

ANTIBIOTIC SUSCEPTIBILITY PROFILING OF *S. AUREUS* ISOLATED FROM CLINICAL BOVINE MASTITIS

SONIA TYAGI¹, PAMELA SINGH^{1*} AND VIPIN KHASA²

¹Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology,
Murthal, Sonipat 131 006, Haryana, India

²Department of Animal Husbandary, Sonipat, Haryana, India

(Received 21 March, 2022; Accepted 26 April, 2022)

Keywords: Mastitis, *S.aureus*, Antimicrobial susceptibility

Abstract– Mastitis is among the most common diseases in livestock farming, resulting in substantial financial losses associated with decreased milk yield. 53 milk samples were collected from dairy cows with clinical bovine mastitis from Sonipat region, Haryana to investigate the prevalence and antimicrobial susceptibility of *S. aureus* responsible for bovine mastitis. Bacterial isolation and identification by morphologic inspection and biotyping were performed on all the samples. The most common organism isolated in mastitis cases is *Staphylococcus aureus* (*S. aureus*), which was found in 41.5% of the cases. The present study showed a significant association of resistant pattern with *S.aureus* isolated, particularly to Azithromycin (36.3%), Chloroamphenicol (31.8%), Erythromycin (22%) along with Cefprozime (22%) and levofloxacin (22%). As a result, regular resistance monitoring employing antimicrobial sensitivity tests aids in the selection of effective antimicrobials and the prevention of drug resistance to routinely used antimicrobials.

INTRODUCTION

Mastitis in cattle is a multi-etiological condition characterized by microorganism-induced inflammation of the mammary glands, which is distinguished by physical, chemical, and, most commonly, bacteriological changes in milk (Zenebe *et al.* (2014); Tezera (2021)). Furthermore, it is the most economically significant disease in the production of dairy milk in the world (Zenebe *et al.* (2014); Cheng *et al.* (2010); Sharif *et al.* (2009)). Mastitis is a complex disease whose prevalence is influenced by a variety of factors including the animal, the environment, and the pathogen (Moges *et al.* (2012)). In clinical and subclinical bovine mastitis, the bacteria *Escherichia coli*, *Streptococcus uberis*, *S.aureus*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Staphylococcus epidermis*, and *Staphylococcus saprophyticus* have been identified (Song *et al.*, 2020); Saidi *et al.* (2019). *S.aureus*, a gram-positive bacteria, is the most infectious, ubiquitous, and economically important pathogen in dairy ruminants (Harshit *et al.*, 2018); Jahan *et al.* (2015)).

The increased predominance of this bacterium could be linked to its regular colonisation of teats, ability to survive intracellularly and locate within udder microabscesses, and hence resistance to antibiotic treatment (Zenebe *et al.*, 2014). *S. aureus* isolates are a serious concern for veterinary and health specialists, as well as dairy cattle farmers, due to their detrimental impact on therapy (Abera *et al.*, 2010). Because of the high prevalence of infectious mastitis and the importance of antimicrobial agents in the treatment and management of this infectious pathology, this multi-etiological infection continues to pique the interest of many researchers and is a topic of investigation for a number of organizations.

The main objective of this study was to illustrate the prevalence of clinical mastitis in dairy herds in the Sonipat region, identification of *S.aureus* as a significant aetiological agent as well as the antibiotic sensitivity profile of the isolated *S. aureus*, in order to provide efficient and appropriate treatment for animals suffering from clinical mastitis, as well as therapeutic intervention.

MATERIALS AND METHOD

Animals : From February to August 2020, the research was carried out at the Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, Sonipat. Lactating cows from dairy herds on different small holder farms in the Sonipat region have been examined.

Sampling : A total of 53 raw milk samples were collected from dairy cows with clinical bovine mastitis between October 2019 and January 2020 from Sonipat region, Haryana. Samples were collected under aseptic conditions for bacteriological studies as recommended by standard techniques. The first streams of milk were discarded before sampling, and the teat ends were cleansed with cotton swabs soaked in 70% alcohol and dried.

