

SUSCEPTIBILITY OF MASTITIS-CAUSING PATHOGENS TO ANTIMICROBIAL DRUGS – STUDY WITH MILK PRODUCED IN THE CENTRAL-WEST REGION OF SÃO PAULO

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ABSTRACT

This study aimed to characterize the agents of bovine mastitis in milk samples from dairy farms in the Midwest of São Paulo State and to assess their resistance to the antimicrobial drugs generally used for the treatment of the disease. Milk samples from 80 cows with clinical or subclinical mastitis were analyzed for pathogens using microbiological, morpho-tintorial and biochemical techniques. The species were also analyzed by mass spectrometry. Disk diffusion susceptibility test was performed with Gentamicin (10 µg), Amoxicillin + Clavulanate (20 µg + 10 µg), Tetracycline (30 µg) and Oxacillin (1 µg). Gram-positive bacteria were isolated in 87.5% of the samples, with the prevalence of *Staphylococcus aureus* (54.3%) and 21.2% of the samples had Gram-negative bacteria, with the predominance of *Escherichia coli* (18.6%). Resistance to oxacillin occurred in 48.6% of Gram-positive and in 100% of Gram-negative. For tetracyclines, the resistance levels were 12.8% and 29.4%, respectively. Gram-positive bacteria showed 11.4% of resistance for gentamicin and amoxicillin + clavulanate. Gram-negative bacteria showed 5.9% of resistance for amoxicillin + clavulanate and no resistance for gentamicin. It was found a high incidence of Gram-positive causing contagious mastitis, and most of the isolated agents were resistant oxacillin and sensitive to amoxicillin with clavulanate.

Key words: antibiotics, bacteria, diagnosis, mastitis, dairy cows.

SENSIBILIDADE DE PATÓGENOS CAUSADORES DE MASTITE A DROGAS ANTIMICROBIANAS – ESTUDO COM LEITE PRODUZIDO NA REGIÃO CENTRO-OESTE PAULISTA

RESUMO

Objetivou-se isolar bactérias causadoras de mastite bovina em amostras de leite provenientes de propriedades rurais do Centro-Oeste Paulista e avaliar sua resistência frente aos antimicrobianos utilizados no tratamento. Amostras de leite de 80 vacas com mastite clínica ou subclínica foram analisadas por técnicas microbiológicas, morfo-tintoriais e bioquímicas. A espécie foi determinada por espectrometria de massa. Os microrganismos identificados foram submetidos ao teste de sensibilidade aos antimicrobianos com discos contendo Gentamicina (10 µg), Amoxicilina + Clavulanato (20 µg + 10 µg), Tetraciclina (30 µg) e Oxacilina (1 µg). As bactérias Gram-positivas foram isoladas em 87,5% das amostras, com prevalência de *Staphylococcus aureus* (54,3%) e 21,2% das amostras apresentaram bactérias Gram-negativas, com o predomínio de *Escherichia coli* (18,6%). A resistência para oxacilina ocorreu em 48,6% das Gram-positivas e em 100% das Gram-negativas isoladas e, para tetraciclinas, a resistência

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foi de 12,8% e 29,4%, respectivamente. Dentre Gram-positivas, 11,4% apresentaram resistência para gentamicina e amoxicilina e clavulanato. As Gram-negativas apresentaram 5,9% de resistência para amoxicilina e clavulanato e nenhuma resistência frente à gentamicina. Conclui-se que as bactérias Gram-positivas eram os principais agentes de mastite contagiosa e que a maioria dos agentes isolados apresentava alta resistência à oxacilina e alta sensibilidade à amoxicilina com clavulanato.

Palavras-chave: antibióticos, bactérias, diagnóstico, mastite, vacas leiteiras.

