In vitro EVALUATION OF THE EFFICACY OF COMMERCIAL DISINFECTANTS USED IN PRE AND POST-DIPPING AGAINST *Candida spp*. ISOLATED FROM BOVINE MASTITIC MILK

AVALIAÇÃO IN VITRO DA EFICÁCIA DE DESINFETANTES COMERCIAIS UTILIZADOS NO PRÉ E PÓS-DIPPING FRENTE À Candida spp. ISOLADA DE LEITE MASTITICO BOVINO

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SUMMARY

The objective of this investigation was to evaluate *in vitro* susceptibility of *Candida spp*. to several commercially available disinfectants used for pre and post-dipping. A total of 45 *Candida* isolates were obtained from mastitic milk of dairy cows with clinical or subclinical mastitis from four Brazilian States. The active drugs chlorhexidine (2.0%), iodine (0.8%), iodine glycerin (0.8%) and sodium hypochlorite (1.0%) were used at four specific intervals (15″, 30″, 60″ and 300″). One hundred percent of *Candida* strains were susceptible to chlorhexidine at 30″, 60″, and 300″; to iodine glycerin at 60″ and 300″; to sodium hypochlorite at 60″ and 300″; and to iodine at 300″ only. It can be concluded that the highest *in vitro* disinfectant activity was achieved with chlorhexidine. It was further concluded that it is important to undertake periodic evaluation of microorganism sensitivity to commonly used disinfectants, in order to ensure the efficacy of teat disinfection process.

KEY-WORDS: *Candida*. Yeast. Disinfectant. Mastitis.

RESUMO

O objetivo desta investigação foi de avaliar a sensibilidade in vitro de cepas de *Candida spp*. Frente a vários desinfetantes comercialmente disponíveis, utilizados para o tratamento pré e pós-dipping. Foram obtidos 45 isolados de *Candida* em leite mastitico provenientes de vacas leiteiras acometidas de mastite clinica ou sub clinica em quatro estados brasileiros. As drogas utilizadas foram clorexidina (2,0%), iodo (0,8%), iodo glicerinado (0,8%) e hipoclorito de sódio (1,0%) em 4 intervalos específicos de tratamento (15″, 30″, 60″ e 300″). Cem por cento das cepas de *Candida* foram sensíveis a clorexidina nos tempos de 30″, 60″e 300″, ao iodo glicerinado em 60″ e 300″, ao hipoclorito de sódio em 60″ e 300″ e ao iodo apenas em 300″. Assim se conclui que a eficácia mais alta de desinfecção foi encontrada para a clorexidina. Uma conclusão adicional é que é importante a realização periódica de avaliações de sensibilidade dos microrganismos aos desinfetantes comumente utilizados, de forma a garantir a eficácia da desinfecção dos tetos.

PALAVRAS-CHAVE: *Candida*. Levedura. Desinfetante. Mastite.

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INTRODUCTION

Mammary gland inflammation (mastitis) continues to be the most frequent and expensive disease of dairy cows (BRADLEY & GREEN, 2001). It negatively influences economic effectiveness of farms and milk hygienic quality (HORTET & SEEGERS, 1998). More than 135 different microorganisms have been found as etiological agents of mastitis (BRADLEY, 2002). In addition to bacterial agents, several other groups of microorganisms such as yeast, fungi and algae can cause an inflammatory process and alteration in the udder (WATTS, 1988, KRUKOWSKI et al., 2006).

Yeast and fungi are normal flora of the soil and may colonize udder skin in small numbers (RICHARD et al., 1980). They are considered opportunists (COSTA et al., 1993), that is, they cause disease when natural defense mechanisms are lowered. *Candida* species are the most frequent organisms among the mycotic mastitis agents isolated from infected glands (WATTS, 1988).

Disinfection is one of the most important aspects of disease prevention. Many disinfectants have been developed specifically for the dairy industry to prevent the spread of infectious diseases (PANKEY et al., 1984, OLIVER et al., 1993, AMARAL et al., 2004, MEDEIROS et al., 2009) which can be controlled to a manageable extent by using safe and effective premilking and postmilking teat germicides.

The present investigation was conducted to test in vitro susceptibility of *Candida spp.* obtained from bovine mastitis to commercially available disinfectants commonly used in dairy farms in Brazil.

MATERIAL AND METHODS

Sampled animals

The study was carried out with cows from dairy farms, under intensive husbandry system, distributed in four Brazilian States, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul. The herds were constituted by animals from different races, ages and under different lactation phases.

Criteria for clinical or subclinical mastitis

Clinical mastitis was characterized by clinical signs and/or abnormal milk secretion detected by the strip cup test. Subclinical mastitis was identified by the conventional CMT (California Mastitis Test).

Milk sample

Forty-five milk samples from quarters with clinical or subclinical mastitis were collected between February and December 2009.

The milk samples were always aseptically collected and plated onto Sabouraud dextrose agar (Oxoid). The plates were incubated at 37^0C for 72 hours. The yeast was phenotypically characterized by standard tests (RICHARD et al., 1980, KREGER-VAN RIJ, 1984, BARNETT et al., 1990). After identification, the isolates, one from each plate, were maintained in Sabouraud dextrose agar slants and kept at room temperature.

In vitro efficacy evaluation of disinfectants

For all evaluations, fresh cultures (48 hours) of *Candida* growth in Sabouraud dextrose agar were used. From each of the strains of *Candida*, cell concentration in sterile saline solution (0.85%) was adjusted to tube 1 of the McFarland scale. After that, 1.2 mL of each suspension was transferred to a sterile glass tube containing 0.2 mL of sterile milk, then 0.8 mL of one of the selected disinfectants chlorhexidine (2.0%), iodine (0.8%), iodine glycerin (0.8%) and sodium hypochlorite (1.0%) was added. The samples were subjected to different times of contact between cells and disinfectants: 15, 30, 60 and 300 seconds. After 0.1 mL of each tube were cultured in Sabouraud dextrose broth at 37^0C for 24 hours to check media turbidity and/or pellicle formation or cell precipitation. The test was done in triplicate.

