Ciência Rural, Santa Maria, v.51:4, e20200417, 2021
http://doi.org/10.1590/0103-8478cr20200417
ISSNe 1678-4596
MICROBIOLOGY

In vitro efficacy of iodine in the pre and post *dipping* against coagulase negative *Staphylococcus* isolated in milk of cows with subclinical mastitis

Daniel Dias da Silva[1*] Elizabeth Sampaio de Medeiros[1] Maria Betânia de Queiroz Rolim[1] Ana Virgínia Marinho[1] Karla Danielle Almeida Soares[1] Gledson Luiz Pontes de Almeida[2] Luiz Moreira Pereira Neto[3] Tamyres Izarelly Barbosa da Silva[3]

[1]Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco (UFRPE), 52171-900, Recife, PE, Brasil. E-mail: diasmedvet@gmail.com *Corresponding author.
[2]Departamento de Tecnologia Rural, Universidade Federal Rural de Pernambuco (UFRPE), Recife, PE, Brasil.
[3]Unidade de Ensino e Pesquisa em Medicina Veterinária, Universidade Federal do Acre (UFAC), Rio Branco, AC, Brasil.

ABSTRACT: *The efficiency of the disinfectants used in the milking management is fundamental to the success in the dairy activity, being a critical point to the control of mastitis. The objective was to evaluate the in vitro efficacy of iodine used in pre and post-dipping against coagulase negative Staphylococcus (CNS). Thus, 53 CNS isolates were studied for the action of the 1.0% disinfectant and their serial dilutions of 0.5%, 0.375% and 0.25%, in addition to two commercial presentations of iodine in concentrations of 0.5% and 0.25%. The rate of CNS inhibition achieved by iodine at 0.375%, 0.5% and 1.0% for 60 seconds, was 60.4%. In 30 seconds, iodine at 0.5% and 1.0% showed a microbial inhibition rate of 52.8% and 56.6%, respectively. The other protocols tested were less efficient. It is concluded that the greatest in vitro disinfectant activity for CNS was demonstrated by iodine at 0.375%, 0.5% and 1.0%, for 60 seconds. Microbial susceptibility tests should be carried out periodically, as well as health education practices and corrective training on the property, in order to ensure udder health and mastitis control.*
Key words: *disinfectant, iodized solution, mammary gland, Staphylococcus spp.*

Eficácia *in vitro* do iodo no pré e pós *dipping* frente a *Staphylococcus* coagulase negativa isolados em leite de vacas com mastite subclínica

RESUMO: *A eficiência dos desinfetantes empregados no manejo de ordenha é fundamental no sucesso na atividade leiteira, sendo um ponto crítico ao controle da mastite. Objetivou-se avaliar a eficácia in vitro do iodo utilizado no pré e pós-dipping frente à Staphylococcus coagulase negativa (SCN). Foram estudados 53 isolados de SCN quanto à ação do desinfetante a 1,0% e suas diluições seriadas de 0,5%, 0,375% e 0,25%, além de duas apresentações comerciais nas concentrações de 0,5% e 0,25%. A taxa de inibição de SCN alcançada pelo iodo a 0,375%, 0,5% e 1,0% durante 60 segundos, foi de 60,4%. Em 30 segundos, o iodo a 0,5% e 1,0% apresentaram taxa de inibição microbiana de 52,8% e 56,6%, respectivamente. Os demais protocolos testados foram menos eficientes. Conclui-se que a maior atividade desinfetante in vitro para SCN foi demonstrada pelo iodo a 0,375%, 0,5% e 1,0%, durante 60 segundos. Testes de susceptibilidade microbiana devem ser realizados periodicamente, assim como práticas de educação em saúde e treinamentos corretivos na propriedade, visando garantir a saúde do úbere e o controle da mastite.*
Palavras-chave: *desinfetante, solução iodada, glândula mamária, Staphylococcus spp.*

Mastitis is an inflammation of the mammary gland, caused mainly by bacteria, in clinical or subclinical forms (ADKINS & MIDDLETON, 2018). The disease is responsible for limitations in dairy farms across the planet (WEN et al., 2019; MBINDYO et al., 2020), leading to a reduction in the quantity and quality of milk, changing its main components and resulting in economic losses in production (HEIKKILÄ et al., 2018). Thus, hygienic-sanitary practices and periodic monitoring tests are necessary in order to reduce losses (AGHAMOHAMMADI et al., 2018; GUSSMANN et al., 2019).

