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Identification and in vitro antimicrobial susceptibility of *Staphylococcus* species isolated from goat mastitis in the Northeast of Brazil

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Abstract

Seventy strains of staphylococci isolated from goat clinical and subclinical mastitis in Brazilian dairy herds were submitted to identification and antimicrobial susceptibility tests. *Staphylococcus aureus* represented 37% of the isolates from subclinical mastitis and coagulase-negative staphylococci (CNS) 60%. *Staphylococcus warneri*, *Staphylococcus caprae*, *Staphylococcus capitis* subsp. *ureolyticus*, *Staphylococcus sciuri*, *Staphylococcus simulans*, *Staphylococcus chromogenes* and *Staphylococcus saccharolyticus* were the CNS species identified in decreasing order of occurrence. Penicillin G was the drug that demonstrated the highest in vitro resistance rates when tested against both *S. aureus* and CNS. The difference observed in the efficacy of the drugs tested shows the importance of carrying out the identification and antimicrobial susceptibility tests of the mastitis agents.

Keywords: Goat; mastitis; *Staphylococcus aureus*; Coagulase-negative staphylococci; Antimicrobial susceptibility

1. Introduction

Members of the genus *Staphylococcus* are the main aetiological agents involved in all forms of mastitis in goats. Although *S. aureus* has been considered the major pathogen, coagulase-negative staphylococci (CNS) are the most commonly observed, principally in subclinical mastitis (Castro et al., 1992; Lima Júnior et al., 1993; Contreras et al., 1995, 1999; Bedidi-Madani et al., 1998).

The control and preventive measures against caprine mastitis are similar to those used for bovine mastitis, including pre- and post-milking teat desinfection, clinical mastitis therapy, dry therapy and proper use of the milking equipment (Poutrel et al., 1997; Menzies and Ramanoon, 2001). Antibiotic treatment is one of the recommended approaches in order to reduce intramammary infection (IMI) and, consequently, the prevalence of mastitis in the herd (Brito and Brito, 1998). However, the indiscriminate use of antimicrobial agents either for treatment of mastitis or any other

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infection might generate an increase of the resistance level of many microrganisms to these drugs (Contreras et al., 1995). Thus, knowledge of the bacterial agents responsible for mastitis cases as well as their profile of susceptibility to drugs should improve rates of IMI cure and reduce bacterial resistance.

Therefore, the aim of the present study was to identify the *Staphylococcus* species responsible for intramammary infections in Brazilian dairy herds and to determine their susceptibility to different drugs.

2. Materials and methods

2.1. Bacterial strains

Seventy *Staphylococcus* strains isolated from goat clinical and subclinical mastitis were used. The strains were obtained from milk samples collected from animals belonging to dairy herds of the Brazilian Agency of Agricultural Research—National Goat Research Center (Embrapa, Caprinos) located in the district of Sobral, Ceará. The collection of milk samples and bacteriological analyse were carried out according to National Mastitis Council (1990). Subclinical mastitis was considered when three or more colonies of the same bacterial type were observed in the primary cultures of two consecutive milk samples of the same animal (Deinhofer and Pernthaner, 1995). The bacterial genus was determined on the basis of Gram staining, colony morphology and catalase test. The colonies identified as *Staphylococcus* were submitted to the tube coagulase test using rabbit plasma (Quinn et al., 1994). The isolates were kept frozen at −20 °C in skim milk containing 15% (v/v) glycerol, until species identification and antimicrobial susceptibility tests were carried out.

2.2. Identification of Staphylococcus species

For the species identification the key proposed by Kloos (1990) was used. Coagulase-negative staphylococci were grouped according to the susceptibility profile towards Novobiocin (5 μg). The Novobiocin resistant group was submitted to tests of sucrose, D-mannose, D-cellobiose, D-xylose, L-arabinose and raffinose fermentation, reduction of nitrate and urease activity. For the susceptible group, arginine utilization, urease activity and D-trehalose, maltose, D-mannitol, D-mannose and sucrose fermentation tests were used. The coagulase-positive staphylococci were identified by the thermonuclease test, acetoin production and aerobic fermentation of sucrose, D-mannose, D-cellobiose, D-xylose, L-arabinose, raffinose, D-trehalose, maltose and D-mannitol. All tests were performed as described by Quinn et al. (1994) and MacFaddin (2000).

