International Journal of Biomolecules and Biomedicine (IJBB)
ISSN: 2221-1063 (Print), 2222-503X (Online)
http://www.innspub.net
Vol. 18, No. 1, p. 20-25, 2024

RESEARCH PAPER OPEN ACCESS

Staphylococcus aureus in cow milk: Prevalence, antibiotic resistance and hygiene implications

Syed Jawad Ali Shah[1], Adeena Nazir[1], Atif Bilal[1], Falak Niaz[2], Muhammad Asim[3], Muhammad Ilyas[*2,4]

[1]*Department of Health and Biological Sciences, Abasyn University, Peshawar, Pakistan*

[2]*Faculty of Rehabilitation and Allied Health Sciences, Riphah International University, Malakand Campus, Pakistan*

[3]*Allied Health Sciences, NCS University System, Peshawar, Pakistan*

[4]*University Institute of Biochemistry and Biotechnology, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan*

Key words: *S. aureus*, Antibiotic resistance, Penicillin, Dairy product safety, Farm hygiene

Article Published: 05 February 2024

Abstract

Staphylococcus aureus is a prominent human pathogen that is typically found in a variety of human anatomical positions. It is a Gram-positive, non-motile, and facultative anaerobic bacteria. A study was carried out to determine the prevalence of and antibiotic susceptibility pattern of *S. aureus* isolated from milk. In Peshawar, 100 samples of cow milk were collected from various farms and processed. *S. aureus* was isolated and identified by inoculation onto Mannitol Salt Agar, gram staining and biochemical analysis. The Kirby Bauer disc diffusion method was used to assess the isolated *S. aureus* strains for antibiotic susceptibility. By measuring zone sizes and interpreting the data in accordance with predetermined standards. *S. aureus* prevalence was identified and its patterns of antibiotic susceptibility were evaluated in samples of cow milk. *S. aureus* was found in 32 of the total 100 samples. Penicillin showed 100% full resistance, followed by Vancomycin (81.2%), Tetracycline (78.1%), and Erythromycin (75%), according to an antimicrobial susceptibility analysis that revealed substantial resistance trends. Contrarily, Gentamicin (84.3%), Trimethoprim-Sulfamethoxazole (75%), Chloramphenicol (59.3%), and Cefoxitin (56.2%) showed the lowest efficacy levels, whereas Ciprofloxacin (90%), which had the highest efficacy. In conclusion, *S. aureus* was detected in 32% of the 100 samples. Resistance to key antibiotics like Penicillin and Vancomycin was pronounced, highlighting the need for improved farm hygiene. Ciprofloxacin's efficacy suggests its potential use, while overall, maintaining stringent sanitation practices is crucial for ensuring safe dairy products.

***Corresponding Author:** Dr. Muhammad Ilyas ✉ milyas.frahs@riphah.edu.pk

Introduction

Staphylococcus aureus, a Gram-positive bacterium belonging to the *Staphylococcaceae* family within the Bacillales order, is a significant microorganism that has garnered substantial attention due to its diverse impact on human and animal health (Cheung *et al.*, 2021). *S. aureus* is classified as a gram-positive, non-spore forming, non-motile, and facultative anaerobic organism (Abd El Halem and Technology, 2019). This bacterium was first identified by German scientist Friedrich Julius Rosebach in 1884, displays distinct characteristics under microscopic observation, appearing as spherical cocci that cluster together resembling grapes (Foster, 2017).

The presence of *S. aureus* in various contexts, ranging from environmental niches to the human body, holds both beneficial and detrimental implications. While it is recognized as normal human flora and typically remains innocuous in healthy individuals, its transition into the bloodstream or internal tissues can lead to severe infections (Guo *et al.*, 2020). Indeed, *S. aureus* stands as a prominent causative agent of numerous human infections, encompassing conditions such as infective endocarditis, skin and soft tissue infections, septic arthritis, and urinary tract infections (Abd El Halem and Technology, 2019; Hiramatsu *et al.*, 2014).