Isolation and identification of *S. aureus* from milk samples : A loopful of bacterial suspension was streaked on mannitol salt agar, which is differential media for *S.aureus* from each pre-cultured sample. Plates were kept inverted in an incubator at 37°C for 24 to 48 hours. Growth on selective agar and broth, colony morphology, and Gram's reaction, various biochemical tests (Catalase, Coagulase, IMViC) findings were used to identify the bacteria.

Antibiotic Sensitivity Profile : Antibiotic sensitivity testing of bacterial species was examined using Disc Diffusion Method (Bauer-Kirby)(Kirby *et al.* (1996)) for nine antimicrobial agents, including Erythromycin (15mcg), Ceftriaxone (30 mcg), Ampicillin/Sulbactam(10/10mcg), Amoxycylav (30mcg), Amikacin (30 mcg), Levofloxacin (5 mcg), Gentamycin (10 mcg), Azithromycin (15 mcg), Chloramphenicol (30 mcg).

RESULTS AND DISCUSSION

Haryana is one of the country's top milk producers, and raising cattle and buffalo helps farmers stay afloat, thrive as entrepreneurs, and contribute to the country's food security. Mastitis losses in terms of milk loss and treatment costs are one of the issues that this industry is facing. Among 53 samples, 30 yielded the bacterial growth on culture. Based on the morphological and biochemical features of colonies obtained on culture from these 30 samples, *Staphylococcus* was found to be the most common bacteria responsible for bovine mastitis. *Staphylococcus* growth was indicated by the production of white and golden colonies on nutrient agar, as well as a distinctive grape-like bunches pattern on gram staining. The yellow colored colonies on Mannitol Salt Agar also confirmed the

Table 1. Biochemical analysis of *S.aureus* isolates

Sample id	Catalase	Coagulase	Indole	Methyl-Red	Voges Proskeur	Citrate
SA1	+	+	-	+	+	+
SA2	+	+	-	+	+	+
SA3	+	+	-	+	+	+
SA6	+	+	-	+	+	+
SA7	+	+	-	+	+	+
SA9	+	+	-	+	+	+
SA10	+	-	-	+	+	+
SA11	+	-	-	+	+	+
SA12	+	+	-	+	+	+
SA15	+	+	-	+	+	+
SA17	+	+	-	+	+	+
SA18	+	+	-	+	+	+
SA21	+	-	-	+	+	+
SA25	+	+	-	+	+	+
SA27	+	+	-	+	+	+
SA28	+	+	-	+	+	+
SA33	+	+	-	+	+	+
SA36	+	-	-	+	+	+
SA41	+	+	-	+	+	+
SA47	+	-	-	+	+	+
SA49	+	+	-	+	+	+
SA53	+	-	-	+	+	+

Table 2. Resistance of *S.aureus* isolates to different antimicrobials (n=22)

Antimicrobial agents	Interpretations		
	Susceptible (%)	Intermediate (%)	Resistant (%)
Azithromycin	14.4	49.3	36.3
Erythromycin	30.8	47.2	22.0
Chloramphenicol	21.0	47.2	31.8
Ceftizoxime	30.8	47.2	22.0
Gentamycin	30.6	51.3	18.1
Levofloxacin	25.7	52.3	22.0
Amikacin	47.2	48.3	4.5
Amoxiclav	26.6	55.3	18.1
Ampicillin/sulbactam	43.2	43.2	13.6

presence of *S.aureus*. Biochemical characterisation demonstrating the representative nature of the samples was used to confirm the findings (Table 1).