Staphylococcus spp. are the main agents of subclinical mastitis in cattle and are associated with the formation of biofilms, due to the adhesion and fixation of bacterial cells on surfaces. These biofilms cause greater difficulty in the penetration of antimicrobials, due to the formation of a three-dimensional polymeric structure, and their control is a challenge in the food industry and public health

Received 05.05.20 Approved 10.02.20 Returned by the author 11.14.20
CR-2020-0417.R1

worldwide (UNLU et al., 2018; GIAOURIS et al., 2020). In addition, the indiscriminate use of drugs also contributes to lower microbial susceptibility (HOEKSTRA et al., 2019).

Several sanitary measures must be adopted during the milking process to minimize the transmission of pathogens, such as *Staphylococcus*, as well as the risks to consumers (BELAGE et al. 2017; JANS et al., 2017; JANSEN et al., 2019). Disinfection of teats before and after milking with antimicrobial products, such as iodine, chlorine, chlorhexidine or quaternary ammonia, is a widely used practice in the prevention of mastitis (RUEGG, 2017; ROWE et al., 2018). The efficiency of these disinfectants is fundamental to the success in the dairy activity, being a critical point in the control of the disease (SKOWRON et al., 2019).

In this context, the objective of this study was to determine the *in vitro* efficacy of iodine used in pre and post-dipping against coagulase negative *Staphylococcus* (CNS), isolated from the milk of cows with subclinical mastitis.

Were analyzed 53 CNS isolates, identified in the milk of different cows with subclinical mastitis, from the municipalities of Batalha, Major Isidoro, Monteirópolis and Palmeira dos Índios, from the dairy basin of the state of Alagoas. Herds were made up of crossbred bovine females, of different age groups and stages of lactation, raised in a semi-intensive manner, with mechanical milking systems.

The milk samples were collected after previous washing of the ceiling with soap and water, drying with paper towels and antisepsis of the ceiling ostium with alcohol at 70 ° GL. Were collected 5mL of milk, in sterile tubes and previously identified with the number of the animal and the mammary quarter. The samples were sent refrigerated in isothermal boxes at the Meat and Milk Inspection Laboratory, at the Federal Rural University of Pernambuco.

Milk aliquots were seeded on base agar enriched with 5% defibrinated sheep blood, incubated at 37 °C and readings were taken at 24 and 48 hours. For the bacterial identification, the morphological characteristics of the colonies were observed, such as diameter, texture, color and hemolytic activity. In microscopy, the cells' disposition and morphotintorial characteristics in the *Gram* test were observed. To differentiate *Staphylococcus* spp. in negative coagulase, the production of free coagulase in a tube was tested (MURRAY et al., 2017).

To evaluate the action of disinfectants, 1.0% iodine was used and the following serial dilutions of 0.5%, 0.375% and 0.25%, according to concentrations used in previous works (MEDEIROS et al., 2009; LOPES et al., 2013; SILVA et al., 2015; DUTRA et al., 2017). Two commercial iodine-based disinfectants were also tested at concentrations of 0.5% and 0.25%.

Homogeneous bacterial suspensions were prepared in sterile saline solution (5.5 mL), corresponding to tube 1 of the McFarland scale. The suspension consisted of a disinfectant (0.8 mL) in the tested concentration and sterile milk (0.2 mL). The bacterial suspension (1.2 mL) was added and the exposure period (30 and 60 seconds) was timed, followed by raising the suspension in Brain Heart Infusion broth. The mixture was incubated at 37 °C for 24 hours to observe the turbidity of the medium, the formation of a film on the surface or a precipitate on the bottom of the tubes. After incubation, the suspension was streaked on a solid medium (5% blood agar) to confirm the presence or absence of CNS against different iodine concentrations and exposure periods. The rates of inhibition of bacterial growth indicated the effectiveness of the protocol in question (SANTOS et al., 2018). For data evaluation, the Tukey test was applied at the level of 5% probability (SILVA & AZEVEDO, 2016).

