms present in the milk. The faster the reduction, the higher the bacterial load, indicating poorer quality milk.

Result and discussion

In this study, the hygiene of milk product containers was significantly associated with the occurrence of *S. aureus* due to the poor-quality milk product containers used (30.77%). The prolonged use of poor-quality plastic materials for handling milk products was common in milk collection centers and among farmers presenting milk products to the markets. Utensils used for milking and storage determine the safety of milk and milk products.

- **Frequency Distribution of bacteria found in Milk samples**

Sr no	Organisms	No of isolates (out of 25)	Percentage (%)
1	*S. aureus*	15	60%
2	*E.coli*	6	24%
3	*Lactobacillus*	4	16%



From 20 Milk samples, frequently occurring organisms were *S. aureus, E.coli, Lactobacillus. S. aureus* was the most frequently isolated organism, accounting for 60% of the total isolates. This high prevalence suggests that *S. aureus* is a predominant opportunistic pathogen studied. Other bacterial isolates *E.coli* (16%), *Lactobacillus* (24%). Frequent use of milk product containers without enough cleaning may increase contamination of the product by *S. aureus*. The use of plastic and traditional containers (clay pots) can be a potential source for the contamination of milk because they allow the multiplication of bacteria on milk contact surfaces during the interval between milking processes.

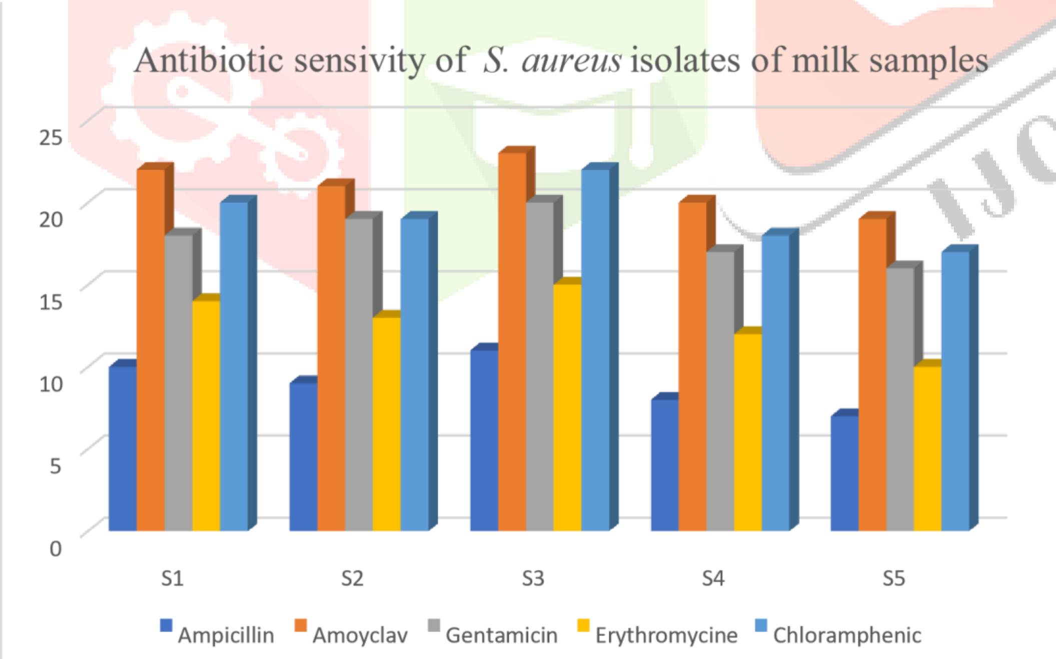
Smith et al. (2018), reported that the prevalence of *Staphylococcus aureus* in our study (40%) is consistent with findings from studies in other regions, where *S. aureus* has been identified as one of the most common contaminants in milk . However, the prevalence of *E. coli* (25%) in our study is slightly higher where the prevalence was around 15%.

The collected milk sample were further analysed Methylene Blue Reduction test to check the quality of milk . it was observed that most of te samples were of poor quality and rest of other samples were of fair quality indicating that before comsumption of milk sample , all samples were needed the pasterurisation to kill the micro flora present in it.

Antimicrobial Sensitivity Test

The antibiotic susceptibility patterns of the isolated bacteria were determined using the disc diffusion method. The results, expressed in terms of the zone inhibition (mm), provide insights into the resistance and sensitivity of each isolate to different antibiotics.

