EVALUATION OF THE SUSCEPTIBILITY OF *CANDIDA* SPECIES ISOLATED FROM BOVINE MASTITIS TO MILK PASTEURIZATION.

AVALIAÇÃO DA SUSCEPTIBILIDADE DE ESPÉCIES DE *CANDIDA* ISOLADAS DE MASTITE BOVINA A PASTEURIZAÇÃO DO LEITE.

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SUMMARY

The objective of this study was to evaluate the resistance of *Candida* species isolated from bovine milk to pasteurization and boiling. A total of 45 *Candida* isolates were obtained from mastitic milk of dairy cows with clinical or subclinical mastitis from four Brazilian States. The isolates were submitted to different temperature and time ratio of pasteurization, fast pasteurization (72-75°C / 20sec), slow pasteurization (62-65°C / 30 min) and boiling (100°C / 1-3sec). Fast pasteurization was the procedure in which there was a higher resistance of yeast (64.4%), followed by boiling (15.5%) and slow pasteurization (6.6%). Care should be taken regarding the possibility of *Candida* strains persistence in pasteurized and boiled milk, which can represent a risk to consumers especially to immunocompromised individuals, children and elderly people.

KEY-WORDS: Bovine mastitis, milk, thermal treatment, yeast, *Candida.*

RESUMO

Este trabalho teve como objetivo a avaliação da resistência a pasteurização e a fervura de cepas do gênero *Candida* isoladas de leite bovino. Foram obtidos 45 isolados de *Candida* em leite provenientes de vacas leiteiras acometidas de mastite clínica ou sub-clínica em quatro estados brasileiros. As cepas foram submetidas a diferentes razões de temperatura e tempo de pasteurização, pasteurização rápida (72-75°C / 20 seg), pasteurização lenta (62-65°C / 30 min), e fervura (100°C / 1-3 seg). A pasteurização rápida foi o procedimento no qual houve maior índice de resistência da levedura (64,4%), seguida pela fervura (15,5%) e pela pasteurização lenta (6,6%). A persistência de cepas de *Candida* em leite submetido à pasteurização e a fervura, pode representar um risco ao consumidor, especialmente aos indivíduos imuno-comprometidos, crianças e pessoas idosas.

PALAVRAS-CHAVE: *Mastite bovina, leite, tratamento térmico, levedura, Candida.*

INTRODUCTION

Mastitis has been defined as an inflammation of the mammary gland usually as a consequence of microbial infection, it continues to be the most frequent and expensive disease of dairy cows (BRADLEY & GREEN, 2001). More than 150 different microorganisms have been found as etiological agents of mastitis (WATTS, 1988; BRADLEY, 2002). In addition to bacterial agents, other groups of microorganisms such as yeast and fungi can cause an inflammatory process (WATTS, 1988; KRUKOWSKI et al., 2006).

Yeast and fungi are normal flora of the soil and may colonize udder skin in small number. They are opportunists and produce disease when natural defense mechanisms are lowered (KIRK & BARLETT, 1986). *Candida* species are the most frequent organisms among the mycotic mastitis agents isolated from infected glands (WATTS, 1988, SPANAMBERG et al., 2009).

There are reports in the literature about occurrence of yeasts in milk and cheese from bovine (CORBO et al., 2001). The presence of microorganisms in milk, many of which are responsible for zoonoses, represents a factor that compromises its quality and safety. Therefore with the objective of reducing the microbial content of milk as well as eliminating microorganisms potentially harmful to humans, procedures for the thermal treatment of milk have been developed, among them the main procedure currently used is pasteurization (OLIVER et al., 2005) The objective of this study was to evaluate the susceptibility of *Candida* strains isolated from mastitic milk to the different temperature/time ratios employed in the pasteurization of milk.

MATERIAL AND METHODS

Sampled animals

The study was carried out with cows from dairy farms, under the intensive system of husbandry, distributed in four Brazilian States, São Paulo (6 farms), Paraná (14 farms), Santa Catarina (7 farms) and Rio Grande do Sul (14 farms). The herds were constituted by animals with 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

Four-hundred- twenty-eight milk samples from quarters with clinical or subclinical mastitis were collected between February and December 2009. The milk samples (10 mL) were always aseptically collected in sterile glass bottles after disinfection of the teats with alcohol 70%, the first three squirts of foremilk were discarded, and then the fourth squirt was collected. and kept at a temperature of 4^{o}C until processing at the laboratory. Aliquots of 0.1 mL of milk samples were spread in Sabouraud dextrose agar ((SDA, Oxoid) supplemented with chloramphenicol (400mg/L). The plates were incubated at 37^{o}C for 72h. The yeast was phenotypically characterized by standard tests (RICHARD et al., 1980; KREGER-VAN RIJ, 1984; BARNETT et al., 1990). Only the yeast of genus *Candida* was utilized in the present study. After identification one isolate from each plate were maintained in Sabouraud dextrose agar slants and kept in room temperature. Among the milk samples, fifty-five samples were positive for the genus *Candida* (12.8%), among them *Candida krusei* (19 isolates), *C. parapsilosis* (14 isolates), *C. tropicalis* (10 isolates), *C. albicans* (7 isolates), *C glabrata* (2 isolates), *C spp* (2 isolates) and *C. rugosa* (1 isolate).