SENSIBILIDAD DE PATÓGENOS CAUSANTES DE MASTITIS A FÁRMACOS ANTIMICROBIANOS – ESTUDIO CON LECHE PRODUCIDA EN LA REGIÓN CENTRO-OESTE PAULISTA

RESUMEN

El objetivo fue aislar bacterias causantes de mastitis bovina en muestras de leche provenientes de propiedades rurales del Centro-Oeste Paulista y evaluar su resistencia frente a los antimicrobianos utilizados en el tratamiento. Se analizaron muestras de leche de 80 vacas con mastitis clínica o subclínica mediante técnicas microbiológicas, morfo-tintoriales y bioquímicas. La especie fue determinada por espectrometría de masas. Los microorganismos identificados fueron sometidos a la prueba de sensibilidad a los antimicrobianos con discos que contenían Gentamicina (10 µg), Amoxicilina + Clavulanato (20 µg + 10 µg), Tetraciclina (30 µg) y Oxacilina (1 µg). Las bacterias Gram positivas fueron aisladas en el 87,5% de las muestras, con prevalencia de *Staphylococcus aureus* (54,3%), y el 21,2% de las muestras presentaron bacterias Gram negativas, con predominio de *Escherichia coli* (18,6%). La resistencia a la oxacilina ocurrió en el 48,6% de las Gram positivas y en el 100% de las Gram negativas aisladas; para las tetraciclinas, la resistencia fue del 12,8% y 29,4%, respectivamente. Entre las Gram positivas, el 11,4% presentó resistencia a gentamicina y amoxicilina con clavulanato. Las Gram negativas presentaron un 5,9% de resistencia a amoxicilina con clavulanato y ninguna resistencia frente a la gentamicina. Se concluye que las bacterias Gram positivas fueron los principales agentes de mastitis contagiosa y que la mayoría de los agentes aislados presentaron alta resistencia a la oxacilina y alta sensibilidad a la amoxicilina con clavulanato.

Palabras clave: antibióticos, bacterias, diagnóstico, mastitis, vacas lecheras.

INTRODUCTION

Dairy production is important for food security as it results in the generation of a highly nutritious food, made of proteins, fat, vitamins and minerals, that is among the essential foods for the nutrition of the world population (1). For these reasons, there is a growing demand to increase the productivity in dairy farms well as the milk quality. However, several factors can influence the quality of the milk, such as the nutrition and the health of the herd (2).

Mastitis is one of the main diseases that affect dairy cattle, decreasing milk production and quality and requiring expenses with the drug treatment which can, sometimes, be ineffective and lead to the culling off dairy cows with chronic infections (3). Defined as the inflammation of the mammary gland, mastitis is a complex and multifactorial condition, responsible for physical and chemical changes in milk, which can cause 10 to 20% decrease in production (4).

Antimicrobial drugs are widely used to treat this disease. However, their indiscriminate use can contribute to reducing the effectiveness of the active ingredient and to the emergence of antimicrobial resistance, reducing the antibiotic's ability to control diseases in both veterinary and human medicine (6, 7).

In addition to prevention strategies such as good agricultural and animal welfare practices, it is essential that an accurate diagnosis of mastitis-causing agents in the herd be carried out, associated with antimicrobial susceptibility tests, to develop effective methods for the control of the disease (8).

The current study aimed to characterize the bacterial agents of bovine mastitis in milk samples from dairy farms located in the Midwest of São Paulo State and to assess their susceptibility to the antimicrobial drugs commonly used in the treatment of the disease.

MATERIAL AND METHODS

This study was approved by the Ethics Committee on the Use of Animals of São Paulo State University (UNESP) with certificate number 0546-2022.

Milk samples from 80 cows in normal lactation period, with clinical or subclinical mastitis, from farms located in the Center-West of São Paulo State (Figure 1) were analyzed. The herds were composed of either Holstein or Girolando or Jersey cows, either primiparous or multiparous and the production system was semi-intensive with mechanic milking. The animals had not been treated with antimicrobials previously.