Milk, a nutritional staple produced by mammary glands of mammals, holds paramount importance in human diets. Rich in essential nutrients including calcium, magnesium, riboflavin, and vitamins, milk consumption is associated with several health benefits, including the prevention of osteoporosis and maintenance of a healthy weight (Jamali *et al.*, 2015). *S. aureus* being found in milk, however, highlights potential health risks, particularly when contamination arises from inadequate hygiene or mammary gland infections (Lee *et al.*, 2018). Due to challenges with food safety, antibiotic resistance, and the potential for human-to-human transmission, this bacterium, which can cause mastitis in cattle and goats, poses threats to both the dairy industry and the general public's health (Macharia, 2018).

This essay explores the various aspects of *S. aureus*, including both its beneficial and harmful effects on human health. It examines the bacterium's prevalence, the importance of the pathogen as an opportunistic one in milk, and its effects on both human and animal health. A serious issue facing the entire world is a surge in antibiotic resistance in *S. aureus* (Makinde *et al.*, 2019; Umaru *et al.*, 2013). Due to the versatility and developing resistance mechanisms of this bacterium, previously treatable diseases become more difficult to treat, resulting in more severe illness, higher healthcare expenses, and a higher fatality rate (Pollitt *et al.*, 2018; Rainard *et al.*, 2018). The increasing worry over *S. aureus's* antibiotic resistance, as demonstrated by the emergence of methicillin-resistant strains, only emphasizes how urgent it is to comprehend and deal with this microbiological threat (Regasa *et al.*, 2019). Action must be taken right away because this problem transcends places and the context of healthcare (Usman *et al.*, 2016).

Materials and methods

Sample collection

Following standardized microbiological methods, a total of 100 milk samples were obtained from diverse farms throughout the Peshawar KP district. The samples were transported aseptically to the Microbiology Research Laboratory (MRL) of Abasyn University.

Sample collection and transportation

In order to ensure the quality milk samples were collected from cows using aseptic procedures. For the collecting process, a disposable plastic container with a tightly sealed lid was used to maintain sterility. Strict hygienic precautions were taken before milking, including complete hand washing and the use of sterile milking gloves. To reduce the possibility of infection, alcohol swabs were used to disinfect the cows' teat ends. After collection samples were transported in ice box to the Microbiology Research Laboratory (MRL) at Abasyn University aseptically.

Sample processing

Upon arrival at the laboratory, the collected milk samples were subjected to processing. Aseptic techniques were maintained throughout the procedure. Each sample was streaked onto Mannitol Salt Agar (MSA) using a sterile wire loop. Subsequently, the samples were incubated aerobically at 37°C for a period of 18 to 24 hours.

Growth observation and identification

After the designated incubation period, the plates were carefully examined for the presence of growth. Colonies showing characteristics indicative of *S. aureus* were further processed for bacteriological identification.

Bacteriological identification

Gram staining, along with a battery of biochemical tests, was performed for the precise identification of *S. aureus*. These tests collectively provided key insights into the bacterium's characteristics, aiding in accurate differentiation and confirmation.

Antibiotic susceptibility testing

Antibiotic Susceptibility Testing (AST) was conducted on *S. aureus* strains isolated from milk samples. Adhering to the 2020 guidelines by the Clinical and Laboratory Standards Institute (CLSI), our study focused solely on investigating the antibiotic susceptibility patterns of *Staphylococcus aureus*. To achieve this, we employed the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA), using carefully selected antibiotics: Vancomycin, Tetracycline, Chloramphenicol, Trimethoprim-Sulfamethoxazole, Cefoxitin, Ciprofloxacin, Penicillin, Gentamicin, and Erythromycin shown in the table 1. This approach helped us better understand how these antibiotics interacted with the *Staphylococcus aureus* bacteria.

Results

The study involved the collection of 100 cow milk samples from various farms in the Peshawar district. These samples were expeditiously transported to the Microbiology Research Laboratory (MRL) at Abasyn University Peshawar, within a period of two hours, while maintaining a controlled temperature using ice bags. The identification of the samples was accomplished through a combination of cultural characteristics, biochemical tests, and Gram staining techniques. Among the collected samples, growth indicative of *S. aureus* was observed in 32 instances, while the remaining samples exhibited no evidence of *S. aureus* growth (Fig. 1).

