

Prevalence of selected virulence factors of *Staphylococcus aureus* isolated in bovine mastitis in Chaharmahal and Bakhtiari province- Iran

Behnam Rozbahan¹, Naser Shams Esfandabadi^{2*}, Ali Kadivar³, Azam Mokhtari⁴ and Najmeh Davoodian⁵

¹ DVSc Student of Theriogenology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

² Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

³ Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

⁴ Associate Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

⁵ Associate Professor, Research Institute of Animal Embryo Technology, Shahrekord University, Shahrekord, Iran

Received: 11.09.2023

Accepted: 08.01.2024

Abstract

It has been determined that there is a direct relationship between the severity of mastitis and the virulence factors produced by bacterial agents. Identifying bacterial virulence factors is necessary for designing suitable vaccines against mastitis. The aim of the present study was molecular diagnosis of selected virulence factors of endemic isolates of *S. aureus* involved in bovine mastitis. A total of 180 milk samples were collected from cows with clinical (37 samples, 20.6%) and subclinical (143 samples, 79.4%) mastitis from 8 semi-industrial dairy farms of Chaharmahal and Bakhtiari province, Iran. After culture and purification, coagulase, catalase and oxidase tests were performed. DNA was extracted from *S. aureus* suspected colonies. Final confirmation was performed using PCR test on the specific 23S rRNA gene of the bacteria. Thirty one (17.22%) out of the 180 collected samples were found to be positive for *S. aureus* by PCR, of which 2 cases were related to clinical mastitis and 29 cases were related to subclinical mastitis. The highest frequency of virulence genes was related to the Coa gene (90.32%), followed by ClfB (87.09%), LukD, and fnbB (80.64%), LukE (77.41%), fnbA (74.19%), Hla (48.38%). The lowest frequency was related to the Hlb gene (45.16%). Based on the obtained results, the diagnosis of the virulence factors of *S. aureus* has the potential of being used in the development of vaccines for the prevention of mastitis.

Key word: Mastitis, *Staphylococcus aureus*, Virulence factors

* **Corresponding Author:** Naser Shams Esfandabadi, Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
E-mail: drn_shams@yahoo.com



© 2020 by the authors. Licensee SCU, Ahvaz, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0 license) (<http://creativecommons.org/licenses/by-nc/4.0/>).