Isolates	Ampicillin		Amoxyclav		Gentamicin		Erythromycin		Chloramphenicol	
	Zone of Inhibition (mm)		Zone of Inhibition (mm)		Zone of Inhibition (mm)		Zone of Inhibition (mm)		Zone of Inhibition (mm)	
S1	10.0	R	22.0	S	18.0	S	14.0	S	20.0	S
S2	9.0	R	21.0	S	19.0	S	13.0	S	19.0	S
S3	11.0	R	23.0	S	20.0	S	15.0	S	22.0	S
S4	8.0	R	20.0	S	17.0	S	12.0	S	18.0	S
S5	7.0	R	19.0	S	16.0	S	10.0	R	17.0	S
S6.	10.0	R	24.0	S	21.0	S	15.0	S	21.0	S
S7	9.0	R	22.0	S	18.0	S	14.0	S	20.0	S
S8	8.0	R	23.0	S	19.0	S	12.0	S	19.0	S
S9	7.0	R	25.0	S	22.0	S	16.0	S	23.0	S
S10	10.0	R	20.0	S	18.0	S	13.0	S	20.0	S
S11	9.0	R	21.0	S	17.0	S	14.0	S	18.0	S
S12	8.0	R	22.0	S	19.0	S	12.0	S	17.0	S
S13	11.0	R	24.0	S	20.0	S	15.0	S	22.0	S
S14	7.0	R	19.0	S	16.0	S	10.0	R	17.0	S
S15	9.0	R	23.0	S	21.0	S	15.0	S	21.0	S



The observation showed susceptibility of each bacterial isolate, highlighting their response to commonly used antibiotics.

In the case of ampicillin against *Staphylococcus aureus,* isolate 3 and isolate 13 exhibited the maximum zone of inhibition, measuring 11.0mm.this indicates that these isolate were more susceptible to ampicillin, leading to greater bacterial inhibition conversely, isolate 5, isolate 9,and isolate 14 respectively showed the minimum zone of inhibition, measuring only 7.0 mm. this suggests that these bacterial strains had lower susceptibility to ampicillin, resulting in reduced effectiveness of the antibiotic.

Gentamicin showed the maximum zone of inhibition on Sample 9 (22.0 mm) and Sample 15 (21.0 mm), indicating strong antibacterial activity. It exhibited the minimum zone of inhibition on Isolate 16 (16.0 mm) and Isolate 17 (17.0 mm), suggesting lower effectiveness against these Isolate.

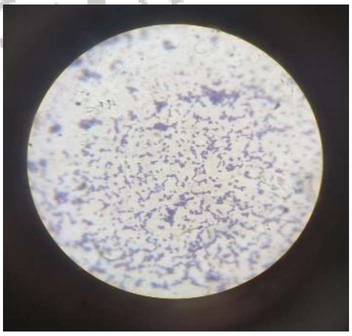
Chloramphenicol demonstrated the maximum zone of inhibition for isolate 15, isolate 9, and isolate, respectively showing its highest antibacterial effect on these samples. Conversely, it exhibited the minimum zone of inhibition on isolate 4 and isolate 14 Additionally, isolate 3, isolate 5, and isolate 14 showed the minimum zone of inhibition, suggesting these isolates were less affected by the tested antibiotics. These results highlight that Gentamicin was the most effective antibiotic, particularly against S9 and S15, exhibit the zone size (22.0mm) and (21.0mm) while Chloramphenicol showed strong activity against multiple samples but was less effective on S14 and S4.

Erythromycin displayed sensitivity in 11 isolate, making it relatively effective but not as universally potent as gentamicin or chloramphenicol. These results suggest that Gentamicin and chloramphenicol may be the most reliable antibiotics for treating

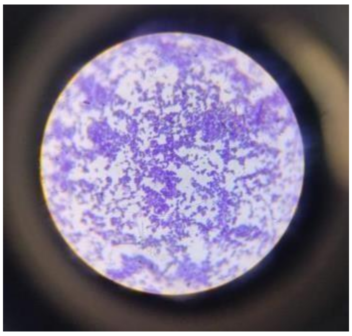
infections caused by the tested bacteria, while Ampicillin and Amoxicillin may have limited effectiveness.

PHOTOPLATE

Collection of Milk Sample



Growth of *S.aureus* on Nutrient agar and its Microscopic view



Growth of *S.aureus* on Mannitol Salt Agar and its Microscopic view

Antibiotic Susceptibility Test

Conclusion

- The current study findings revealed that quality of raw milk and milk products were highly contaminated with *S.aureus* indicating improper hygenic measures.
- *S.aureus* shows resistance to antibiotic commonly used in the therapetic protocols of many human infections.
- So this study highlights the need for continuous surveillance of antibiotic sensitivity pattern of *S.aureus* with a view to select appropriate therapy

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