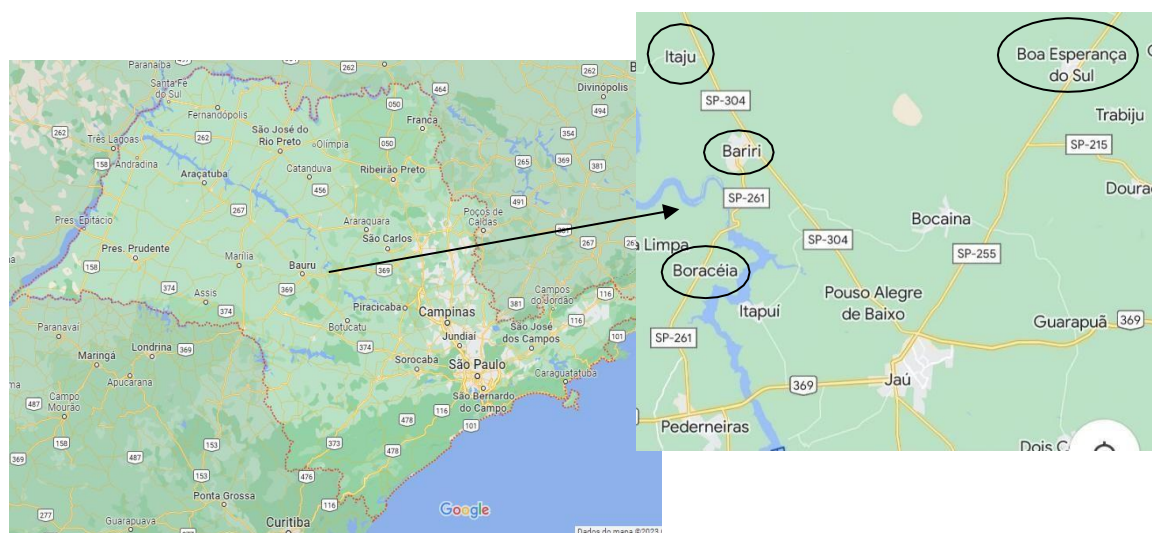


Figure 1 – Geographic distribution of sampled municipalities, obtained from Google Maps

In order to detect mastitis, a clinical inspection of the cows' mammary gland was carried out, checking for inflammatory process and the presence of pus, clots or blood, in the first three jets discarded in a dark-bottomed mug. Cows with no signs of clinical mastitis underwent the California Mastitis Test (CMT) for the diagnosis of subclinical mastitis. Cows with a CMT score ≥ 3 (scale 1 to 5) in at least one teat were included in the sampling. The tests and the examinations were carried out by a veterinarian.

After the diagnosis, 50 mL of the milk were aseptically collected from each cow (including all the mastitic teats) in sterile plastic tubes. The milk samples for microbiological examination were refrigerated at 2 to 4 °C and analyzed in the Clinical Analysis Laboratory – UNIP, campus of Bauru, within 48 hours after sampling.

For the microbiological analysis, each milk sample was plated onto blood agar containing 5% defibrinated sheep blood and onto MacConkey agar and incubated at 37 °C for 24 h. The colonies grown on the blood agar were interpreted according to their aspect, color and hemolysis. The colonies grown on MacConkey agar were interpreted on the basis of their metabolic activity as lactose fermenter or non-lactose fermenter. The morpho-tintorial characteristics of the colonies were checked with Gram staining. For the Gram-positive bacteria, the following biochemical tests were performed: catalase, DNase, coagulase, resistance to Polymyxin B, tolerance to 6.5% NaCl and Camp Test. Gram-negative bacteria were cultured in Rugai's Media with lysine and Triple Sugar Iron Agar (TSI) to check on the following biochemical characteristics: indole reaction, L-tryptophan deamination, glucose, sucrose and sucrose fermentation, H₂S production, gas production, hydrolysis of urea, lysine decarboxylation and motility (9, 10, 11).

In order to confirm the species isolated in the traditional microbiological method, some of the milk samples were also analyzed by mass spectrometry (MALDI-TOF MS) in the Qualileite Laboratory, located in the Department of Nutrition and Animal Production, FMVZ, USP, Pirassununga (12).

The antimicrobial susceptibility test was carried out by the disk diffusion method, following the specifications of the Institute of Clinical and Laboratory Standards (CLSI) (13). The antimicrobials used in this analysis were selected based on the most used to treat and control mastitis in the region. Oxacillin was chosen to search for methicillin-resistant *Staphylococcus aureus*. The inoculum was spread on a plate containing Müller-Hinton Agar, and discs containing the antimicrobials Gentamicin (10 µg), Amoxicillin + Clavulanate (20 µg + 10 µg), Tetracycline (30 µg) and Oxacillin (1 µg) were placed on it. The incubation was carried out at 35 °C for 24 h. According to the diameter of the zones of inhibition, the microorganisms were classified as: 1 – sensitive to the antimicrobial; 2 – with intermediate resistance to the antimicrobial; and 3 – resistant to the antimicrobial, as recommended by CLSI 2021 (13). A descriptive statistic was applied to the results.