2.3. Antimicrobial susceptibility test

Antimicrobial susceptibility was determined by agar diffusion test, according to NCCLS (1999) and using the following drugs: Penicillin G 10 U, Erythromycin 15 μg, Trimethoprim/Sulfamethoxazole 1.25/23.75 μg, Tetracycline 30 μg, Cephalothin 30 μg and Oxacillin 1 μg. The zones of growth inhibition were evaluated as recommended by NCCLS (1999). Reference strains from the American Type Culture Collection (ATCC) were used and included: *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212.

3. Results

A summary of the identified staphylococci species is shown in Table 1. Overall, five (7.14%) of the strains were isolated from clinical mastitis: four were *S. aureus* and one *Staphylococcus warneri*. Of the 65 isolates from subclinical mastitis, *S. aureus* was identified in 37% and CNS in 60%. *Staphylococcus warneri*, *Staphylococcus caprae*, *Staphylococcus capitis* subsp. *ureolyticus*, *Staphylococcus sciuri*, *Staphylococcus simulans*, *Staphylococcus chromogenes* and *Staphylococcus saccharolyticus* were the CNS species identified. We were unable to specifically identify 10% of the isolates: two of the coagulase-positive staphylococci and five CNS.

Antimicrobial susceptibility tests (Table 2) showed that of the *S. aureus* strains, 64% were susceptible to Penicillin G, 86% to Erythromycin, 96% to Oxacillin and 100% were susceptible to Cephalothin, Trimethoprim/Sulfamethoxazole and Tetracycline. Among the coagulase-negative species, the results obtained were: 40% susceptible to Penicillin G, 77.5% susceptible to Oxacillin, 87.5% to

Table 1
Absolute and relative frequency of the *Staphylococcus* species isolated from goat clinical and subclinical mastitis

Species	Absolute frequency	Relative frequency (%)
Clinical mastitis		
S. aureus	4	5.71
S. warneri	1	1.43
Subclinical mastitis		
S. aureus	24	34.30
S. warneri	8	11.42
S. caprae	7	10.00
S. capitis subsp. *ureolyticus*	6	8.57
S. sciuri	5	7.14
S. simulans	4	5.71
S. chromogenes	3	4.29
S. saccharolyticus	1	1.43
Staphylococcus spp.[a]	7	10.00
Total	70	100

[a] Strains not identified to the specie level by the biochemical tests used.

Tetracycline and 97.5% to Erythromycin, Trimethoprim/Sulfamethoxazole and Cephalothin. Of the strains which showed Penicillin G resistance, 14 were also resistant to other drugs: five to Tetracycline/Penicillin G, seven to Oxacillin/Penicillin G, one to Erythromycin/Penicillin G and one to Trimethoprim/Sulfamethoxazole/Cephalothin/Penicillin G.

4. Discussion

Staphylococci are the bacteria most frequently isolated from goat mastitis and are thus considered the most important aetiological agents for this infection. In the early studies, *S. aureus* was isolated in a small number of subclinical mastitis cases (Kalogridou-Vassiliadou, 1991; Deinhofer and Pernthaner, 1995; Contreras et al., 1999). However, in the present investigation, it was identified in 37% of the subclinical mastitits isolates. Failure to apply the dry therapy might have contributed to the maintenance of *S. aureus* in the goat's mammary glands in the herds in this study. Because of its pathogenicity *S. aureus* must be considered an important caprine mastitis agent since it is responsible for chronic, clinical and subclinical IMI as well as for gangrenous mastitis with very high morbidity and mortality rates. Moreover, *S. aureus* is frequently resistant to antibiotic therapy due to its capacity to produce an exopolysaccharide barrier and because of its location within microabscesses, which limit the action of the drugs (Contreras et al., 2000). Since infected animals may pass on this microrganism in milk, they represent source of infection for the herd and a serious danger to public health. Therefore, it is most important to identify those animals harbouring *S. aureus*.

