

SURVEY OF BOVINE MASTITIS IN DAIRY HERDS IN RIBEIRÃO PRETO, SÃO PAULO STATE, BRAZIL

(LEVANTAMENTO SOBRE MASTITE BOVINA EM REBANHOS LEITEIROS
EM RIBEIRÃO PRETO, ESTADO DE SÃO PAULO, BRASIL)

(PESQUISA SOBRE MASTITIS BOVINA EN REBAÑOS LECHEROS EN LA
REGIÓN DE RIBEIRÃO PRETO, ESTADO DE SÃO PAULO, BRASIL)

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SUMMARY

The purpose of this study was to isolate pathogens from the quarter milk of cows udders from different dairy herds and to identify the different genera and species involved in mastitis. A total of 528 milk samples from clinical and subclinical mastitis quarters from five dairy herds of Ribeirão Preto, São Paulo State, Brazil, were included in this survey. *Staphylococci* was the largest group of pathogens isolated. Antibiotic resistance was found to be high between the major pathogens, especially to lincomycin (73.6-100.0%), penicillin (61.9- 100.0%), novobiocin (64.2-100.0%), trimethoprim-sulphadiazin (71.0-100.0%). Multidrug resistance was a common event for the isolates.

KEY-WORDS: Bovine mastitis. Antibiotic resistance. *Escherichia coli*. *Staphylococcus sp.* *Streptococcus sp.*

RESUMO

O objetivo deste estudo foi isolar e identificar os diferentes patógenos causadores de mastite bovina, de úberes de vacas leiteiras. Um total de 528 amostras de leite provenientes de vacas apresentando mastite clínica ou subclínica foram coletadas em cinco rebanhos leiteiros da região de Ribeirão Preto, Estado de São Paulo. O patógeno mais isolado neste estudo foi o *Staphylococcus sp.* Foi verificado que a resistência a antibióticos se mostrou bastante elevada especialmente para a lincomicina (73,6-100,0%), penicilina (61,9-100,0%), novobiocina (64,2-100,0%), trimetoprim-sulfadiazina (71,0-100,0%), entre os principais patógenos causadores da mastite. Também foi verificado que a resistência a múltiplos antibióticos foi um evento comum entre os isolados bacterianos.

PALAVRAS-CHAVE: Mastite bovina. Resistência a antibióticos. *Escherichia coli*. *Staphylococcus sp.* *Streptococcus sp.*

RESUMEN

El objetivo de este estudio fue aislar e identificar los diferentes patógenos causadores de mastitis bovina en vacas lecheras. Un total de 528 muestras de leche provenientes de vacas con mastitis clínica o subclínica fueron colectadas en cinco rebaños lecheros de la región de Ribeirão Preto, Estado de São Paulo, Brasil. El patógeno más aislado en este estudio fue el *Staphylococcus sp.* Se verificó que la resistencia a antibióticos fue bastante elevada, especialmente para lincomicina

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(73,6-100,0%), penicilina (61,9-100,0%), novobiocina (64,2-100,0%), trimetoprim-sulfadiazina (71,0-100,0%), entre los principales patógenos causadores da mastitis. También se observó que la resistencia a múltiples antibióticos fue un evento común entre los aislados bacterianos.

PALABRAS-CLAVE: Mastitis bovina. Resistencia a antibióticos. *Escherichia coli*. *Staphylococcus sp.* *Streptococcus sp.*

INTRODUCTION

Bovine mastitis has been defined as an inflammation of the mammary gland, usually as a consequence of microbial infection. A wide variety of microorganisms have been implicated as causative agents of bovine mastitis (WATTS, 1988, MYLLYS et al., 1998).

Clinical and sub clinical mastitis are one of the major diseases in dairy herds. It induces economic costs, mainly consisting of discarded milk, increased health care costs and reduced milk quality (HOBLET et al., 1991, MORIN et al., 1998). Mastitis also contributes to consumer concerns regarding animal welfare (WILLEBERG, 1994, FEDORKA-CRAY et al., 1998) and concerns regarding the impact of use of antibiotics in animals on efficacy of antibiotics for human health (FEDORKA-CRAY et al., 1998).

Mastitis control programmes are designed to reduce sub clinical mastitis on farms as these provide guidelines for hygiene and management practices to control intramammary infections (IMI) with contagious pathogens (KIRK et al., 1994, HONKANEN-BUZALSKI e MYLLYS, 1996). Because opportunistic pathogens are ubiquitous on farms, eradication of clinical mastitis is an unreasonable goal. Rather, dairy producers must strive to reduce the incidence of mastitis by instituting control measures to minimize exposure of teat ends to bacteria (SMITH e HOGAN, 1993).