RESULTS

The microorganisms isolated by the traditional techniques are listed in Table 1. The results showed a multiple etiology, since Gram-positive bacteria causing contagious mastitis were isolated in 87.50% of the samples, and Gram-negative bacteria related to environmental mastitis were also isolated in 24.3 % of them. In 12.5% of the samples there was no growth of microorganisms.

Table 1. Incidence of etiological agents isolated by traditional microbiology techniques from milk samples of cows with mastitis.

Etiological agent	Incidence
Gram-positive	
<i>Staphylococcus aureus</i>	54.3 %
Coagulase negative Staphylococci (CNS)	31.4 %
<i>Streptococcus</i> spp.	5.7 %
<i>Enterococcus</i> spp.	4.3 %
<i>Streptococcus agalactiae</i>	4.3 %
Gram-negative	
<i>Escherichia coli</i>	18.6 %
<i>Klebsiella pneumoniae</i>	5.7 %

The species identified by MALDI TOF MS are presented in Table 2. *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* and *Klebsiella pneumoniae* identified by the traditional methods were also detected by this technique.

Table 2. Incidence of etiological agents isolated by mass spectrometry from milk samples of cows with mastitis

Etiological agent	Incidence
Coagulase negative Staphylococci (CNS)	
<i>Staphylococcus chromogenes</i>	77.3 %
<i>Staphylococcus simulans</i>	9.1 %
<i>Staphylococcus haemolyticus</i>	9.1 %
<i>Staphylococcus sciuri</i>	4.5 %
Streptococcus spp.	
<i>Streptococcus uberis</i>	5.7 %
Enterococcus spp.	
<i>Enterococcus faecium</i>	4.3 %

The screening for the diagnosis of mastitis showed subclinical mastitis in 76.3% of the animals and clinical mastitis in 23.7% of them. The agents isolated from clinical and subclinical forms of mastitis are presented in Table 3.

Table 3 Etiological agents isolated from milk samples of cows with clinical and subclinical mastitis.

Form	Etiological agent	Incidence (%)
Clinical mastitis	<i>Staphylococcus aureus</i>	6.3
	<i>Staphylococcus chromogenes</i>	1.3
	<i>Staphylococcus simulans</i>	0.0
	<i>Staphylococcus haemolyticus</i>	0.0
	<i>Staphylococcus sciuri</i>	1.3
	<i>Streptococcus uberis</i>	2.5
	<i>Streptococcus agalactiae</i>	0.0
	<i>Enterococcus faecium</i>	0.0
	<i>Escherichia coli</i>	16.3
	<i>Klebsiella pneumoniae</i>	3.8
Subclinical Mastitis	<i>Staphylococcus aureus</i>	41.3
	<i>Staphylococcus chromogenes</i>	20.0
	<i>Staphylococcus simulans</i>	2.5
	<i>Staphylococcus haemolyticus</i>	2.5
	<i>Staphylococcus sciuri</i>	0.0
	<i>Streptococcus uberis</i>	2.5
	<i>Streptococcus agalactiae</i>	3.8
	<i>Enterococcus faecium</i>	3.8
	<i>Escherichia coli</i>	0.0
	<i>Klebsiella pneumoniae</i>	1.3

Table 4 presents the antimicrobial susceptibility of the microorganisms isolated from the milk of cows with mastitis.

Table 4 Degrees of antimicrobial susceptibility of the etiological agents of mastitis isolated from the milk by traditional microbiological techniques.

Etiological agent	Antimicrobial drug											
	Tetracycline (30 µg)			Gentamicin (10 µg)			Amoxicillin and Clavulanate (20 µg + 10 µg)			Oxacillin (1 µg)		
	Degree of susceptibility											
	1	2	3	1	2	3	1	2	3	1	2	3
GRAM-POSITIVE BACTERIA (%)												
<i>S. aureus</i>	89	0	11	89	0	11	87	0	13	66	0	34.2
CNS	72.8	13.6	13.6	77	9	14	87	0	13	13.6	13.6	72.8
<i>S. chromogenes</i>	64.7	17.6	17.6	70.6	11.8	17.6	82.4	17.6	0	17.6	17.6	64.7
<i>S. simulans</i>	100	0	0	100	0	0	100	0	0	100	0	0
<i>S. haemolyticus</i>	100	0	0	100	0	0	100	0	0	100	0	0
<i>S. sciuri</i>	100	0	0	100	0	0	0	0	100	0	0	100
<i>S. uberis</i>	75	0	25	100	0	0	100	0	0	50	0	50
<i>E. faecium</i>	66.7	0	33.3	0	66.7	33.3	100	0	0	33.3	0	66.7
<i>S. agalactiae</i>	67	0	33	0	67	33	100	0	0	66.7	0	33.3
GRAM-NEGATIVE BACTERIA (%)												
<i>E. coli</i>	54	8	38	100	0	0	77	15	8	0	0	100
<i>K. pneumoniae</i>	100	0	0	100	0	0	75	0	25	0	0	100