According to the results (Table 1), there was a greater *in vitro* disinfectant activity of iodine at concentrations of 0.375%, 0.5% and 1.0% for 60 seconds against CNS, with a microbial inhibition rate of 60.4%, no statistic difference between them.

In the 30-second exposure period, 0.5% and 1.0% iodine concentrations were more efficient against CNS, with a microbial inhibition rate of 52.8% and 56.6%, respectively, with no statistic difference between both.

However, the use of disinfectant in a concentration greater than or equal to 0.375% for 60 seconds demonstrated greater bacterial susceptibility, when compared to the period of 30 seconds. Regarding diluted and commercial iodine at 0.25%, there was less effectiveness against SCN, regardless of the exposure period.

Preliminary studies in Brazil have demonstrated the efficacy of iodine for CNS at different concentrations, periods of exposure and rates of microbial inhibition (MEDEIROS et al., 2009; RAMALHO et al., 2012; SANTOS et al., 2018). According to the work of Fonseca & Santos (2000), iodine 0.3 to 1.0% used in the handling of milking showed satisfactory results, similar to that found in the present study, which demonstrates that this disinfectant is still effective and can be used in

Table 1 - Efficacy of iodine at different concentrations and exposure period to control bovine mastitis caused by coagulase negative *Staphylococcus*.

Concentration of disinfectant	Exposure period (seconds)	----------Microbial inhibition (n=53)----------	
		AF*	RF (%)
Iodine 0.25%	30”	7^{dB}	13.2
	60”	16^{cA}	30.2
Iodine 0.375%	30”	24^{bB}	45.3
	60”	32^{aA}	60.4
Iodine 0.5%	30”	28^{aB}	52.8
	60”	32^{aA}	60.4
Iodine 1.0%	30”	30^{aB}	56.6
	60”	32^{aA}	60.4
Commercial iodine 0.25%	30”	8^{dB}	15.1
	60”	12^{dA}	22.6
Commercial iodine 0.5%	30”	18^{cB}	34
	60”	21^{bA}	39.6

Subtitle: AF – absolute frequency; RF – relative frequency; *Different letters show statistically significant differences in Tukey’s test ($P<0.05$).

mastitis control protocols, given the sensitivity of the evaluated CNS strains.

The most suitable disinfectant, which aims to guarantee satisfactory results in subclinical mastitis control programs, must have a better cost-benefit ratio, stability, validity, chemical safety and a high rate of microbial inhibition. Iodine, specifically, is effective in controlling *Staphyloccocus* spp., but its use in low concentrations is recommended, preferably less than or equal to 0.5%, given the residues in the milk and the chemical aggression to the teats (LOPES et al., 2013; BACH et al., 2019).

Thus, depending on the results obtained, it is believed that the use of iodine at 0.375% or 0.5% under 60 seconds of exposure would be the most viable for use against CNS in the evaluated region, for having a microbial inhibition rate similar to 1.0% iodine, low risk of residues in milk, low potential for aggression to the skin of the teats and is still more economically viable to the producer, presenting better cost-benefit.

Another aspect to highlight is that, according to Lopes et al. (2013), commercial iodine solutions in Brazil are the best options in milking management. However, a lower index of sensitivity of commercial iodine for CNS was observed, a fact of concern, since the use of commercial presentation is a reality adopted by most producers, due to its low cost and practicality.

The production of good quality milk in the country, within hygienic-sanitary standards, is a challenge for producers (DATTA et al., 2019). In the case of dairy industries, biofilms can form on surfaces, being associated with hygiene failures in milking (GARVEY et al., 2017). These biofilms represent a critical point in the sector, since bacterial cells can detach themselves from the matrix and contaminate food, leading to early product deterioration and risks to Unique Health, as it is a potential source of pathogenic microorganisms to dairy consumers (UNLU et al., 2018; GIAOURIS et al., 2020). Pre and post dipping is essential to reduce contamination of teats before and after milking, as well as the formation of biofilms (BACH et al., 2019).