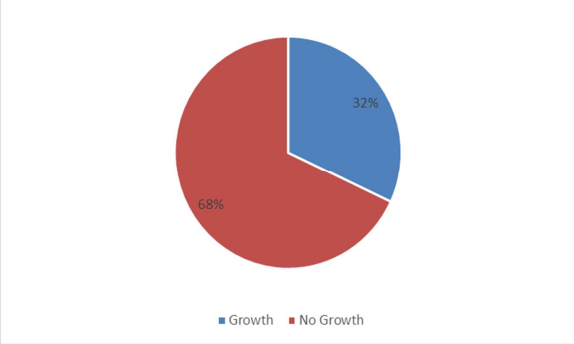

Fig. 1. Prevalence of *S. aureus* isolated from Milk samples

Isolation and identification of Staphylococcus aureus from milk samples

Through a thorough series of microbiological and biochemical assays, *Staphylococcus aureus* was isolated and identified from milk samples. Incubation at 37°C for 24 hours after inoculation onto Mannitol Salt Agar (MSA) produced noticeable yellow colonies that were consistent with *S. aureus*. The unique arrangement of Gram-positive cocci, with cells forming clusters resembling grapes, was subsequently verified by Gram staining. When a colony was introduced to a catalase reagent, bubbles formed, indicating that *S. aureus* was catalase positive. The coagulase test, wherein colonies were added to plasma-containing tubes, resulted in clot formation, confirming *S. aureus* as coagulase positive. These results collectively underscore the successful identification of *Staphylococcus aureus* in milk samples, highlighting the significance of employing a multi-faceted approach to ensure accurate detection within intricate matrices like milk.

Antibiogram analysis of Staphylococcus aureus isolated from milk samples

The analysis of *Staphylococcus aureus* antibiogram in this study revealed concerning resistance rates, with

Penicillin, Tetracycline, and Erythromycin showing 100%, 68.7%, and 75% resistance, respectively, against *Staphylococcus aureus* isolated from milk samples (Fig. 2).

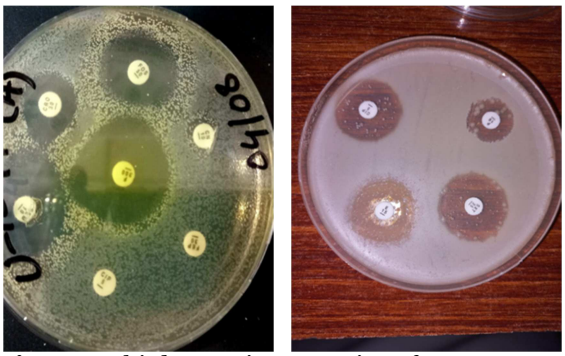

Fig. 2. Multi drug resistant strains of *S. aureus* on MHA isolated from milk

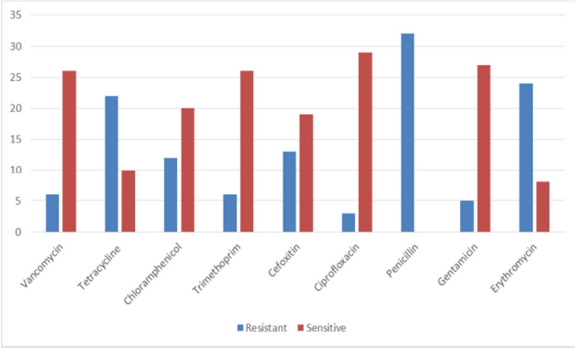

Fig. 3. Antibacterial susceptibility pattern of *Staphylococcus aureus*

However, Ciprofloxacin demonstrated notable efficacy at 90.6%, followed by Gentamicin (84.3%), Trimethoprim-Sulfamethoxazole and Vancomycin (both 81.2%). Chloramphenicol exhibited moderate effectiveness (62.2%), while Cefoxitin displayed lower efficacy at 59.3%. These findings highlight critical patterns of antibiotic resistance and emphasize the potential of Ciprofloxacin and other select antibiotics for effective treatment against *Staphylococcus aureus* infections in the study region. Efforts to combat rising antibiotic resistance remain imperative (Fig. 3).