CNS: Coagulase negative Staphylococci. 1 –sensitive to the antimicrobial; 2 –intermediate resistance to antimicrobials; and 3 –resistant to the antimicrobial.

DISCUSSION

A high incidence of *Staphylococcus aureus* was found in milk samples from cows with mastitis (54.3%), especially in those with subclinical mastitis (41.3%). These results are in line with the recent data reported for the State of Minas Gerais (6). The authors found that 28 to 93% of this microorganism were isolated from cows with the udder infection thus confirming *S. aureus* as the main causal agent of mastitis in the world (14), which raises great concerns, since the cure rate for the disease caused by this bacterium is relatively low.

The presence of this microorganism in milk represents a risk to consumers due to the existence of virulence factors such as hemolysins, α -toxin (hla), which has dermonecrotic and neurotoxic effects, and β -toxin (hly), responsible for increasing the adherence of *S. aureus* to bovine mammary epithelial cells (15).

This microorganism is also capable of producing TSST-1 (toxic shock syndrome toxin) and 24 types of heat-stable enterotoxins, such as staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB) and staphylococcal enterotoxin I (SEI), that can remain viable after a heat treatment and cause poisoning and other fatal illnesses (16) due to the

ingestion of contaminated milk or dairy products. As it comes from outside the mammary gland, its eradication on the property is very challenging (17).

Coagulase-negative Staphylococci (CNS) were the second group of microorganisms with the highest incidence found in this study (31.4%). The same was found in a study that related mastitis to the reproductive efficiency of dairy cows, in which 40.7% of the isolated agents belonged to this group (18). Close values (48.7%) were also found in studies that addressed etiological aspect, cellularity and bacterial counts in the milk of cows with mastitis in farms of São Paulo State (19).

CNS is a heterogeneous group of microorganisms which includes 47 species, like *S. chromogenes*, *S. xylosus*, *S. haemolyticus*, *S. epidermidis*, *S. simulans*, *S. sciuri* and *S. hyicus*, besides others (5, 20). These microorganisms are typically used as starter cultures in the production of fermented foods and, for several years, were identified only at the genus level, since they were considered non-pathogenic (21, 22). However, several outbreaks of food poisoning involving immunosuppressed people caused by CNS species from raw foods such as milk (22, 23) have been reported. This is due to the ability of these microorganisms to produce enterotoxins such as the staphylococcal enterotoxin C (SEC) and the staphylococcal enterotoxin D (SED) by *S. warneri* and *S. chromogenes* (21, 22).

Among the species belonging to this group, *S. chromogenes* was found in 77.3% of the samples, mainly in those from animals with subclinical mastitis (20%). The high rate of *S. chromogenes* among the coagulase-negative staphylococci is consistent with current studies, in which this species of CNS was the most frequently isolated in cases of mastitis (24).

Regarding the CNS isolates, *S. haemolyticus* represented 9.1% of the identified species. This microorganism may cause nosocomial infections, meningitis, skin and eye infections in humans. The species *S. simulans*, isolated in 9.1% of the samples, can cause endocarditis, osteomyelitis and soft tissue infections, although infections in humans are rare (25, 26).

In milk samples evaluated in this study, *Streptococcus uberis* was isolated in 5.7% of the cases of both clinical and subclinical mastitis. This microorganism is described as an environmental pathogen responsible for causing approximately one third of cases of clinical and subclinical mastitis, mainly in herds under intensive management (6, 27). This microorganism presents high genetic variety among its lineages, with the ability to adapt to different environments, in addition to having broad nutritional flexibility, which leads to difficulties in the establishment of effective control strategies on dairy properties (28).