In present study, the overall prevalence of *S.aureus* with regard to the bacteriological analysis of milk samples in mastitic dairy herds was 41.5%. This result was in line with a prevalence of 42.8 as reported by Duguma *et al.*, 2018. Besides this result was in coherence with the results of Verma *et al.*, 2018 (Prevalence of *S. aureus* : 42.55%). This was closely comparable with the findings of Algammal *et al.* (2020) and Tessema and Soloman (2017) who reported 35.9% and 35.3% prevalence respectively in their studies. However, the current prevalence is lower when compared with the previous findings as 56.3% was reported by Tassew *et al.* (2017) and lower in comparison with Srednik *et al.* (2018). This variability of mastitis between different reports could be attributed to differences in farms management practices, herd size, genetic differences and environmental factors. The high prevalence of *S.aureus* could be due to its contagious nature and it has been better adapted to survive in the udder and establish chronic infections.

A total of 22 samples were tested for antibiotic susceptibility using 9 antimicrobial agents after testing positive for *S. aureus*. The overall antibiogram profile indicated Amikacin (47.2%), Ampicillin/Sulbactam (43.2), Erythromycin (30.8%), Ceftizoxime (30.8) and Gentamicin (30.6) to be the most effective antimicrobials against *S.aureus* mediated bovine mastitis in the study. The isolates were found to be resistant against Azithromycin (36.3%), Chloroamphenicol (31.8%), Erythromycin along with Ceftizoxime and levofloxacin (22%). These findings are not in agreement with those of Jahan *et al.* (2015) who depicted a higher resistance to erythromycin (75%) and Hazari *et al.*, 2018 with

resistance of Amoxiclav (68.29), Gentamycin (17.6) and Erythromycin (11.58). The results of the present study are not in agreement with the findings of Liu *et al.* (2017) who reported a lower resistant against Gentamicin (11.1%) and Chloramphenicol (7.41). The bacteria showed less sensitivity to commonly used antibiotics Azithromycin and chloramphenicol due to frequent use of these antibiotics. The ineffectiveness of these antibiotics against *S.aureus* could be due to their indiscriminate and frequent usage in animals. In this study Gentamicin, Chloramphenicol and Amikacin proved to be the highly sensitive to mastitic agents which is partially in agreement of the study conducted by Al-Ashmawy *et al.* (2016). The findings are consistent with Verma *et al.* (2018) previous reports from other states' studies suggesting that extended and indiscriminate use of some antimicrobials could lead to the development of resistance. This can be related to the inappropriate use of antibiotics, antimicrobial selection pressure on pathogens, or resistant strain colonisation of the mammary gland, as well as a lack of bacteriological investigation of mastitis-suspected milk. It is therefore critical to use an in vitro antibiotic susceptibility test in a systemic manner prior to the administration of antibiotics in the treatment and prevention of intra-mammary infections.

CONCLUSION

The majority of the tested isolates were shown to be resistant to various antimicrobial agents which are Azithromycin (36.3%), Chloramphenicol (31.8%), Erythromycin along with Ceftizoxime and levofloxacin (22%). As a result, regular antimicrobial sensitivity testing is required to select effective medications and to alter drugs as needed.