References

- Abad, H. E. K., Sadeghi, J., Aghazadeh, M., Ahangarzadeh Rezaee, M., Samadi Kafil, H., & Ahangar Oskouee, M. (2020). Frequency of *fnbA*, *fnbB*, *hla* and *cna* genes in *Staphylococcus aureus* isolates obtained from blood cultures and their antimicrobial susceptibility pattern in Tabriz, Iran. *Archives of Pharmacy Practice*, *11*(S1), 137-43.
- Bennett, M. R., & Thomsen, I. P. (2020). Epidemiological and clinical evidence for the role of toxins in *S. aureus* human disease. *Toxins*, *12*(6), 408.
- Bohach, G. A. (2006). *Staphylococcus aureus* exotoxins. *Gram-Positive Pathogens*, 464-477.
- Divyakolu, S., Chikkala, R., Ratnakar, K. S., & Sritharan, V. (2019). Hemolysins of *Staphylococcus aureus*—An update on their biology, role in pathogenesis and as targets for anti-virulence therapy. *Advances in Infectious Diseases*, *9*(2), 80-104.
- Dubin, G., Koziel, J., Pyrc, K., Wladyka, B., & Potempa, J. (2013). Bacterial proteases in disease—role in intracellular survival, evasion of coagulation/fibrinolysis innate defenses, toxicoses and viral infections. *Current pharmaceutical design*, *19*(6), 1090-1113.
- Effendi, M. H., Hisyam, M. A. M., Hastutiek, P., & Tyasningsih, W. (2019). Detection of coagulase gene in *Staphylococcus aureus* from several dairy farms in East Java, Indonesia, by polymerase chain reaction. *Veterinary World*, *12*(1), 68-71.
- Fowler, T., Wann, E. R., Joh, D., Johansson, S., Foster, T. J., & Höök, M. (2000). Cellular invasion by *Staphylococcus aureus* involves a fibronectin bridge between the bacterial fibronectin-binding MSCRAMMs and host cell β 1 integrins. *European journal of cell biology*, *79*(10), 672-679.
- Geoghegan, J. A., & Foster, T. J. (2017). Cell wall-anchored surface proteins of *Staphylococcus aureus*: many proteins, multiple functions. *Staphylococcus aureus: Microbiology, Pathology, Immunology, Therapy and Prophylaxis*, 95-120.
- Gill, S.R., Fouts, D.E., Archer, G.L., Mongodin, E.F., DeBoy, R.T., Ravel, J., & et al. (2005). Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. *Journal of bacteriology*, *187*(7), 2426-2438.
- Holtzhauer, M. (2006). *Basic methods for the biochemical lab*. Berlin, Heidelberg: Springer Berlin Heidelberg.
- Jain, V. K., Singh, M., Joshi, V. G., Chhabra, R., Singh, K., & Rana, Y. S. (2022). Virulence and antimicrobial resistance gene profiles of *Staphylococcus aureus* associated with clinical mastitis in cattle. *Plos one*, *17*(5), e0264762.
- Jung, H. R., & Lee, Y. J. (2022). Characterization of Virulence Factors in Enterotoxin-Producing *Staphylococcus aureus* from Bulk Tank Milk. *Animals*, *12*(3), 301.
- Lacey, K. A., Mulcahy, M. E., Towell, A. M., Geoghegan, J. A., & McLoughlin, R. M. (2019). Clumping factor B is an important virulence factor during *Staphylococcus aureus* skin infection and a promising vaccine target. *PLoS pathogens*, *15*(4), e1007713.
- Lee, I. W., Kang, L., Hsu, H. P., Kuo, P. L., & Chang, C. M. (2010). Puerperal mastitis requiring hospitalization during a nine-year period. *American journal of obstetrics and gynecology*, *203*(4), 332-e1.
- Leitão, J. H. (2020). Microbial virulence factors. *International journal of molecular sciences*, *21*(15), 5320.
- Magro, G., Biffani, S., Minozzi, G., Ehricht, R., Monecke, S., Luini, M., & Piccinini, R. (2017). Virulence genes of *S. aureus* from dairy cow mastitis and contagiousness risk. *Toxins*, *9*(6), 195.
- McAdow, M., Missiakas, D. M., & Schneewind, O. (2012). *Staphylococcus aureus* secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infections. *Journal of innate immunity*, *4*(2), 141-148.
- Mohammadi, M., & Faghri, J. (2019). Genetic diversity of *Staphylocoagulase* genes (Coa) among methicillin-resistant *Staphylococcus aureus* isolates at clinical specimens of blood and urinary infections. *Tehran University Medical Journal*, *77*(4), 208-215.
- Mulcahy, M. E., Geoghegan, J. A., Monk, I. R., O'Keefe, K. M., Walsh, E. J., Foster, T. J., & McLoughlin, R. M. (2012). Nasal colonisation by *Staphylococcus aureus* depends upon clumping factor B binding to the squamous epithelial cell envelope protein loricrin. *PLoS pathogens*, *8*(12), e1003092.

