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# *In vitro* efficacy of teat antiseptics against *Staphylococcus aureus* strains isolated from bovine mastitis

# Eficácia *in vitro* de antissépticos utilizados no controle da mastite bovina frente a isolados de *Staphylococcus aureus*

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## Abstract

The process of teat disinfection is a widely accepted component of successful mastitis control programs by reducing the number of bacteria on the teat skin and healing teat lesions. For contagious pathogens such as *Staphylococcus aureus*, post-milking teat disinfection remains a simple, effective and economical practice for prevention of new intramammary infections (IMIs) of lactating dairy cows. Despite the universal acceptance of teat dipping as a method of mastitis control, variations in the susceptibility and resistance profile of mastitis pathogens among antiseptics have been described. Thus, here we sought to explore the *in vitro* efficacy of the followings antiseptics against *S. aureus* isolated from IMIs: chlorhexidine (2.0%), chlorine (2.5%), quarternary ammonium (4.0%), lactic acid (2.0%) and iodine (0.6%). We used 50 *S. aureus* strains isolated from bovine IMIs from 50 dairy herds located at Minas Gerais, São Paulo, Paraná and Rio Grande do Sul States (Brazil). The antiseptics were evaluated at four different specific intervals (15, 30, 60 and 300 s). We found a higher activity for quarternary ammonium and chlorhexidine against *S. aureus* at all time-points, followed by iodine and then chlorine. Lactic acid treatment produced the worst results for all time-points and strains. Due to variations in the sensitivity and resistance profile of antiseptics against *S. aureus* isolated from IMIs, the effectiveness of the antiseptics against the major mastitis pathogens should be periodically evaluated in dairy farms in an attempt to reduce the rate of new IMIs in the herd.

**Key words**: Dairy cow. Intramammary infection. Milking. Teat dip. Antiseptics.

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## Resumo

O processo de antissepsia dos tetos é uma prática amplamente recomendada no controle da mastite por reduzir o número de bactérias na pele do teto e curar lesões de teto pré-existentes. Para patógenos contagiosos como o *Staphylococcus* aureus, a antissepsia dos tetos após a ordenha permanece como uma prática simples, efetiva e economicamente viável na prevenção de novas infecções intramamárias (IMIs). No entanto, variações do perfil de susceptibilidade de patógenos causadores de mastite aos antissépticos têm sido descritas. Desta forma, o presente estudo objetivou avaliar a eficácia *in vitro* dos antissépticos a base de iodo (0,6%), cloro (2,0%), clorexidine (2,5%), compostos quartenários de amônio (4,0%) e ácido láctico (2,0%) utilizados no pré- e pós-*dipping* em isolados de *Staphylococcus aureus* de casos de IMIs. Utilizou-se 50 isolados de *S. aureus* provenientes de 50 propriedades leiteiras localizadas nos Estados de Minas Gerais, São Paulo, Paraná e Rio Grande do Sul – Brasil. Os antissépticos foram avaliados em quatro tempos distintos (15 s, 30 s, 60 s e 300 s). Os resultados apontaram para maior eficácia dos compostos quartenário de amônio e do clorexidine, seguido pelo iodo e cloro contra os isolados de *S. aureus* nos momentos avaliados. O ácido láctico apresentou a menor atividade antisséptica em todos os momentos avaliados. Desta forma, devido a variações no perfil de susceptibilidade e resistência dos isolados de *S. aureus* isolados de casos de mastite bovina aos antissépticos, a eficácia dos antissépticos contra os patógenos primários causadores de mastites devem ser periódicamente avaliada na tentativa de reduzir a taxa de novas infecções intramamárias no rebanho.

**Palavras-chave**: Bovinos leiteiros. Desinfetante de teto. Infecção intramamária. Ordenha.

Mastitis is one of the most common and detrimental diseases that effect the entire milk production chain. Mastitis threatens the income of farmers as well as the image of the dairy sector, largely because of animal welfare issues and issues related to milk quality and public health due to an increased risk of antimicrobial residues and the emergence of resistant bacteria (LANGONI, 2013). Among the mastitis pathogens, *Staphylococcus aureus* remains the most important cause of bovine mastitis worldwide (BARKEMA et al., 2006).