Discussion

Staphylococcus aureus is commonly found as a commensal bacterium on the skin, within the nasal cavity, mucous membranes, pharynx, armpits, groin, and various other regions of the human body. Typically, it does not induce infections in healthy individuals. However, if it gains entry into the bloodstream or internal tissues, it can lead to severe infections. Notably, *S. aureus* ranks among the primary causative agents of bacterial infections in humans, encompassing a wide array of conditions such as osteomyelitis, septic arthritis, infections related to prosthetic devices, pulmonary infections (including pneumonia and empyema), meningitis, gastroenteritis, toxic shock syndrome, and urinary tract infections.

Staphylococcus aureus is an opportunistic bacterium frequently found in milk, thriving due to milk's bacteriological suitability. Contamination of milk can stem from mammary gland infections, suboptimal hygiene practices during milk handling, and human-associated transmission via nasal colonization by *S. aureus*.

In this study, the culture analysis of 100 samples revealed that (32%) were positive for *S. aureus*, while (68%) tested negative. These findings align with the prevalence rates reported in prior research. For instance, in a study conducted by (Halem *et al.,* 2019) in Alexandria, the prevalence of *S. aureus* in raw milk and dairy products was noted as 22.5%. Similarly, (Agban and Ahmed, 2013) detected *S. aureus* in 28% of raw milk samples collected from rural areas in Assiut governorate, corroborating our results.

Discrepancies in prevalence rates across regions are evident. For instance, the study by (Mansour e*t al.*, 2017) reported a lower *S. aureus* prevalence of 16.3% in raw milk samples from Cairo, Giza, and Kafr El-Sheikh governorates. This variation could be linked to disparities in farm development and agricultural practices, possibly influenced by more advanced techniques in that country.

In contrast to (Salem e*t al.*, 2016), who found 12.6% prevalence in Alexandria, our study revealed a higher *S. aureus* prevalence in milk samples (32%). This elevated contamination could stem from unhygienic milking and handling practices, potentially compromising milk quality.

The lack of proper training for milk handlers may contribute to this higher prevalence. This highlights the need for improved hygiene measures and training to mitigate contamination risks and ensure dairy product safety.

In contrast to (Usman e*t al.*, 2016) reporting 42.6% and 66.7% vancomycin resistance in Kaduna and Zaria, our study yielded different results. This divergence could arise from potential contamination of milk with vancomycin-resistant *S. aureus* from human sources. Notably, our study's high penicillin resistance echoes (Billa e*t al.*, 2014) in India, where a 100% resistance rate was observed. The notable prevalence of tetracycline and penicillin resistance may stem from their extensive use in dairy farm infection management. These variations highlight the intricate relationship between resistance, location, and agricultural practices, underscoring the need for robust surveillance and management strategies to counter antimicrobial resistance and ensure effective treatment options.

Machria (2016) reported susceptibility of all *S. aureus* isolates to Ciprofloxacin, Gentamicin, and Vancomycin, in contrast to our study where resistance to Penicillin (56%) and Tetracycline (22%) was observed. This discrepancy could arise from antibiotic misuse or unrestricted access. *S. aureus*, a common inhabitant of the respiratory tract and skin, poses significant challenges due to prevalent antibiotic resistance, particularly MRSA.

Conclusion

In conclusion, our study highlights the persistent prevalence of *S. aureus* contamination in cow milk within regional dairy farms. The concerning presence of multi-drug resistant isolates emphasizes the urgent need to address this challenge. The potential spread of antibiotic-resistant *S. aureus* poses risks to animals, caregivers, processors, and the wider population. Identifying hygiene deficiencies among milk handlers and the environment underscores the necessity for stringent mitigation measures. Enhancing hygiene practices, promoting judicious antibiotic use, and adopting comprehensive approaches are crucial to ensure both dairy product quality and public health.

Recommendations

Dissemination of information regarding the health risks associated with consuming contaminated raw milk is crucial. Public awareness campaigns should be conducted to discourage the consumption of untreated or inadequately treated raw milk.

Rigorous personal hygiene practices for milkers and proper hygiene maintenance for animals are imperative. Additionally, enhancing and sustaining the overall sanitary conditions of dairy farms are essential steps to prevent contamination.

Regular monitoring of antimicrobial susceptibility in pathogenic bacteria among animals is advised. This proactive approach can aid in early detection of emerging antibiotic resistance patterns and inform appropriate interventions.

