ISAH 2005 - Warsaw, Poland
Vol 1

CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF CATALASE-NEGATIVE GRAM-POSITIVE COCCI ISOLATED FROM BOVINE MASTITIS IN BRAZIL

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Key words: *Streptococcus, Enterococcus, Lactococcus,* bovine mastitis, microbiological diagnostic, minimal inhibitory concentration.

Introduction

In dairy farms, mastitis is within the primary sanitary problems to be controlled, once its presence results in significant economic losses for the producer and the dairy industry, in function of the effects on quality and composition of the milk produced by the sick animals. Amongst the etiological agents of bovine mastitis, noted is the catalase-negative gram-positive cocci. Members of this group include the contagious pathogens, *Streptococcus agalactiae*, and the environmental pathogens: *S. uberis*, *S. bovis*, *S. dysgalactiae dysgalactiae*, *Enterococcus* spp., *Lactococcus* spp.

Streptococcus agalactiae is an oligate intramammary bacteria that can be eradicated from dairy herds. Most infections are subclinical, with transmission thought to occur most often at the time of milking (1, 2, 3, 6, 10, 13). The primary reservoir of environmental pathogens is the dairy cow's environment and exposure of uninfected udder quarts to environmental pathogens is not limited to the milking process (16, 17, 19, 20).

Due to the diversity of genera and species found internationally in this group it is necessary to evaluate which of them are present in the Brazilian herds. The aim of this work was to characterize catalase-negative gram-positive cocci isolated from bovine intramammary infections in Brazil.

Material and methods

A total of 163 gram-positive catalase-negative cocci isolated from the milk of cows with clinical or subclinical mastitis were characterized. The samples were isolated from 60 herds in South-eastern Brazil throughout the time period from 1995 to 2004. The strains were

identified using conventional tests described previously (5, 7, 8, 9, 11). Twenty-six biochemical and Lancefield serological grouping tests were performed: cell arrangement, hemolysis, vancomycin susceptibility test, LAP and PYR tests, growth at 10 and 45°C, esculin and sodium hippurate hydrolysis, growth in bile-esculin agar, in 6,5% NaCl and in selective medium for *Enterococcus* (Slanetz-Bartley medium), CAMP test, motility test, Voges-Proskauer test, tellurite tolerance test, pyruvate utilization test, and fermentation of arabinose, inulin, lactose, mannitol, raffinose, ribose, sorbitol and trehalose. Lancefield groups were determined using Oxoid – Diagnostic Reagents Streptococcal Grouping, containing groups A, B, C, D, F and G antisera.

The minimum inhibitory concentration (MIC) was determined for the following antimicrobial agents: ampicillin, cephalothin, gentamicin, oxacillin, penicillin, tetracycline and tylosin. The reference strains *Staphylococcus aureus* American Type Culture Collection ATCC (29213), *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 e *Pseudomonas aeruginosa* ATCC 27853 recommended by National Committee for Clinical Laboratory Standards (2002) were included in the study. All MIC determinations were performed according to the method described by NCCLS (12). The interpretative criteria used to categorize an isolate as susceptible or resistant to an antimicrobial agent are defined by NCCLS (12).

Results

The following genera and species were identified: 43 (26,4%) *S. agalactiae*, 25 (15,4%) *S. uberis*, 24 (14,7%) *S. bovis*, 15 (9,2%) *S. dysgalactiae* subsp. *dysgalactiae*, two (1,2%) *S. pluranimalium*, two (1,2%) *S. porcinus* and one (0,6%) *S. canis*; 31 (19,1%) *Enterococcus*; and two (1.2%) *Lactococcus*. Eighteen samples (11%) could not be classified in genera. Seven *Enterococcus* strains were classified as *E. Faecalis*. Seventy-nine isolates (48%) agglutinated with one or more of the grouping sera: B (43 isolates); C (18); D (10); C and D (4); G (3) and C, D and G (1 isolate).

All values obtained with control strains in MIC tests were within the expected ranges for all antimicrobial agents tested. The concentration that inhibits 90% (MIC_{90}) of the analyzed strains, reported in micrograms per milliliter, for the enterococci were 2, 1, 8, 32, 32, 32, 32 to ampicillin, penicillin, oxacillin, cephalothin, gentamicin, tetracycline and tylosin, respectively. Streptococci had different values for ampicillin (1), penicillin (0,5), oxacillin (4), tetracycline (64) and tylosin (64).

Discussion

The Lancefield grouping may aid in identification of *Streptococcus* species. However, serogroups do not exactly match species in the veterinary important streptococci, except in the case of *S. agalactiae*. For this reason, serogrouping is less useful in veterinary than in human medicine. Furthermore, a great number of strains fail to react with Lancefield groups antisera (4).

Simplified physiological tests are very useful in the diagnostic mastitis routine, because they are able to separate *S. agalactiae* from the other environmental streptococci species. However, did not offer enough information about other species of *Streptococcus* and other catalase-negative Gram-positive cocci isolated from bovine mastitis. The complete information should be obtained using a large number of tests who needs a lot of medium and reagents, technical assistance and time. The molecular methods for bacteria identification should be an alternative to the characterization of this group of organism.

The determination of the antimicrobials susceptibility patterns showed that all strains of *Enterococcus* spp. were susceptible to penicillin. This antibiotic is the recommended class representative for the enterococci (18). On the other hand, oxacillin (29%), cephalothin (34%) and tylosin (34%) demonstrated moderate in vitro activity against *Enterococcus* spp. This agree with results from monitoring studies done in California, USA, where the enterococci population isolated from mastitis were highly susceptible to penicillin, but such more resistant to oxacillina and cephalothin (14).

The MIC_{50} of cephalothin, oxacillin and penicillin G for the streptococci were 2.0, 1.0 e 0.125 μgmL^{-1}, respectively. Results from New Zealand and Denmark disagree with our findings (15). The MIC_{50} values to cephalothin, oxacillin and penicillin G found by Salmon et al. (1998) were 0.25, 0.13 e 0,06 μgmL^{-1}, respectively. These results indicated differences in the susceptibility patterns for the streptococci and enterococci isolated from mastitis in various regions.

Conclusion

The results showed that different genera and species were present in the group of catalase-negative gram-positive cocci that are generally classified as *Streptococcus* spp., and that their identification is important in order to appropriately apply control measures.

The culture from samples of milk derived from clinical and subclinical mastitis for the identification of etiological agents and the understanding of the susceptibility patterns for these agents to the antimicrobials may lead to the development of a rational and effective program for the control of mastitis, contributing to the reduction of the costs with the therapy and significant improvement in the milk quality.

Acknowledgements

Financial support: Empresa Brasileira de Pesquisa Agropecuária (Embrapa) e Conselho Nacional de Desenvolvimento Científico e Tecnologico (CNPq).

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