ANTIMICROBIAL SUSCEPTIBILITY OF STRAINS OF *Staphylococcus aureus* AND *Staphylococcus* COAGULASE-NEGATIVE ISOLATED FROM COWS' MILK WITH MASTITIS IN THE WEST OF PARANÁ, BRAZIL

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ABSTRACT

Mastitis, inflammation of the mammary gland, is one of the most common diseases of dairy cattle, and the important bacterial infectious agent is *Staphylococcus* sp., which commonly presents resistance to the antibiotics used in the treatment of mastitis. For this reason, this study was aimed at drawing the profile of anti-microbial sensibility of 60 strains of coagulase-negative *Staphylococcus* (CNS) and 45 strains of *Staphylococcus aureus* isolated from samples of dairy cattle with mastitis. The tests of anti-microbial sensibility were accomplished through a disk-diffusion technique for 14 antibiotics, it being verified that the most effective *in vitro* drugs was cefepime (97.9%) for CNS and for *S. aureus* the most effective was norfloxacin (89.1%). The least effective *in vitro* drug was penicillin, its susceptibility was 7.3% and 7.5% respectively for CNS and *S. aureus.* The strains that presented resistance for three different classes of antibiotic were considered multi-resistance. Among CNS, 28.5% were multi-resistant and 64.4% for *S. aureus.* The conclusion is that penicillin, which is widely used in veterinary medicine, is not a good option for treatment of bovine *Staphylococcus* mastitis, since this study showed a low *in vitro* susceptibility. The analysis of the anti-microbial susceptibility *in vitro* should be considered by the veterinarians and owners, especially before taking decisions about the choice of the appropriate treatment in order to reduce losses in milk production and improve the control programs.

KEYWORDS: Antibiotics treatment, staphylococci; mammary gland infection, resistance

SUSCETIBILIDADE ANTIMICROBIANA DE CEPAS DE *Staphylococcus aureus* E *Staphylococcus* COAGULASE-NEGATIVA ISOLADAS DE VACAS DE LEITE COM MASTITE NO OESTE DO PARANÁ, BRASIL

RESUMO

A mastite, inflamação da glândula mamária, é uma das principais doenças de bovinos de leite, e tem um importante agente infeccioso, a bactéria *Staphylococcus*

spp., que comumente apresenta resistência aos antibióticos usados no tratamento da mastite. Por essa razão, este estudo visou traçar o perfil de sensibilidade antimicrobiana de 60 cepas de *Staphylococcus* coagulase negativa (SCN) e 45 cepas de *Staphylococcus aureus* isolados de amostras de vacas leiteiras com mastite. O teste de sensibilidade antimicrobiana foi realizado por meio da técnica de disco-difusão de 14 antibióticos, sendo verificado que a mais efetiva droga *in vitro* foi cefepime (97,9%) para SCN e para *S. aureus* a mais efetiva foi norfloxacina (89,1%). A droga menos efetiva *in vitro* foi a penicilina, com suscetibilidade de 7,3% e 7,5% respectivamente para SCN e *S. aureus.* As cepas que apresentaram resistência para três diferentes classes de antibióticos foram considerados multi-resistentes. Entre os SCN, 28,5% foram multirresistentes e 64,4% para *S. aureus*. Conclui-se que a penicilina, que é amplamente utilizada em medicina veterinária, não é uma boa opção para tratamento de mastite bovina por *Staphylococcus* spp., visto que este estudo mostrou uma baixa suscetibilidade *in vitro*. A análise da suscetibilidade antimicrobiana *in vitro* deve ser considerada pelos veterinários e proprietários, especialmente antes da tomada de decisões sobre a escolha do tratamento adequado a fim de reduzir as perdas na produção de leite e melhorar os programas de controle.
PALAVRAS-CHAVE: Antibioticoterapia, estafilococos, infecção da glândula mamária, resistência

INTRODUCTION

Bovine mastitis is a common infection of the mammary gland in dairy cattle and one of the main infectious agents are bacteria from *Staphylococcus* spp. genus (BEZERRA et al., 2009).

It is one of the main reasons for economic loss in dairy farming due to the decreased in milk production, spends with medication and medical care, disposal of contaminated milk after treatment and early discarding of sick animals (GENTILINI et al., 2002; FREITAS et al., 2005; GUILLOUX et al., 2008).

Most cases are empirically treated with antibiotics via intramammary (BOTREL et al., 2010). The therapeutic failures are often related to multiple resistances to antibiotics and the increased antimicrobial resistance has become a global concern (WITTE, 1998).