Finally, it is concluded that the greatest in vitro disinfectant activity for SCN was demonstrated by iodine at 0.375%, 0.5% and 1.0%, during 60 seconds of exposure. However, the use of these compounds has not completely eliminated the contamination. Microbial susceptibility tests should be carried out periodically, as well as health education practices and corrective training on the property, in order to ensure udder health and mastitis control.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Animal Use Ethics Committee of the Universidade Federal Rural de Pernambuco, under license number 37/2018.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest. The funding entities had no influence on the study design; nor in the collection, analysis or interpretation of the data; in the writing of the manuscript, nor in the decision to publish the results.

ACKNOWLEDGEMENTS

For the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil, for the scholarship funding.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

ADKINS, P. R. F.; MIDDLETON, J. R. Methods for diagnosing mastitis. **Veterinary Clinics of North America**: Food Animal Practice, v.34, n.3, p.479-491, 2018. Available from: <https://doi.org/10.1016/j.cvfa.2018.07.003>. Accessed: Apr. 29, 2020.

AGHAMOHAMMADI, M. et al. Herd-level mastitis-associated costs on canadian dairy farms. **Frontiers in Veterinary Science**, v.5, p.1-12, 2018. Available from: <https://doi.org/10.3389/fvets.2018.00100>. Accessed: Apr. 29, 2020.

BACH, A. T. et al. Efficacy of disinfectants in the management of dairy cows in the control of mastitis and its infectious agents - Review Article. **Revista Científica Rural**, v.21, n.1, p.188-204, 2019. Available from: <https://doi.org/10.30945/rcr-v21i1.326>. Accessed: May, 03, 2020.

BELAGE, E. et al. Adoption and consistency of application of premilking preparation in Ontario dairy herds. **Journal of Dairy Science**, v.100, n.5, p.3902-3911, 2017. Available from: <https://doi.org/10.3168/jds.2016-11698>. Accessed: May, 01, 2020.

DATTA, A. K. et al. Economic analysis of dairy farming in Bangladesh. **Tropical Animal Health and Production**, v.51, n.1, p.55-64, 2019. Available from: <https://doi.org/10.1007/s11250-018-1659-7>. Accessed: May, 02, 2020.

DUTRA, M. M. et al. Influence of different pre-dipping solutions in the microbial population of extensive dairy crops. **Colloquium Agrariae**, v.13, n.2, p.116-122, 2017. Available from: <https://doi.org/10.5747/ca.2017.v13.n2.a166>. Accessed: Aug. 13, 2020.

GARVEY, M. et al. Efficacy testing of teat dip solutions used as disinfectants for the dairy industry: Antimicrobial properties. **International Journal of Dairy Technology**, v.70, n.2, p.179-187, 2017. Available from: <https://doi.org/10.1111/1471-0307.12344>. Accessed: Aug. 30, 2020.

GIAOURIS, E. et al. The Role of biofilms in the development and dissemination of microbial resistance within the food industry. **Foods**, v. 9, n. 816, p. 1-5, 2020. Available from: <https://doi.org/10.3390/foods9060816>. Accessed: Aug. 30, 2020.

GUSSMANN, M. et al. Economic and epidemiological impact of different intervention strategies for subclinical and clinical mastitis. **Preventive Veterinary Medicine**, v.166, p.78-85, 2019. Available from: <https://doi.org/10.1016/j.prevetmed.2019.03.001>. Accessed: May, 02, 2020.

HEIKKILÄ, A. M. et al. Pathogen-specific production losses in bovine mastitis. **Journal of Dairy Science**, v.101, n.10, p.9493-9504, 2018. Available from: <https://doi.org/10.3168/jds.2018-14824>. Accessed: May, 02, 2020.

HOEKSTRA, J. et al. Differences between *Staphylococcus aureus* lineages isolated from ovine and caprine mastitis but not between isolates from clinical or subclinical mastitis. **Journal of Dairy Science**, v.102, n.6, p.5430-37, 2019. Available from: <https://doi.org/10.3168/jds.2018-16196>. Accessed: May, 01, 2020.

JANS, C. et al. East and West African milk products are reservoirs for human and livestock-associated *Staphylococcus aureus*. **Food Microbiology**, v.65, p.64-73, 2017. Available from: <https://doi.org/10.1016/j.fm.2017.01.017>. Accessed: Apr. 29, 2020.