REFERENCES

- Abera, M., Demie, B., Aragaw, K., Regassa, F. and Regassa, A. 2010. Isolation and identification of *S.aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. *Journal of Veterinary Medicine and Animal Health*. 2(3) : 29-34.
- Al-Ashmawy, M. A., Sallam, K. I., Abd-Elghany, S. M., Elhadidy, M. and Tamura, T. 2016. Prevalence, molecular characterization, and antimicrobial susceptibility of methicillin-resistant *S.aureus* isolated from milk and dairy products. *Foodborne Pathogens and Disease*. 13(3): 156-162.
- Algammal, A. M., Enany, M. E., El-Tarabili, R. M., Ghobashy, M. O. and Helmy, Y. A. 2020. Prevalence, antimicrobial resistance profiles, virulence and enterotoxins-determinant genes of MRSA isolated from subclinical bovine mastitis in Egypt. *Pathogens*. 9(5) : 362.
- Kirby, W. M., Bauer, A. W., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 45(4) : 493-6.
- Cheng, D., Zhu, S., Yin, Z., Ding, W., Mu, Z., Su, Z. and Sun, H. 2010. Prevalence of bacterial infection responsible for bovine mastitis. *African Journal of Microbiology Research*. 4(11) : 1110-1116.
- Duguma, A., Wirtu, A. and Abunna, F. 2018. Isolation and identification of *S.aureus* from dairy farms in Bishoftu town, Ethiopia. *JOJ Pub Health*. 3(1): 555604.
- Hazari, R., Hirpurkar, S. D., Sannat, C., Rawat, N. and Kushwaha, K. 2018. Antimicrobial drug resistance of *S.aureus* from clinical bovine mastitis in Chhattisgarh State.
- Jahan, M., Rahman, M., Parvej, M. S., Chowdhury, S. M. Z. H., Haque, M. E., Talukder, M. A. K. and Ahmed, S. 2015. Isolation and characterization of *S.aureus* from raw cow milk in Bangladesh. *Journal of Advanced Veterinary and Animal Research*. 2(1) : 49-55.
- Liu, H., Li, S., Meng, L., Dong, L., Zhao, S., Lan, X. and Zheng, N. 2017. Prevalence, antimicrobial susceptibility, and molecular characterization of *S.aureus* isolated from dairy herds in northern China. *Journal of Dairy Science*. 100(11) : 8796-8803.
- Moges, N., Hailemariam, T., Fentahun, T., Chanie, M. and Melaku, A. 2012. Bovine mastitis and associated risk factors in small holder lactating dairy farms in Hawassa, Southern Ethiopia. *Global Veterinaria*. 9(4): 441-446.
- Saidi, R., Mimoune, N., Baazizi, R., Benaissa, M. H., Khelef, D. and Kaidi, R. 2019. Antibiotic susceptibility of Staphylococci isolated from bovine mastitis in Algeria. *Journal of Advanced Veterinary and Animal Research*. 6(2): 231.
- Sharif, A., Umer, M. U. H. A. M. M. A. D. and Muhammad, G. H. U. L. A. M. 2009. Mastitis control in dairy production. *J. Agric. Soc. Sci*. 5 : 102-105.
- Song, X., Huang, X., Xu, H., Zhang, C., Chen, S., Liu, F. and Wu, C. 2020. The prevalence of pathogens causing bovine mastitis and their associated risk factors in 15 large dairy farms in China: an observational study. *Veterinary Microbiology*. 247: 108757.
- Srednik, M. E., Usongo, V., Lépine, S., Janvier, X., Archambault, M. and Gentilini, E. R. 2018. Characterisation of *S. aureus* strains isolated from mastitis bovine milk in Argentina. *Journal of Dairy Research*. 85(1) : 57-63.
- Tassew, A., Aki, A. and Legesse, K. 2017. Isolation, Identification and Antimicrobial Resistance Profile of *S. aureus* and Occurrence of Methicillin Resistant *S. aureus* Isolated from Mastitic Lactating Cows in and around Assosa Town, Benishangul Gumuz Region, Ethiopia. *J. Dairy Vet. Anim. Res*. 6 : 180.
- Tessema, D. and Tsegaye, S. 2017. Study on the Prevalence and Distribution of *S. aureus* in Raw Cow Milk Originated from AlageAtvet College Dairy Farm, Ethiopia. *Journal of Nutrition & Food Sciences*. 7(2) : 2-5.
- Tezera, M. and Aman Ali, E. 2021. Prevalence and associated risk factors of Bovine mastitis in dairy cows in and around Assosa town, Benishangul Gumuz Regional State, Western Ethiopia. *Veterinary Medicine and Science*. 7(4) : 1280-1286.
- Verma, H., Rawat, S., Sharma, N., Jaiswal, V., Singh, R. and Harshit, V. 2018. Prevalence, bacterial etiology and antibiotic susceptibility pattern of bovine mastitis in Meerut. *J Entomol Zool Stud*. 6(1) : 706-709.
- Zenebe, N., Habtamu, T. and Endale, B. 2014. Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia. *African Journal of Microbiology Research*. 8(4) : 327-331.