The process of teat disinfection is a widely accepted component of successful mastitis control programs by reducing the number of bacteria on the teat skin and healing teat lesions. For contagious pathogens such as *S. aureus*, post-milking teat disinfection remains a simple, effective and economical practice for the prevention of new intramammary infections (IMIs) of lactating dairy cows (AMARAL et al., 2004; BARKEMA et al., 2006; PICCININI et al., 2009; WILLIAMSON; LACY-HULBERT, 2013).

Despite the universal acceptance of teat dipping as a method of mastitis control, a number of limitations are associated with most teat dips currently available. The most significant restriction is that teat dips do not provide equal protection against the huge amount of bacteria that cause bovine mastitis. Furthermore, prolonged *in vitro* exposure to germicidal teat dips has been shown to enhance the resistance of some bacteria to chemical antiseptics (OLIVER et al., 1990; AZIZOGLU et al., 2013). Several passages of bacterial isolates with sub-lethal concentrations of antiseptics were found to either induce resistance or select for resistant variants (BEHIRY et al., 2012). As a result, variations in the susceptibility and resistance profile of mastitis pathogens among antiseptics have been reported (MEDEIROS et al., 2009; COUTINHO et al., 2012; RAMALHO et al., 2012; AZIZOGLU et al., 2013).

The present study evaluated the *in vitro* efficacy of chlorhexidine (2.0%), chlorine (2.5%), quarternary ammonium (4%), lactic acid (2.0%) and iodine (0.6%) against *S. aureus* isolated from mastitic milk.

Previous studies have investigated the susceptibility of *S. aureus* isolated in Brazil against antiseptics, however, these studies have used a limited number of *S. aureus* strains (PEDRINI; MARGATHO, 2003; RAMALHO et al., 2012) or dairy herds, and covered bacteria strains isolated

from a relatively small region (MEDEIROS et al., 2009).

In the present study, we analyzed fifty *S. aureus* strains isolated from IMIs. Investigations into the ability to spread within quarters and cows, and cause persistent IMIs, revealed that one strain of *S. aureus* predominates in each individual herd; although the predominant *S. aureus* strain usually coexists with other *S. aureus* strains (SMITH et al., 1998; ZADOKS et al., 2000; BARKEMA et al., 2006).

Targeting these specific strains may be beneficial in controlling mastitis within a given herd. Thus, in this study, in an attempt to achieve a representative sampling, we selected one strain of *S. aureus* per herd totaling 50 *S. aureus* strains from 50 dairy herds to determine the antiseptic efficacy. Furthermore, the *S. aureus* strains used here covered a large geographical region, having been obtained from the Minas Gerais (60%), São Paulo (26%), Paraná (10%) and Rio Grande do Sul (4%) States (Brazil).

The effectiveness of chlorhexidine (2.0%), chlorine (2.5%), quarternary ammonium (4.0%), lactic acid (2.0%) and iodine (0.6%), in concentrations commonly used in teat disinfection aimed at controlling bovine mastitis, were tested as previously described (MEDEIROS et al., 2009; COUTINHO et al., 2012). Briefly, 0.8 mL of each disinfectant in the concentrations stated above, 0.2 mL of sterile milk and 1.2 mL of a bacteria suspension standardized in 1.0 MacFarland scale were evaluated at different times of 15, 30, 60 and 300 sec. After these intervals, the bactericidal activity of the antiseptics against *S. aureus* were verified by the absence of bacteria growth at 37°C for 24 h in brain-heart infusion broth (BHI; cat. n. CM1135B, Oxoid, UK). The bactericidal activity of antiseptics against *S. aureus* were confirmed by plating 0.1 mL of the BHI broth culture onto 5% ovine blood agar plates aerobically at 37°C for 24 h.

The statistical analyses were performed by the Cochran's Q test using the Medcalc statistical software (Medcalc software, Brussels, Belgium). The bacteria growth was scored as either one (bacteria growth) or zero (no bacterial growth) for statistical analysis. The significance was set at $P \leq 0.05$.

