TIME COURSE OF BIOFILM FORMATION BY STAPHYLOCOCCI SUBCLINICAL MASTITIS ISOLATES

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Staphylococci are recognised MASTITIS pathogens, often responsible for subclinical infections and chronic bovine MASTITIS. Biofilm formation is considered a selective advantage for staphylococci MASTITIS isolates, facilitating bacterial persistence in the udder and contributing to the evasion of immunological defences and hampering pathogen eradication. Biofilm formation requires attachment to mammary epithelium, proliferation and accumulation of cells in multilayers and enclosing in a polymeric matrix, being regulated by several loci. As biofilm formation can proceed through different pathways, its detection may differ according to the time of observation.

This study aimed at evaluating the time course evolution of biofilm production in Staphylococcus epidermidis (n=50), S. chromogenes (n=16), S. simulans (n=14) and S. aureus (n=5) isolated from subclinical bovine MASTITIS by Fluorescent In Situ Hybridisation.

After incubation for 24h, 48h and 72h to allow biofilm formation, a FISH protocol was applied, as follows: fixation with paraformaldehyde 4% in PBS (2h); dehydration using ethanol serial concentrations (50%, 80%, 96%, 3min); permeabilization with 0,01 mg/ml lysostaphin (4 min); hybridization with 10µl of hybridization buffer (0,9M NaCl, 20mM Tris-HCl, 0,01% SDS, 5ng/µl of a 16S rRNA fluorescent probe, Sta) (3h, 45ºC); stringency washes with washing buffer (0,9M NaCl, 20mM Tris-HCl and 0,1% SDS) (15min, 45ºC); and observation under fluorescence microscopy in the x1000 amplification (Objective HCX PLAN APD) in a Leica DMR microscope.

It was possible to detect biofilm formation in isolates from the species under study. Biofilm-forming ability increased with incubation time for all species. The percentage of biofilm-positive isolates at 24h, 48h and 72h were 48%, 80% and 86% for S. epidermidis; 44%, 63% and 75% for S. chromogenes; 57%, 64% and 79% for S. simulans; and 60%, 80% and 100% for S. aureus.

Bacterial biofilms may impair eradication of chronic MASTITIS, rendering antibiotherapy less effective. Detection of biofilm-forming ability in MASTITIS isolates may provide useful information for the establishment of a more adequate therapeutic regimen, in view of the antimicrobial concentrations required for bacterial control. However, it is essential that biofilm formation time course is taken into consideration. Further studies are required, aiming at better simulating in vivo conditions, thus providing a better model for biofilm formation in the udder.