Identification of bacteria that cause mastitis in cows and their antibiotic susceptibility is necessary to select appropriate antibiotics for treatment. With variability in antibiotic susceptibility patterns for bacteria isolated from animals (BEZEK, 1998, FITZGERALD et al., 2000, TOLLERSRUD et al., 2000), it is prudent to periodically monitor bacterial isolates from cows with mastitis.

The purpose of this study was to isolate pathogens from the mammary glands of cows from five dairy herds and examined the isolates for antibiotic susceptibility to eliminate ineffective antibiotics from the treatment regimen, thus resulting in more successful antibiotic treatments.

MATERIAL AND METHODS

Samples and Pathogens Examinations: Five dairy farms in the Ribeirão Preto region, São Paulo State, with 800

crossbred cows were examined, from March 2002 to July 2002. Milk sample from each cow were submitted to the California Mastitis Test (CMT) according to the method proposed by Schalm e Noorlander (1957) on a scale of 1-5 (KLASTRUP, 1975). The teat ends were cleaned with alcohol (70%), moistened swabs and allowed to dry. After discarding the first few streams, a milk sample was submitted to CMT. From the CMT-positive quarters (only one from each cow) a sample of 2-4mL was aseptically collected into sterile 10mL glass flasks and kept refrigerated at about 4°C. The samples were dispatched to the laboratory without delay, and plated as soon as possible on MacConkey agar, Baird-Parker agar, Acetate-crystal violet blood agar and Sabouraud dextrose agar. The plates for bacterial isolation were incubated for 72 h at 37°C and the Sabouraud dextrose agar plates were kept at 25°C for at least three weeks before being discarded. Bacterial strains were identified by standard bacteriological methods (KONEMAN et al., 1997) and yeast examination was performed according to Barnett et al. (1983).

Sensitivity Testing: Antimicrobial agent susceptibility testing with the bacterial isolates was performed using the Kirby-Bauer disk diffusion method with Mueller Hinton agar plates (CRAIG, 1993). The following disks (CEFAR) were tested: ampicillin (AMP, 10µg), cephalotin (CEP, 30µg), erythromycin (ERY, 30µg), gentamycin (GEN, 10µg), lincomycin (LIN, 50µg), kanamycin (KAN, 10µg), nalidixic acid (NAL, 10µg), neomycin (NEO, 25µg), novobiocin (NOV, 30µg), penicillin (PEN, 30µg), streptomycin (STR, 30µg), tetracycline (TET, 30µg), trimethoprim-sulphadiazin (TRI, 25µg). After 16 to 18 h of incubation at 37 °C, zones of inhibition around all disks were measured using reflected light and interpreted according to the NCCLS- National Committee for Clinical Laboratory Standards (2000) recommendations.

RESULTS AND DISCUSSION

A total of 528 milk samples (66.0%) from clinical (497 cows) and sub clinical (31 cows) mastitis, detected by the CMT applied to 800 crossbred cows were submitted to a bacteriological culture. Only one quarter from each cow was examined. Two hundred seventy-two samples were CMT-negative.

Table 1 shows the distribution of organisms

cultured from milk samples. From the 528 milk samples examined, 75 yeasts were isolated as pure culture (14.20%). The percentage of yeast isolations in surveys carried out in many countries varies considerably thus, Awad et al. (1980) registered 6.1% isolation in Egypt, Yeo e Choi (1982) registered 1.3% in Korean, Krukowski et al (2000) reported 9.6%, while Costa et al. (1993) recorded a 12.07% occurrence in a survey in the São Paulo State, Brazil, what is almost the same recorded by this study.

Staphylococci coagulase-positive was the largest group of pathogens isolated, 173 strains (32.70%) (Table 1) what agree with the results described by Sargeant et al. (1998) and Waage et al. (1999). Coagulase-positive *staphylococci* presently include *Staphylococcus aureus*, *S. intermedius*, and some strains of *S. hyicus* (WATTS, 1988), but *S. aureus* is the major mastitis pathogen within this group (ROBERSON et al., 1996).

Table 1 - Distribution of organisms isolated from 528 samples of mastitic milk from dairy herds in Ribeirão Preto, São Paulo State, Brazil (2002).

Organism	CMT-positive (%)
<i>Staphylococcus</i> sp	173 (32.76)
<i>Escherichia coli</i>	31 (5.87)
<i>Streptococcus</i> sp	7 (1.32)
<i>Candida</i> sp	75 (14.20)
No identified agents	242 (45.83)
Total	528

The family *Streptococcaceae* have long been recognized as causative agents of bovine mastitis, *Streptococcus agalactiae*, *S. dysgalactiae* and *S. uberis* have been reported as the 3 most commonly isolated species (SCHALM et al., 1971, MORIN et al., 1998). In this study only 7 isolates (1.32%-Table 1) were *Streptococcus* sp what represent a low frequency as compared with Bezek (1998) 26.6% and Morin et al. (1998) 25.8%.