Table 2
Antimicrobial susceptibility of the *Staphylococcus* species isolated from goat clinical and subclinical mastitis

Species	Frequency (%) of the susceptible (S) and resistant (R) strains											
	Penicillin G		Erythromycin[a]		Trim/Sulfa[b]		Tetracycline		Cephalothin		Oxacillin[c]	
	S	R	S	R	S	R	S	R	S	R	S	R
S. aureus	64	36	86	–	100	–	100	–	100	–	96	–
S. warneri	56	44	100	–	100	–	100	–	100	–	100	–
S. caprae	57	43	100	–	100	–	57	43	100	–	100	–
S. capitis subsp. *ureolyticus*	–	100	100	–	100	–	100	–	100	–	67	33
S. sciuri	–	100	100	–	100	–	100	–	100	–	–	100
S. simulans	75	25	100	–	100	–	75	25	100	–	75	25
S. chromogenes	–	100	100	–	100	–	67	33	100	–	100	–
S. saccharolyticus	100	–	100	–	100	–	100	–	100	–	100	–
Staphylococcus spp.[d]	71	29	86	14	86	14	100	–	86	14	71	29

[a] From the *S. aureus* strains, 14% presented intermediate susceptibility to this drug.
[b] Trimethoprim/Sulfamethoxazole.
[c] From the *S. aureus* strains, 4% presented intermediate susceptibility to this drug.
[d] Strains not identified to the specie level by the biochemical tests used.

In the present study, CNS were the most frequently isolated organisms in the subclinical IMI. This finding is consistent with other studies (Castro et al., 1992; Lima Júnior et al., 1993; Contreras et al., 1995, 1999; Bedidi-Madani et al., 1998). Some of these subclinical mastitis isolates were human-associated CNS as described by many investigators (Kalogridou-Vassiliadou, 1991; Maisi and Riipinen, 1991; Castro et al., 1992; Contreras et al., 1995, 1999; Bedidi-Madani et al., 1998). Besides its association with subclinical mastitis, *S. warneri* was identified as the only agent in hyperacute clinical mastitis case, indicating that CNS may express pathogenic factors capable of causing mastitis with similar clinical features to *S. aureus* mastitis. The presence of this and other human-associated CNS indicate that humans must be considered as a possible source of infection for the goats mammary gland.

Among the six antibiotics tested against the identified species, Penicillin G was the least effective. Most of the CNS species demonstrated high resistance levels to this drug, with *S. capitis* subsp. *ureolyticus*, *S. sciuri* and *S. chromogenes* strains showing 100% of resistance. Resistance of goat CNS isolates to penicillin G was also described by Lima Júnior et al. (1993) and Corrales et al. (1995) who observed resistance rates of 41.18 and 75%, respectively. The Penicillin G also showed low efficacy against *S. aureus* that was similar to results reported by Lima Júnior et al. (1993) who found 41.67% of Penicillin G resistance. The resistance to Penicillin G observed in this research must be of concern since this drug represents the main antibiotic group recommended for staphylococcal mastitis treatment.

In this investigation it was also observed that some of the CNS species had low susceptibility to penicilinase-resistant penicillins (PRPs). The Oxacillin resistance demonstrated by *S. capitis* subsp. *ureolyticus*, *S. sciuri* and *S. simulans* indicates that the CNS group has a potential capacity to develop resistance mechanisms to this drug. Hinckley et al. (1985) testing CNS isolated from goat's milk reported 82% of PRPs resistance using Cloxacillin as representative of that group. Oxacillin resistant CNS might compromise the IMI elimination, since these species are the main agents of goat subclinical mastitis and Oxacillin and related drugs are the main components of the antibiotic formulations for the dry therapy.

In conclusion, this study showed that CNS species are the agents most commonly involved in subclinical mastitis in the National Goat Research Center herds. In addition, the differences observed in the efficacy of the drugs tested shows the importance of antimicrobial susceptibility tests, performed together with identification of the bacterial agents.

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