After the incubation, an aliquot of 0.1 mL of each tube was cultured in Sabouraud dextrose agar using the spread-plate technique to check the absence or not of *Candida* growth (COSTA et al., 1998).

RESULTS

The results of the susceptibility tests of 45 strains of *Candida spp*. exposed to several disinfectants for different periods of time are shown in Figure 1.

Except for iodine (0.8%), all other disinfectants were hundred per cent efficient and *Candida* cells were destroyed after a 60-second contact. All tests were carried out in triplicate for each isolate.

DISCUSSION

Yeasts are microorganisms found in nature and frequently isolated from diseased humans and animals. Among yeast types, *Candida* species are eukaryotic pathogens most commonly isolated from infections of dairy cow mammary glands. Generally, they are considered opportunists and mastitis occurs when a high number of microorganisms are present on the teat, breaking down udder defenses. Also, udder skin, milker hands, milking machines, straw, feed, dust, soil, drugs and sanitizing solutions are contributing factors to development of mastitis (QUINN et al., 2002). Among them, sanitizing solutions have been studied because they can play an important role helping decrease mammary gland inflammation, but they can also produce teat skin irritation depending on the used product and its concentrations.

Good disinfectants should be effective against major mastitis pathogens, be economical, easy to apply and should maintain or promote good hygiene conditions. Most of the studies on the subject are related to *Staphilococcus aureus* and *Streptococcus agalactiae*. (PANKEY et al., 1984, BOODIE et al.,

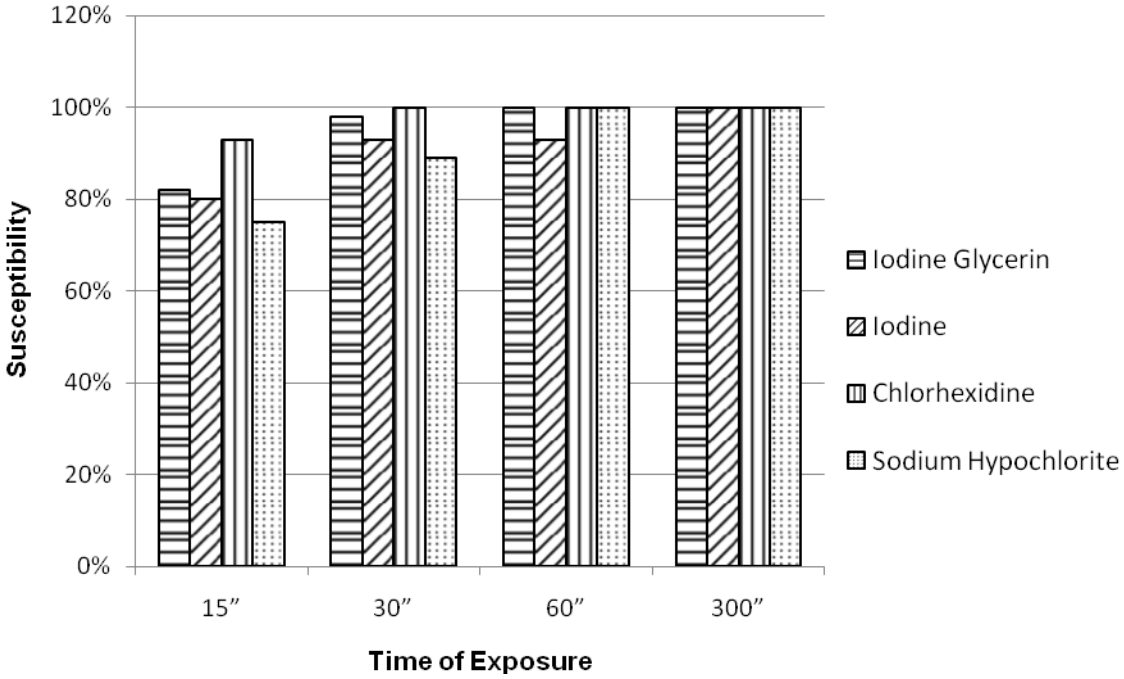

Figure 1- *In vitro* percent efficacy of disinfectants used in pre and post-dipping against *Candida spp*. strains isolated from bovine mastitic milk from dairy farms in four Brazilian States in 2009.

1997, PEDRINI & MARGATHO, 2003, AMARAL et al., 2004, MEDEIROS et al., 2009); therefore, results with other microorganisms including *Candida* are scarce (LOPES et al., 2008).

In the present work, four disinfectants to control mastitis caused by bacterial species were tested, chlorhexidine (BOODIE et al., 1997, MEDEIROS et al., 2009) sodium hypochlorite (AMARAL et al., 2004), iodine (BOODIE et al., 1997), iodine glycerin (GOLDBERG et al., 1994). The results demonstrate that infected cell exposure time to disinfectants is crucial and should be at least 60 seconds.

The best results were obtained for chlorhexidine (2.0%), which is also not irritating to teat skin. The *in vitro* results obtained for microorganism sensitivity to disinfectants does not represent exactly what occurs *in vivo*. It should be considered only as an indication of the best product available. After that an extensive field work should be done under different environmental conditions and using different herds to check the efficacy of the disinfectant to control mastitis pathogens.

It can be further concluded that it is important to undertake periodic evaluation of the sensitivity of the microorganisms to the disinfectants to ensure an effective process of udder disinfection.

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