Our results demonstrated that there is an effect of time exposure on the efficacy of teat antiseptics against *S. aureus* strains, except for quarternary ammonium (4.0%) (Table 1). Here, we found higher activity of quarternary ammonium (4.0%) and chlorhexidine (2.0%) against *S. aureus* isolated from IMIs at all time-points, followed by iodine (0.6%) and chlorine (2.5%), respectively (Table 1). Lactic acid (2.0%) treatment produced the worst results in all cases (Table 1).

**Table 1.** Susceptibility (%) of *S. aureus* strains isolated from bovine intramammary infections against antiseptics commonly used in pre- and post-dipping.

| Antiseptics | Susceptibility (%) | | | |
|---|---|---|---|---|
| | Time (s) | | | |
| | 15 | 30 | 60 | 300 |
| Lactic acid | $4^{Bc*}$ | $4^{Bc}$ | $8^{ABd}$ | $14^{Ac}$ |
| Quarternary ammonium | $98^{Aa}$ | $98^{Aa}$ | $98^{Aa}$ | $98^{Aa}$ |
| Chlorhexidine | $84^{Ba}$ | $90^{Aba}$ | $94^{Aab}$ | $96^{Aa}$ |
| Chlorine | $30^{Cbc}$ | $40^{BCb}$ | $48^{ABc}$ | $64^{Ab}$ |
| Iodine | $46^{Cb}$ | $58^{BCb}$ | $66^{ABbc}$ | $78^{Aab}$ |

*Different lower case letters designate the means with statistically significant differences ($P \leq 0.05$) among lines (antiseptics). Capital letters indicate statistically significant differences ($P \leq 0.05$) among columns (time).

In agreement with our results, Kassaif et al. (2007) have reported lower *in vitro* bactericidal activity of iodophor-based antiseptics than antiseptics containing quarternary ammonium in a panel of common dairy associated bacteria, including *S. aureus*. Additionally, Azizoglu et al. (2013) observed that the response among bovine *S. aureus* strains selected as genotype group representatives based on their pulsed-field gel electrophoresis patterns to iodine varied significantly, whereas all isolates were susceptible to chlorhexidine.

Ramalho et al. (2012) also identified chlorhexidine as having the highest *in vitro* disinfectant activity against *S. aureus* isolated from bovine IMIs in dairy herds from the Wasteland and Forest Zone of Alagoas State (Brazil), followed by chlorine, iodine and quarternary ammonium. Medeiros et al. (2009) showed that iodine and chlorhexidine had the greatest *in vitro* disinfectant activity against *S. aureus* isolated from bovine IMIs in dairy herds located at the Metropolitan Recife, the Agreste and Zona da Mata regions of Pernambuco State (Brazil), followed by quarternary ammonium, lactic acid and chlorine. In light of this, the observed variation among studies into susceptibility patterns of these antiseptic against *S. aureus* isolated from bovine mastitis support the idea that it is crucial to undertake a periodic evaluation of antiseptics commonly used in dairy farms, while also considering their implications for occurrence of *S. aureus* mastitis in the herd/region studied.

In most of dairy farms, the antiseptics that are applied are commonly chosen based on the price, consumer habits and/or ease of application, and the efficacy of antiseptics against major mastitis pathogens are not usually regarded (PEDRINI; MARGATHO, 2003). Furthermore, many aspects of each antiseptic should be carefully regarded. For instance, quarternary ammonium compounds do not volatilize readily and persist on the skin, probably leading to protection between milkings. Chlorhexidine has a lesser reduction of its germicidal activity by organic material than some other common antiseptics, such as chlorine (SPINOSA et al., 2011). Improper choice of antiseptic or insufficient low concentrations can lead to natural selection of resistant bacterial isolates in the microbial population (AZIZOGLU et al., 2013), with important implications for mastitis control programs. Thus, further investigations should address antiseptic activities against the mastitis pathogens under various management strategies and across regions, and should also be focused on the appropriate minimum contact time between the antiseptics and bacteria (i.e., 30 to 60 s), as well as the optimal preparation lag time in conventional milking (i.e., 60 to 90 s) (LANGONI, 2013).

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