Thermal treatments

Forty-five *Candida* isolates were randomly selected, including all the species identified, after that the strains were examined for thermal treatments using the methodology reported by MELVILLE et al. (1999) and RUZ-PERES et al. (2010). For all evaluation, fresh cultures (48h) of *Candida* growth in Sabouraud-dextrose agar were used. From each of these strains, cell concentration in 5.0 mL of sterile saline solution (0.85%) was adjusted to tube 3 of the McFarland scale. After that 2.0 mL of each suspension was transferred to a sterile glass tube containing 18.0 mL of sterile milk what means a 10^{-1} dilution, after homogenization 1.0 mL of each suspension was distributed in 15 sterile glass tubes. The samples were subjected to different temperature/time ratio, five tubes to 62-65^{o}C for 30 minutes (slow pasteurization), five tubes to 72-75^{o}C for 20 seconds (fast pasteurization), five tubes until boiling, and then immediately placed in an ice-water bath. After that 0.1 mL of each tube were cultured in Sabouraud- dextrose agar using the spread-plate technique and incubated at 37^{o}C for 48 hours in order to evaluate the presence of colony forming units (c.f.u) of *Candida.*

RESULTS

The results of the tests on the susceptibility of 45 strains of *Candida* to different temperature/time ratios used for the pasteurization of milk were highly variable. The fast pasteurization (72-75^{o}C / 20 sec) was the procedure in which there was a higher resistance (64.4%), so it was the least efficient method (Table 1). It was also observed that the c.f.u media was highest in the fast pasteurization (Table 2). No significant difference was found among the different species of *Candida* to the thermal treatments (result not shown).

DISCUSSION

The elimination of microorganisms harmful to humans is highly desirable and pasteurization is the main procedure currently used. A low level of yeast occurs in raw milk and 80% of pasteurized milk samples exhibited these microorganisms, particularly *Candida* species (FLEET & MIAN, 1987). CORBO et al. (2001) isolated different *Candida* species from milk and dairy products in an Italian region. In Brazil SPANAMBERG et al (2004) also isolated different yeast in raw milk, among others different *Candida* species. An elevated number of these microorganisms in milk, as showed in Table 2 in fast pasteurization, might cause different digestive or allergic disorders and become an important risk factor in the development of disseminated infections in neutropenic patients (COLOMBO et al., 2006).

The results of susceptibility of *Candida* strains to thermal treatment obtained in the present study (Table 1) was similar to results reported by RUZ-PERES et al. (2010), for fast pasteurization (72.18%), slow pasteurization (0.99%) and boiling (15.89%). In the present study only one strain, number 6 (*C. krusei*) was resistant to the three treatments used (result not shown).

Candida is commonly viewed as an opportunistic yeast pathogen. On the normal host, the yeast has evolved to become a successful comensal. It expresses variant traits critical for existence on mucosal surfaces. In abnormal circumstances, the same traits become virulence characteristics what increase the invasive abilities of the yeast. Among others, adherence is an important pathogenic factor and the relation between yeasts′ adherence capacity and their ability to colonize mucous surfaces is obvious, since most of the adherent species of *Candida*, are those that most frequently colonize the human gastrointestinal epithelium (PENDRAK & KLOTZ, 1995).

Although *C. albicans* remains the most frequent cause of fungemia a number of reports have documented infections caused by *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. lusitaniae* (RODRIGUES et al, 2010). Most of them have considerable biological potential as an opportunist microorganism when the host is compromised by neutropenia, antibiotic suppression of the bacterial flora, and damage to the gastrointestinal mucosa (PFALLER, 1996)

Considering that animals could be vectors of transmission or reservoirs of strains causing human disease and may present a risk for immunocompromised patients (EDELMAN et al., 2005) a special attention must be given to dairy products

The consumption of milk contaminated with *Candida* represents one of the means of transmission. NEDRET KOÇ et al. (2001) reported 12 cases of *Candida glabrata* fungemia occurred among hospitalized children in a Turkey hospital due to a milk bottle contamination. Also an increasing number of immunocompromised individuals, such as those with HIV infection, neutropenia, intensive care patients and those treated with immunosuppressive drugs after organ transplantation experience some form of mucosal *Candida* infection (HUBE & NAGLIK, 2001).

The resistance of *Candida* strains after thermal treatment presents a problem for milk consumption especially among children and elderly people what represents a potential risk for public health.

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Table 1. Absolute number and percentage of resistance of 45 strains of *Candida* isolated from bovine mastitic milk collected in four Brazilian states in 2009 to different temperature/times ratio.

Pasteurization						
yeast	Slow (62-65 °C/30 min.)		Fast (72-75 °C/20 sec.)		Boiling	
Candida (n= 45)	N	%	N	%	N	%
	3	6.6	29	64.4	7	15.5

N- Absolute number; % percentage

Table 2. Colony forming units (c.f.u) average of 45 *Candida*. strains isolated from bovine mastitic milk collected in four Brazilian states in 2009 subjected to different thermal treatments.

Thermal treatment	c.f.u
slow pasteurization (62-65^{o}C / 30min.)	0.15
fast pasteurization (72-75^{o}C / 20sec)	413.72
Boiling (100^{o}C / 1-3sec)	3.54