Whenever is possible, the choice of antimicrobials should be based on susceptibility testing. GÜLER et al., (2005) affirm that the determination of antimicrobial susceptibility of clinically isolated strains is not only useful for drug therapy, control programs and strategies for decision, but also to monitor resistant strains in a population and its possible spread.

In dairy cattle farming, the use of antibiotics has been implicated as a possible source of spread of resistant bacteria to animals and also humans by the consumption of dairy food (PIDDOCK, 1996; KORB et al., 2011; MARSHALL & LEVY, 2011).

The purpose of this study was to evaluate the antimicrobial susceptibility profile of strains of *Staphylococcus* spp. of milk samples from cows with clinical mastitis in the West of Paraná State, Brazil.

MATERIAL AND METHODS

The bacterial isolates (60 strains of coagulase negative *Staphylococcus* -CNS and 45 strains of *Staphylococcus aureus*) were obtained from a study in different

herds in the west of Parana between the months of November, 2006 to march, 2007. With those strains was performed the antimicrobial susceptibility testing (AST) on Mueller Hinton agar by disk diffusion method on solid media, according to the Clinical Laboratory Standards Institute (CLSI, 2012).

The following antibiotic disks were tested: amikacin, 30 µg; ampicillin, 10 µg; cephalexin, 30 µg; cefepime, 30 µg; clindamycin, 2 µg; chloramphenicol, 30 µg; erythromycin, 15 µg; streptomycin, 10 µg; gentamicin, 10 µg; norfloxacin,10 µg; oxacillin, 1 µg; penicillin G, 10 IU; co-trimoxazole, 25 µg and tetracycline, 30 µg.

The choice of antibiotics was based on the most used active principles in national veterinary therapy, however, the use of oxacillin/methicillin it is usually not used in veterinary practice and it was included in this study only for epidemiology purposes.

The plates were incubated at 35℃ for 24 hours. Aft er this period, the isolates were examined and the inhibition halos were measured. Then, they were classified as susceptible or resistant based on the criteria of interpretation developed by the CLSI (2012). Intermediate strains were considered as resistant.

In order to control were also analyzed the following standard strains: *Staphylococcus epidermidis* (ATCC12228) and *Staphylococcus aureus* (ATCC25923).The multidrug resistance was considered positive when a strain showed resistance to three or more different classes of antibiotics.

Proportion of isolates exhibiting resistant phenotypes was calculated, together with their 95% confidence intervals in the statistic program BioEstat 5.0 (AYRES et al., 2007).

RESULTS AND DISCUSSION

The antibiotic that showed better efficacy for *Staphylococcus aureus* was norfloxacin (n = 41, 89.1%) followed by co-trimoxazole (n=38, 82.6%) (Table 1).

FREITAS et al., (2005), although with a higher percentage of susceptibility for norfloxacin, found 96%, which reassures the continued use of norfloxacin in the treatment of animals affected with mastitis. Similar results were also found by ZANETTE et al., (2010) who found that norfloxacin showed 94.87% efficacy for strains of *Staphylococcus aureus* isolated from bovine mastitis in Santa Catarina State, Brazil. For norfloxacin, despite being a widespread drug in veterinary therapy, it still proved to be efficient, showing the importance of regional surveys about antibiotic sensitivity as guidance in implementing treatment.

From 96 strains tested for methicillin, twenty eight oxacillin resistant strains were isolated and classified as methicillin-resistant staphylococcus (Table 1).

Methicillin-resistant *staphylococcus* strains (20.6% for *S.aureus* and 35.1% for CNS) found in this study highlights the importance of constant monitoring of these isolates in animals to follow a possible spread of MRSA (methicillin-resistant *Staphylococcus aureus*) in the community.

According to GUÉRIN-FAUBLÉE et al., (2003) the presence of MRSA in veterinary medicine is rare, although recent studies have shown the opposite (JUHÁSZ-KASZANYITZKY., 2007; GRAVELAND et al., 2010; DIAS et al., 2011). In a research developed by JUHÁSZ-KASZANYITZKY et al., (2007) the authors reported that several cases of subclinical mastitis in Hungary are caused by MRSA and 27 strains of MRSA were isolated from dairy cows treated with intramammary infusions of penicillin, aminopenicillin or cephalosporins. According to them, using such drugs may have contributed to the emergence of these strains on the property. In this same research (JUHÁSZ-KASZANYITZKY et al., 2007) was possible to isolate MRSA from people (veterinarians, handlers or producers) who have had contact with these

animals, suggesting that these people can become colonized with MRSA and spread to the community. Methicillin-resistant staphylococci have also frequently shown multiply resistant to several classes of antimicrobial agents, including aminoglycosides, clindamycin, macrolides, quinolones, sulfonamides and tetracycline (CLSI, 2012), leaving only vancomycin as the drug of choice against these agents.