Escherichia coli was another organism isolated in this study (5.87%-Table 1) that has been reported as a causative agent of mastitis (MILTENBURG et al., 1996, BARKEMA et al., 1998, HAAS et al., 2002).

Bacterial resistance to antimicrobial agents varies widely and depends on country use of drugs and infection control practice (FEDORKA-CRAY et al., 1998, BEZEK, 1998). However, the extensive use of antimicrobial agents has led to a heavy increase in antibiotic resistance in animal production worldwide (McKELLAR, 1998).

Table 2 summarizes the data about antimicrobial resistance to thirteen agents found between the three major pathogens (*Staphylococcus* sp, *Streptococcus* sp and *E. coli*). Costa et al. (1985) examined *Staphylococcus* sp and *Streptococcus* sp strains isolated from bovine mastitis in the São Paulo State, and they found a high level of resistance to lincomycin (62.0-60.0%), penicillin (92.0-60.0%), tetracycline (76.0-55.0%) and streptomycin (65.0-91.0%) as those reported in this study. Also Pereira e Siqueira-Junior (1995) and Cardoso et al. (2000) reported high level of resistance to penicillin (80.4% / 64.9%) and streptomycin (47.8% / 37.5%) between the *staphylococci* coagulase-positive isolates, agreeing with the results of this study. Schoken-Iturrino et al. (1996) reported the

Table 2 - Results of antimicrobial agent susceptibility testing on bacteria isolated from milk samples obtained from cows with mastitis in Ribeirão Preto, São Paulo State, Brazil (2002).

Antimicrobial Agent	<i>E. coli</i>		<i>Staphylococcus</i> sp		<i>Streptococcus</i> sp	
	Nº/Total	%	Nº/Total	%	Nº/Total	%
Novobiocin	31/31	100.0	111/173	64.2	7/7	100.0
Lincomycin	30/31	96.8	127/173	73.6	7/7	100.0
Penicillin	30/31	96.8	107/173	61.9	7/7	100.0
Trimethoprim / Sulphadiazin	22/31	71.0	152/173	87.9	7/7	100.0
Erythromycin	28/31	90.3	66/173	38.1	7/7	100.0
Cephalotin	26/31	83.9	34/173	19.6	4/7	57.1
Streptomycin	12/31	38.7	62/173	35.8	5/7	71.4
Ampicillin	10/31	32.3	95/173	54.9	7/7	100.0
Neomycin	10/31	32.3	57/173	32.9	2/7	28.6
Tetracycline	6/31	19.4	88/173	50.9	7/7	100.0
Nalidixic Acid	6/31	19.4	164/173	94.8	6/7	85.7
Gentamycin	0/31	0.0	11/173	6.3	1/7	14.3
Kanamycin	0/31	0.0	37/173	21.4	4/7	57.1

penicillin (100.0%) and tetracycline (68.2%) as the most commonly found antimicrobial resistance between the *staphylococci* coagulase-positive, the resistance rates reported here to these antibiotics are also high (Table 2). In Sweden about 25% of clinical mastitic cases are caused by *Staphylococcus*, and about 5 to 10 % of recent isolates are resistant to penicillin due to penicillinase production. In some countries outside Scandinavia (UK 60.0%, Ireland 90.0%), penicillin can no longer be used in the treatment of mastitis caused by *S. aureus* (FRANKLIN, 1999), situation that we are quickly reaching.

The frequency of recovery of strains resistant to each antimicrobial agent reflects the local environmental pressure, as we can see with *E. coli* isolates (Table 2). A high level of resistance to novobiocin (100.0%), lincomycin (96.8%), penicillin (96.8%) and erythromycin (90.3%) were found in this study, while Bezek (1998) reported small resistance level to novobiocin (0.0%), lincomycin (0.0%), penicillin (2.0%) and erythromycin (2.0%). However, while Bezek (1998) reported a high level of resistance to gentamycin (95.0%) and tetracycline (44.0%), in this study was found 0.0% to gentamycin and 19.4% to tetracycline (Table 2). In Norway, it is recommended that mastitis caused by coliform bacteria should not be treated by antibiotics. The self-cure rate of mastitis caused by coliform bacteria is close to 90.0% and it has been suggested that therapy of *E. coli* mastitis should focus on anti-inflammatory therapy, whereas antibiotics should be used rationally (YAZDANKHAH et al., 2001).

It has been well established that a heavy antimicrobial drug selective pressure in overcrowded populations of production animals creates favorable environments both for the emergence and the spread of antibiotic resistance genes (FRANKLIN, 1999), so it is prudent periodically to monitor bacterial isolates from cows with mastitis to determine the antimicrobial susceptibility patterns.

In conclusion, this study found the *Staphylococci* as the largest group of pathogens isolated from mastitic milk and showed high levels of resistance to lincomycin, penicillin, novobiocin and trimethoprim-sulphadiazin between the isolates.

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