JANSEN, W. et al. Foodborne diseases do not respect borders: zoonotic pathogens and antimicrobial resistant bacteria in food products of animal origin illegally imported into the European Union. **The Veterinary Journal**, v.244, p.75-82, 2019. Available from: <https://doi.org/10.1016/j.tvjl.2018.12.009>. Accessed: May, 01, 2020.

LOPES, L. O. et al. Efficiency of disinfectants in milking management in dairy cows in preventing mastitis. **Revista Científica Eletrônica de Medicina Veterinária**, ano XI, n.21, p.1-9, 2013. Available from: <https://doi.org/10.1590/S0100-736X2012001200012>. Accessed: Apr. 29, 2020.

MBINDYO, C. M. et al. Prevalence, etiology, and risk factors of mastitis in dairy cattle in Embu and Kajiado counties, Kenya. **Veterinary Medicine International**, v.2020, p.1-12, 2020. Available from: <https://doi.org/10.1155/2020/8831172>. Accessed: Aug. 30, 2020.

MEDEIROS, E. S. et al. *In vitro* evaluation of the effectiveness of commercial disinfectants used in pre and post-dipping against samples of *Staphylococcus* spp. isolated from bovine mastitis. **Pesquisa Veterinária Brasileira**, v.29, n.1, 2009. Available from: <http://dx.doi.org/10.1590/S0100-736X2009000100011>. Accessed: Aug. 13, 2020.

MURRAY, P. et al. **Microbiologia Médica**. 8 ed. Rio de Janeiro: Guanabara Koogan, 2017. 831p.

RAMALHO, A. C. et al. *In vitro* efficacy of commercial disinfectants used in pre-and post-dipping against *Staphylococcus* spp. isolated from dairy cattle. **Pesquisa Veterinária Brasileira**, v.32, n.12, p.1285-1288, 2012. Available from: <https://doi.org/10.1590/S0100-736X2012001200012>. Accessed: Apr. 25, 2020.

ROWE, S. M. et al. Effect of pre-milking teat disinfection on clinical mastitis incidence in a dairy herd in Northern Queensland, Australia. **Australian Veterinary Journal**, v.96, n.3, p.69-75, 2018. Available from: <http://dx.doi.org/10.1111/avj.12674>. Accessed: Apr. 29, 2020.

SANTOS, I. C. et al. Eficácia in vitro de desinfetantes utilizados no pré dipping frente a amostras de *Staphylococcus* spp. **Jornal Interdisciplinar de Biociências**, v.3, n.1, p.1-4, 2018. Available from: <https://doi.org/10.26694/jibi.v3i1.6155>. Accessed: Apr. 29, 2020.

SILVA, F. A. S.; AZEVEDO, C.A.V. The Assistat Software Version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research - Academic Journals**, v.11, n.39, p.3733-3740, 2016. Available from: <http://doi.org/10.5897/AJAR2016.11522>. Accessed: Apr. 29, 2020.

SILVA, L. C. A. et al. In vitro evaluation of the sensitivity of strains of *Staphylococcus* spp. isolated from goat mastitis against commercial disinfectants. **Arquivos do Instituto Biológico**, v.82, p.1-4, 2015. Available from: <https://doi.org/10.1590/1808-1657000262013>. Accessed: Aug. 13, 2020.

SKOWRON, K. et al. Comparison of the effectiveness of dipping agents on bacteria causing mastitis in cattle. **Annals of Agricultural and Environmental Medicine**, v.26, n.1, p.39-45, 2019. Available from: <https://doi.org/10.26444/aaem/82626>. Accessed: Apr. 29, 2020.

UNLU, A. et al. Biofilm formation by *Staphylococcus aureus* strains and their control by selected phytochemicals. **International Journal of Dairy Technology**, v.71, n.3, p.637-646, 2018. Available from: <https://doi.org/10.1111/1471-0307.12520>. Accessed: Aug. 30, 2020.

WEN, J. et al. The status of *Mycoplasma bovis* infection in clinical mastitis cases in China. **International Journal of Dairy Technology**, v.72, n.3, p.350-356, 2019. Available from: <https://doi.org/10.1111/1471-0307.12602>. Accessed: Aug. 30, 2020.