Title: Effect of thymol, carvacrol and peracetic acid against *Salmonella* Typhimurium biofilm on stainless steel

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Resume:

Salmonella spp. is one of the etiologic agents identified in outbreaks of foodborne diseases and has the ability to adhere and form biofilms onto various surfaces, becoming extremely resistant to antibiotics and disinfectants. Based on these problems, the aim of this study was to evaluate the effect of thymol, carvacrol and peracetic acid against *Salmonella* Typhimurium ATCC 14028 biofilm on stainless steel. Antimicrobial activity of these compounds was evaluated by determining the Minimum Inhibitory and Minimum Bactericidal concentration (MIC and MBC) using the broth microdilution assay. The effect of different concentrations of these compounds on biofilm was evaluated by colony counts and scanning electronic microscopy (SEM). Two-day-old *S*. Typhimurium biofilm was left to be formed on steel coupons at 35 ºC and coupons were treated with thymol and carvacol at sub-MIC, MIC and 2 x MIC and peracetic acid at MIC. Cells were detached using ultra-sonic bath (25 kHz/ 5 min) diluted, plated on MHA and incubated at 35 ºC for 24 h. MIC and MBC of thymol and carvacrol were 312 µg/mL and 156 µg/ml, respectively. MIC and MBC of peracetic acid was 100 ppm. Number of *S*. Typhimurium cells recovered from steel surface was approximately 7 log CFU/cm^2. SEM images demonstrated the presence of microcolonies, cellular multilayer and exopolysaccharide production, which are biofilm characteristics. Treatment with 234 µg/mL of thymol and 117µg/ml of carvacrol (sub-MIC concentrations) during biofilm formation reduced approximately 0.8 and 1 log UFC/cm^2 of *S*. Typhimurium, respectively. The greatest reductions in mature biofilm was observed with thymol at 624 µg/mL and carvacrol at 312 µg/mL (2 x MIC concentrations), which reduced bacterial counts approximately 2.7 log and 2.3 log, respectively. Peracetic acid-treatment drastically reduced *S*. Typhimurium biofilm at MIC concentration, where no viable cells were recovered. Thus, this study demonstrate that substances tested were able to reduce *S*. Typhimurium on stainless steel and could be used in conjunction with other treatments as new strategy to biofilm control.

Keywords: Biofilm, Carvacrol, *Salmonella* Typhimurium, stainless steel, Thymol.

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