The use of specific vaccines is among the control strategies used to provide protection against the predominant strains of *S. uberis*. However, there still remain difficulties in its control due to the various virulence factors produced by the microorganism, such as proteoglycans and proteins (28, 29).

Detection of *Streptococcus agalactiae* occurred in 4.3% of the samples, with a prevalence in the animals affected by subclinical mastitis (3.8%). This is an important causal agent of mastitis in Brazil, Colombia and Uruguay, with herd-level prevalence of 60%, 42% and 11%, respectively (30). *S. agalactiae* is a contagious pathogen that survives for short periods of time outside the mammary gland, but once inside it, it can remain for long times (5).

This species belongs to group B in the Lancefield classification and is responsible for causing clinical or subclinical mastitis in dairy cattle (31) and serious diseases in newborns, pregnant women, immunocompromised adults and the elderly, such as meningitis and septicemia (31, 32). Studies suggest a possible genetic similarity between strains of bovine and human origin and some cite the possibility of interspecies transmission; however, there is still much to be elucidated about this relationship (33, 34).

Environmental mastitis can also be caused by species of the genus *Enterococcus*, particularly *Enterococcus faecalis* and *Enterococcus faecium*. Although it was detected in small quantities in this study (4.3%), with a prevalence in subclinical mastitis (3.8%), *Enterococcus*

faecium represents a risk to public health due to its ability to resist to high temperatures (35, 36). More than 50% of *Enterococcus* spp. in raw food resist to rapid pasteurization and some of them can survive 85 °C for 16 seconds (35). This thermal resistance can explain its presence in pasteurized milk or dairy products, causing harm to immunocompromised consumers (36). Moreover, *Enterococcus* spp. has a high capacity to adapt to adverse conditions and survive in the environment for long periods (37).

Among the Gram-negative bacteria detected in cases of clinical mastitis, *Escherichia coli* and *Klebsiella* spp. were identified in 16.3% and 3.8% of the cases, respectively. They are opportunistic agents and may cause environmental and clinical mastitis (38). The occurrence of these microorganisms as causes of mastitis is mostly related to the association between the host and exposure factors (animal health and environmental hygiene) than to a set of mammary pathogenic strains (3). Their high incidence in samples of milk from cows with mastitis has been previously reported, similarly to this study: 76.4% for *E. coli* and 23.6% for *Klebsiella* spp. (39).

The presence of pathogenic strains of *E. coli* in raw milk and dairy products made with unpasteurized milk is a major Public Health concern, because this microorganism is capable of producing important toxins, such as Shiga toxin (STEC), and causing serious enteric disorders in people of any age (40).

Among the genus *Klebsiella*, *K. pneumoniae* is one of the most common causes of bovine mastitis (41). It is generally associated with foodborne diseases outbreaks, causing intestinal symptoms; however, it is not an important foodborne pathogen (42).

In this study, high resistance to oxacillin (55%) by Gram-positive and Gram-negative bacteria was found. This resistance may be associated with the production of beta-lactamases enzymes, originated from plasmids or transposon, which degrade beta-lactams antibiotics, such as penicillin (43).

Oxacillin is a semi-synthetic antibiotic belonging to the penicillin group, resistant to acids and enzymes. As a stable penicillin, it is effective against Gram-positive cocci and Gram-negative bacilli penicillinase producers (44). This antimicrobial is related to methicillin resistance, also called resistance to penicillin or oxacillin, and is used in the detection of methicillin-resistant *S. aureus* (MRSA), which can be confirmed by the *mecA* genes (45).

MRSA is an important human pathogen and its occurrence in cases of bovine mastitis may be associated with the contact between cattle and humans or other hosts, such as pigs. Most of the CNS carry antimicrobial resistance determinants, which can be transferred to *S. aureus* and make it resistant to methicillin (46).

It is a matter of concern that 34.2% of the *S. aureus* isolated in this study were considered MRSA, due to their resistance to oxacillin. This group is resistant to practically all β -lactam antibiotics, due to the presence of β -lactamases enzymes (16). The increase of *S. aureus* in bovine mastitis with multidrug-resistant characteristics is a major problem for Public Health, as it increases treatment costs and morbidity (47). *S. agalactiae* is a commensal parasite of the bovine mammary gland with a low resistance to antimicrobials (17, 41), as we found in this study, with sensitivity percentage of 58.4%. However, the resistance of the microorganisms to tetracycline (33%) is worrying because it is one of the main antimicrobials used to control intramammary infection in dairy cattle (31). This resistance seems to be associated with a reduction in the permeability of the microorganism's membrane, resulting from spontaneous chromosomal mutations during drug absorption due to intense use (43).

