

TITLE: INHIBITORY ACTION OF SANITIZERS IN BIOFILM FORMATION OF LISTERIA MONOCYTOGENES ISOLATED IN EXPANSION TANK MILK ASSESSED IN ALAGOAS STATE COUNTIES, BRAZIL

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ABSTRACT:

Biofilms can interfere with the bacterial resistance to sanitizers used in pre- and post-*dipping*, besides favoring the persistence of microorganisms in the milk and derivatives production chain. *Listeria monocytogenes*, a psychrotrophic pathogen, represents a danger to contamination in milks stored in refrigerated tanks. The objective of this study was to evaluate the ability of sanitizers used in pre- and post-*dipping* to inhibit the formation of *Listeria monocytogenes* biofilms isolated in expansion tank milk assessed in Alagoas State counties, Brazil. Four biofilm-forming strains isolated from individual expansion tank milks in counties of the dairy Alagoas State were used, stored at the UFRPE Meat and Milk Inspection Laboratory. Colonies of *L. monocytogenes* were inoculated in 3mL of Tryptone Soy Broth until 0.5 turbidity on the McFarland scale and incubated at 37°C/ 24 hours. So were added 100µl of this solution to 96-well microdilution plates and then 100µl of the sanitizer to be tested (2.5% chlorine and 2% chlorhexidine commercially purchased) were added, followed by incubation (37°C/24h). The contents of each well were aspirated and three washes were performed with sterile distilled water. After drying the plate at room temperature, 200µl of 0.25% gentian violet was added for 5 minutes and the wells were again washed and subjected to drying. Then, 200µl of alcohol: acetone (80:20) was added and reading of the optical density was followed. To determine the degree of adherence under the action of the sanitizing was measured by spectrophotometry at 620nm the mean optical density (DO) of the negative control (DO_{CN}) and compared to the mean DO of the isolates (DO_{IS}), and the adhesion was classified as: negative (DO_{IS}< DO_{CN}); weak (DO_{CN}<DO_{IS}<2.DO_{CN}); moderate (2.DO_{CN}<DO_{IS}<4.DO_{CN}); and strong (4.DO_{CN}<DO_{IS}). It was observed that in relation to chlorine 50% (2/4) of the strains the adhesion was negative and in 50% (2/4) there was weak adherence. With clorexidine, there was moderate adherence in 100% of the strains. In this way, chlorine presented a more effective action in relation to the ability to interfere in the formation of biofilm by the strains tested. Thus, in addition to the proper use of sanitizers in pre- and post-*dipping*, it is necessary to regularly evaluate the efficiency of the products to favor the control of *L. monocytogenes* in the herd, to prevent the contamination of milk by pathogenic microorganisms and to avoid the formation of biofilms.

Keywords: foodborne diseases, pathogenic microorganisms, public health, listeriosis, pre- and post-*dipping*.

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