Only one strain (2.2%) of *S. aureus* was sensitive to all antibiotics tested and ten (22.2%) were sensitive to at least nine drugs. The other results can be seen in Table 1.

Regarding the isolates which were susceptible to all antibiotics in this study, we found very close results in a study from Pernambuco by FREITAS et al., (2005), in which was found that the overall percentage of sensitivity among isolates of coagulase positive *Staphylococcus* was 28.5%.

High levels of resistance in *S. aureus* for two drugs were found in the group of beta-lactam (penicillin n = 37, 92.5% and ampicillin n = 37, 86.1%) and a macrolide (erythromycin with n = 37, 90.2%) (Table 1).

Staphylococcus aureus has developed resistance to several drugs in different regions around the world (CDC, 2012), though these rates can vary from region to region or even from one property to another (WAAGE et al., 2002). In Argentina, resistance to penicillin was 40% (GENTILINI et al., 2000), in Swedish only 7.1% (BENGTSSON et al., 2009) and Turkey 63.3% resistant samples to penicillin and ampicillin (GÜLER et al., 2005).

In this study was also found a high incidence of drug resistance in *S. aureus* to the beta-lactam group (penicillin and ampicillin with 92.5% and 86.1%, respectively). Remarkable discrepancies can be found about susceptibility results for penicillin/ampicillin around the world. Relatively, these drugs have been on the Brazilian market for a long period of time as compared to countries such as Swedish, where they have a restrict use of such drugs, and therefore may not have been subjected to high use and or misuse. In Brazil, CHAGAS et al., (2012) found only 67.7% *S. aureus* resistant to penicillin. However FREITAS et al., (2005) found 80% of strains resistant to penicillin and 75% resistant to ampicillin. Already, ZANETTE et al., (2010) found 46.15 % *S. aureus* resistant to penicillin.

The resistance results obtained for beta-lactams tested (penicillin and ampicillin) was very high in this study. This high resistance may be related to several factors, such as the difference in the animal production system, considering the samples were received from, in most cases, household production properties without an adequate technical assistance.

The abusive or incorrect use of antimicrobials has been implicated as the major selective force for the development of resistance (LEVY, 2002). The high resistance rate found in this study also may reflect the misuse of antibiotics in the studied area. Evidently these are the most widely used drugs in bovine mastitis (GÜLER et al., 2005) and other diseases.

Twenty-nine isolates (64.4%) were resistant to three or more classes of antibiotics and classified as multidrug resistant.

With respect to multidrug resistance, ZANETTE et al.,(2010) in a study in Santa Catarina State found only 23.07% multiresistant *S. aureus* isolates. Similar figures were also found near to the Kenya (SHITANDI & STERNESJO, 2004) in which the incidence for small properties was 34.3% and 17.9% for large-scale properties.

MEDEIROS et al., (2009) found that the total of 291 *Staphylococcus* spp. isolated from cows with subclinical mastitis, 181 were multiresistant, and others

(110), sensitive to most part of the antibiotics tested or resistant to only one of them. FREITAS et al., (2005), in a city of Pernambuco, found that 60% of strains were resistant for six to nine antibiotics simultaneously. The high percentage of multidrug resistance revealed in this research is worrying since many of the antibiotics commercially available would have no effect on these strains, making it difficult or impossible the treatment of animals with mastitis providing greater expenses and losses to the owners. Probably the high frequency of *S. aureus* strains resistant to different groups of antibiotics used to combat bovine mastitis is often dependent among other factors, on the misuse of a product, for example, under dosing application or inadequate period of treatment, associated with mishandling sanitary standards of milking management, providing the existence of carriers of resistant S. *aureus* strains and facilitating the transmission of this bacteria between animals reared in the same environment, including through the milker's hands and utensils used for milking.

Another factor that can be considered for this high level of resistance is that most of the samples submitted to the laboratory were from animals that had been unsuccessfully treated with antimicrobial drugs and it may not faithfully reflect of the true epidemiological prevalence of the region.

Since the products of animal origin may serve as a source of resistant bacteria, there is a worldwide concern about the possibility of spread of these bacteria among animals and humans (WITTE, 1998). However, the risks for consumers directly exposed to resistant bacteria can be minimized by pasteurization of milk thereby reducing the public health problem.