The resistance of *S. agalactiae* to tetracyclines has been increasing since the 1980s due to the excessive use of this antimicrobial. In a study carried out with milk from cows affected by mastitis and strains isolated from human mucosal surfaces, the authors found resistance of 89.6% for bovine strains and 89.2% for the human strain of this microorganism (31). Although the control of *S. agalactiae* is relatively simple, the presence of this microorganism in milk and

dairy products generates important impacts on Public Health, potentially causing serious neonatal infections, such as septicemia and meningitis (48).

High resistance to tetracycline (25%) was also observed in *Streptococcus uberis*. This resistance results from the intense use of the antimicrobial on dairy farms over the years, reaching 80% to 95% of cases (49). This microorganism is highly sensitive to the β -lactam antimicrobials, which are frequently used to treat mastitis; however, the occurrence of subspecies resistant to these antibiotics has been reported in recent years (15, 49).

Despite the different levels of resistance found to tetracyclines, they remain the most recommended for the treatment of infected animals, since the sensitivity (81.6%) of microorganisms for them is greater than the resistance (15.8%), as demonstrated in the results of the present study.

The *Enterococcus faecium* species isolated in this study showed 33.3% resistance to the antimicrobials tested. Such resistance is related to intrinsic and acquired mechanisms, and the consumption of food contaminated with this microorganism, such as milk and dairy products, allows the transposition of *Enterococcus* spp. resistant to antimicrobials for humans due to temporary or permanent colonization in the gastrointestinal tract (35, 36). The genus *Enterococcus* spp has the ability to acquire and transfer resistance-determining genes to other bacteria, thus it is considered a good indicator of antimicrobial resistance in the environment (36).

The CNS group showed 28.4% resistance to the antimicrobials, a significant and worrying value, given that they are capable of harboring several resistance genes to various antimicrobials that can be transferred to *S. aureus* through plasmids and/or or transposons (21, 50). Studies involving the detection of resistance genes, including the Blaz gene, showed that none of the *S. aureus* analyzed possessed more than three resistance genes, while 25% of CNS isolates harbored at least four genes encoding antibiotic resistance (51).

The high antimicrobial resistance of the *E. coli* isolate (36.5%) also represents a problem, since, although the therapy for the mastitis caused by this microorganism is often limited to the reduction of the clinical signs and the maintenance of the cow, with spontaneous healing (44, 52), there is the possibility of infection by toxin-producer strains which can pose risks to the Public Health when contaminated food is ingested.

Unlike what happens with *E. coli*, mastitis caused by *K. pneumoniae* tends to have a low spontaneous cure rate and may also present recurrence of clinical cases and harbor antimicrobial resistance genes (50). The antimicrobial resistance of coliforms is associated to the production of extended-spectrum β -lactamases (ESBL) enzymes, which inactivate the action of antimicrobials from the broad-spectrum β -lactam class (amoxicillin and oxacillin) (53). Furthermore, the entry of antimicrobials into their cells is reduced due to the presence of phospholipids, lipoproteins, proteins and lipopolysaccharides in their cell wall, well as to the peptidoglycan layer (50, 53).

So, it is clear that there is a need to monitor pathogens in the dairy herd by means of diagnosis of mastitis-causing agents, management programs and conscious use of antimicrobials in milk farms, for the effective treatment. It is also important to highlight the need to establish a milking control and management strategy to avoid and/or reduce the occurrence of contagious pathogens and cross-contamination among healthy and sick cows, using good milking practices, such as pre- and post-dipping, hygiene of the milkers and disposal of cows with chronic mastitis.

CONCLUSION

Gram-positive bacteria were the main cause of contagious mastitis in herds of the MidWest region of São Paulo, and both Gram-positive and Gram-negative bacteria isolated

from the milk of affected cows showed high rate of resistance to oxacillin and high rate of sensitivity against amoxicillin + clavulanate.

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