- Momtaz, H., Rahimi, E., & Tajbakhsh, E. (2010). Detection of some virulence factors in *Staphylococcus aureus* isolated from clinical and subclinical bovine mastitis in Iran. *African Journal of Biotechnology*, 9(25), 3753-3758.
- Niehues, H. (2017). *From sequence to function: Understanding of genetic risk factors for psoriasis and atopic dermatitis* (Doctoral dissertation, [SI: sn]).
- Niemann, H. H., Schubert, W. D., & Heinz, D. W. (2004). Adhesins and invasins of pathogenic bacteria: a structural view. *Microbes and infection*, 6(1), 101-112.
- Nickerson, S. C., Saxon, A., Fox, L. K., Hemling, T., Hogan, J. S., Morelli, J., ... & Petersson, L. (2004). National Mastitis Council: Recommended protocols for evaluating efficacy of postmilking teat germicides. In *NMC Annual Meeting Proceedings* (pp. 379-399).
- Oaks Jr, S. C., Shope, R. E., & Lederberg, J. (Eds.). (1992). *Emerging infections: microbial threats to health in the United States*. National Academies Press.
- Oliveira, D., Borges, A., & Simões, M. (2018). *Staphylococcus aureus* toxins and their molecular activity in infectious diseases. *Toxins*, 10(6), 252.
- Pacha PA, Munoz MA, Paredes-Osses E, Latorre AA. Virulence profiles of *Staphylococcus aureus* isolated from bulk tank milk and adherences on milking equipment on Chilean dairy farms. *Journal of dairy science*. 2020 May 1;103(5):4732-4737.
- Parker, D. (2018). A live vaccine to *Staphylococcus aureus* infection. *Virulence*, 9(1), 700-702.
- Peterson, J. W. (1996). *Bacterial pathogenesis. Medical Microbiology. 4th edition*.
- Pollard, B. S., & Pollard, H. B. (2018). Induced pluripotent stem cells for treating cystic fibrosis: state of the science. *Pediatric Pulmonology*, 53(S3), S12-S29.
- Rohmer, C., & Wolz, C. (2021). The role of hlb-converting bacteriophages in *Staphylococcus aureus* host adaptation. *Microbial Physiology*, 31(2), 109-122.
- Salgado-Pabón, W., Herrera, A., Vu, B. G., Stach, C. S., Merriman, J. A., Spaulding, A. R., & Schlievert, P. M. (2014). *Staphylococcus aureus* β -toxin production is common in strains with the β -toxin gene inactivated by bacteriophage. *The Journal of infectious diseases*, 210(5), 784-792.
- Seilie, E. S., & Wardenburg, J. B. (2017, December). *Staphylococcus aureus* pore-forming toxins: The interface of pathogen and host complexity. In *Seminars in cell & developmental biology* (Vol. 72, pp. 101-116). Academic Press.
- Seyedi-Marghaki, F., Kalantar-Neyestanaki, D., Saffari, F., Hosseini-Nave, H., & Moradi, M. (2019). Distribution of aminoglycoside-modifying enzymes and molecular analysis of the coagulase gene in clinical isolates of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. *Microbial Drug Resistance*, 25(1), 47-53.
- Soltani, E., Farrokhi, E., Zamanzad, B., Shahini Shams Abadi, M., Deris, F., Soltani, A., & Gholipour, A. (2019). Prevalence and distribution of adhesins and the expression of fibronectin-binding protein (FnB A and FnB B) among *Staphylococcus aureus* isolates from Shahrekord Hospitals. *BMC Research Notes*, 12(1), 1-5.
- Sommerhäuser, J., Kloppert, B., Wolter, W., Zschöck, M., Sobiraj, A., & Failing, K. (2003). The epidemiology of *Staphylococcus aureus* infections from subclinical mastitis in dairy cows during a control programme. *Veterinary microbiology*, 96(1), 91-102.
- Tam, K., & Torres, V. J. (2016). *Staphylococcus aureus* Secreted Toxins & Extracellular Enzymes Kayan. *Physiology & Behavior*, 176, 139-148.
- Tan, L., Li, S. R., Jiang, B., Hu, X. M., & Li, S. (2018). Therapeutic targeting of the *Staphylococcus aureus* accessory gene regulator (agr) system. *Frontiers in microbiology*, 9, 55.
- Tegegne, D. T., Mamo, G., Waktole, H., & Messele, Y. E. (2021). Molecular characterization of virulence factors in *Staphylococcus aureus* isolated from bovine subclinical mastitis in central Ethiopia. *Annals of Microbiology*, 71(1), 1-8.
- Wu, S. C., Liu, F., Zhu, K., & Shen, J. Z. (2019). Natural products that target virulence factors in antibiotic-resistant *Staphylococcus aureus*. *Journal of agricultural and food chemistry*, 67(48), 13195-13211.

- Yamada, T., Tochimaru, N., Nakasuji, S., Hata, E., Kobayashi, H., Eguchi, M., Kaneko, J., Kamio, Y., Kaidoh, T., & Takeuchi, S. (2005). Leukotoxin family genes in *Staphylococcus aureus* isolated from domestic animals and prevalence of lukM–lukF-PV genes by bacteriophages in bovine isolates. *Veterinary microbiology*, *110*(1-2), 97-103.
- Yazarlu, O., Iranshahi, M., Kashani, H. R. K., Reshadat, S., Habtemariam, S., Iranshahy, M., & Hasanpour, M. (2021). Perspective on the application of medicinal plants and natural products in wound healing: A mechanistic review. *Pharmacological research*, *174*, 105841.
- Zadoks, R. N., Allore, H. G., Barkema, H. W., Sampimon, O. C., Wellenberg, G. J., Gröhn, Y. T., & Schukken, Y. H. (2001). Cow-and quarter-level risk factors for *Streptococcus uberis* and *Staphylococcus aureus* mastitis. *Journal of Dairy Science*, *84*(12), 2649-2663.