TABLE 1 – *In vitro* antibiotic resistance and susceptibility of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* strains isolated from clinical mastitis in dairy cows from Parana, Brazil.

	S. aureus					Coagulase Negative Staphylococci				
Antibiotic	Sensitive	Sensitive (%)	Resistant	Resistant (%)	95% CI	Sensitive	Sensitive (%)	Resistant	Resistant (%)	95% CI
Amikacin	14/38	36.8	24/38	63.2	47.8-78.5	30/47	63.8	17/47	36.2	22.4-49.9
Ampicillin	6/43	13.9	37/43	86.1	75.7-96.4	5/60	8.3	55/60	91.7	---
Cephalexin	23/31	74.2	8/31	25.8	10.4-41.2	40/43	93.0	3/43	7.0	---
Cefepime	23/34	67.6	11/34	32.4	16.6-48.1	46/47	97.9	1/47	2.1	---
Clindamycin	20/40	50.0	20/40	50.0	34.5-65.5	32/54	59.2	22/54	40.8	27.6-53.8
Chloranphenicol	26/45	57.7	19/45	42.3	27.8-56.7	49/55	89.1	6/55	10.9	2.7-19.1
Erythromycin	4/41	9.8	37/41	90.2	---	26/54	48.1	28/54	51.9	38.5-65.2
Gentamicin	26/46	56.5	20/46	43.5	29.2-57.8	47/60	78.3	13/60	21.7	11.2-32.1
Norfloxacin	41/46	89.1	5/46	10.9	---	46/49	93.9	3/49	6.1	---
Oxacillin	31/39	79.4	8/39	20.6	7.8-33.2	37/57	64.9	20/57	35.1	22.7-47.5
Penicillin G	3/40	7.5	37/40	92.5	---	4/55	7.3	51/55	92.7	---
Streptomycin	5/31	16.1	26/31	83.9	---	34/44	77.2	10/44	22.8	---
Co-trimoxazole	38/46	82.6	8/46	17.4	6.4-28.3	55/60	91.7	5/60	8.3	---
Tetracyclin	20/35	57.1	15/35	42.9	26.5-59.3	31/50	62.0	19/50	38.0	---

Among the CNS the most effective antibiotic was the Cefepime (n= 46, 97.9%) (Table 1). The high sensitivity was followed by norfloxacin (n= 46, 93.9%) and cephalexin (n= 40, 93%). Twenty strains of CNS were resistant to oxacillin (35.1%)

Beetwen to the total sample63.3% were sensitive to at least nine or more antibiotics and two strains (3.3%) were sensitive to all drugs and 28.3% of CNS were resistant to seven or more antibiotics.

In the study by AKRAM et al., (2013) sensitivity to norfloxacin was 88.23%, showing that these drugs still have a good index of effectiveness and may be indicated in the treatment of bovine mastitis after previous microbiology testing laboratory. The values related to strains resistant to penicillin (92.7%) found in this study in CNS were relatively higher than those found by GENTILINI et al., (2002) in Argentina (27.6%) and MYLYYS et al. (1998) in Finland (37.2%).

On the other hand, also in Brazil, MACHADO et al.,(2008) found a similar value of in vitro resistance (93.5%) for penicillin. In a study of ROESCH et al., (2006) the high resistance of the isolated CNS was attributed to the fact that the samples were multiparous, considering primiparous tend to have a better profile of susceptibility, since the udder microbiota has not came into contact with different drugs or other resistant bacteria. This same event may have happened in this study since no data from each animal referring to the number of breeding was questioned.

According to GENTILINI et al., (2002) the role of CNS in bovine mastitis is not completely clear. It is known that they can cause some damage in breast tissue with a moderate increase in somatic cell count. When a CNS is isolated, the antimicrobial therapy should be indicated, but when there is detection of multidrug resistance or the emergence of methicillin CNS resistant strains, the animal must be isolated.

The CNS resistant should be monitored continuously in the Brazilian property for susceptibility to establish appropriate treatment protocols and a proper handling of infected animals. The amount of resistant strains of staphylococci is a concern to both veterinary medicine and public health, since milk is a necessary ingredient to the diet of many people and a source of income for many farmers in the area studied.

CONCLUSION

In conclusion, this study found that the most effective *in vitro* drugs for CNS strains isolated from cow was cefepime and for *S. aureus*. is norfloxacin. Also, the findings of this work discourage the use of penicillin for bovine *Staphylococcus* mastitis treatment.

In veterinary practice, this work has contributed as an important tool in the orientation of the best treatment in cases of mastitis, and showed the importance in implementing management programs for optimizing decision strategies and monitoring of strains.

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