Further research should be undertaken to explore the impacts and dynamics of genetic antibiotic determinants in *S. aureus*. Investigating these aspects can provide insights into the mechanisms underlying antibiotic resistance and guide effective control strategies

References

Abd El Halem SGJAJOFS, Technology. 2019. Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from raw milk and dairy products collected from Alexandria, Egypt. **16**(2), 25-33.

Agban MN, Ahmed ASJEJOMM. 2013. Detection and identification of *Staphylococcus aureus* enterotoxins in some milk products and their handlers. **22**(2), 101-112.

Billa G, Thakkar K, Jaiswar S, Dhodi DJAHE, Policy H. 2014. A cross-sectional study to evaluate the awareness and attitudes of physicians towards reducing the cost of prescription drugs, Mumbai. **12**, 125-137.

Cheung GY, Bae JS, Otto MJV. 2021. Pathogenicity and virulence of *Staphylococcus aureus*. **12**(1), 547-569.

Foster TJJFMR. 2017. Antibiotic resistance in *Staphylococcus aureus*. Current status and Future Prospects. **41**(3), 430-449.

Guo Y, Song G, Sun M, Wang J, Wang YJFIC, Microbiology I. 2020. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. **10**, 107.

Hiramatsu K, Kayayama Y, Matsuo M, Aiba Y, Saito M, Hishinuma T, Iwamoto AJJOGAR. 2014. Vancomycin-intermediate resistance in *Staphylococcus aureus*. **2**(4), 213-224.

Jamali H, Paydar M, Radmehr B, Ismail S, Dadrasnia AJFC. 2015. Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. **54**, 383-388.

Lee AS, De Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, Harbarth SJNRDP. 2018. Methicillin-resistant *Staphylococcus aureus*. **4**(1), 1-23.

Macharia JK. 2018. Prevalence, risk factors and antimicrobial resistance of *Escherichia coli* pathotypes isolated from livestock and rats in slums areas of Nairobi. University of Nairobi,

Machria M. 2016. Prevalence, susceptibility patterns and risk factors associated with *Staphylococcus aureus* presence in marketed milk and milk products within Nairobi city country, Kenya.

Makinde TM, Ako-Nai KA, Shittu AJAJOMR. 2019. A study on the isolation and analyzation of *Staphylococcus aureus* obtained from nasal samples of female students (Undergraduates and Graduates) at the Obafemi Awolowo University Campus. **7**(1), 24-27.

Mansour AS, Wagih GES, Morgan SD, Elhariri M, El-Shabrawy MA, Abuelnaga AS, Elgabry EJVW. 2017. Detection of *Staphylococcus aureus* enterotoxigenic strains in bovine raw milk by reversed passive latex agglutination and multiplex polymerase chain reaction, **10**(8), 843.

Pollitt EJ, Szkuta PT, Burns N, Foster SJJPP. 2018. *Staphylococcus aureus* infection dynamics. **14**(6), e1007112.

Rainard P, Foucras G, Fitzgerald JR, Watts J, Koop G, Middleton JJT, Diseases E. 2018. Knowledge gaps and research priorities in *Staphylococcus aureus* Mastitis Control. **65**, 149-165.

Regasa S, Mengistu S, Abraha AJVMI. 2019. Milk safety assessment, isolation, and antimicrobial susceptibility profile of *Staphylococcus aureus* in selected dairy farms of Mukaturi and Sululta town, Oromia Region, Ethiopia. 2019.

Salem H, El-Attar L, Omran EJJOH IOPH. 2016. Microbiological assessment of some parameters of Kariesh cheese sold by supermarkets and street vendors in Alexandria, Egypt. **46**(2), 77-85.

Umaru GA, Kabir J, Umoh VJ, Bello M, Kwaga JKJIJODR, Technology. 2013. Methicillin-resistant *Staphylococcus aureus* (MRSA) in fresh and fermented Milk in Zaria and Kaduna, Nigeria. **3**(3), 67-75.

Usman R, Mustapha B, Mohammed FJFS, Management Q. 2016. Isolation and identification of methicillin resistant *Staphylococcus aureus* (MRSA) from traditionally fermented milk "nono" and yoghurt in Kaduna Metropolis, Nigeria. **2**(2), 1-21.