Biofilm formation by *Salmonella enterica* Enteritidis on regular and antimicrobial incorporated food processing surfaces and subsequent cellular viability

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Aims: Industrial and domestic food-processing environments are one of several areas where biofilm formation has a very negative impact, due to post-processing contamination that leads to lowered shelf-life of products and transmission of diseases. In this context *Salmonella* is one of the most problematic pathogens, causing substantial costs in industrial systems and being responsible for millions of human cases of salmonellosis worldwide every year. A significant portion of *Salmonella* infections occurs in domestic environments, where cross-contamination seems to be more relevant than direct consumption of contaminated food. So, regarding the role of food contact surfaces in the transmission of this pathogen, our aim was to determine biofilm formation of *Salmonella* Enteritidis on regular and antimicrobial incorporated kitchens' surfaces and to evaluate the viability of cells within biofilms.

Methods: Five *Salmonella* Enteritidis strains were used in this work: 1 clinical isolate (355), 3 food isolates (357, 358, CC) and 1 reference strain (NCTC 13349). Materials tested were stainless steel (the most used material in industrial kitchens and present in all domestic kitchens), granite and marble (two commonly used kitchen bench stones) and two silestones (a material mainly made from quartz with Microban® incorporated, also used as kitchen bench stone). Assays were performed in 6-well plates with an incubation period of 48 hours at room temperature (22ºC), with shaking at 120 rpm and using LB + 0.25% glucose as culture medium. Biofilm formation and cellular viability were assessed through crystal violet (CV) staining and colony-forming units (CFU) enumeration (after cell scraping), respectively.

Results: Marble was the surface on which most strains were able to form biofilm with higher biomass amount (OD ≈ 0.12) comparing to the other materials. Granite, both silestones and stainless steel revealed similar OD values, although on each silestone there was one strain displaying the lowest biomass amount of all tested materials. On the other hand, both regular stones (granite and marble) had approximately the same number of viable cells (≈ $1x10^7$ cfu/cm^2) which was significantly higher than those found on both silestones, though the number of viable cells on silestones was still very high (≈ $3x10^6$ cfu/cm^2). Stainless steel had an intermediate number of viable cells when compared with other materials.

Conclusions: In this work *Salmonella* was able to colonize all surfaces tested and has formed biofilms with high numbers of viable cells, which leads to conclude that these materials will need a cautious utilization with appropriate sanitation when used in food processing

environments. Nevertheless, if we had to choose one of these surfaces, silestones would be the best option since, although ineffective in preventing biofilm formation, Microban® seems to have some bacteriostatic activity that has decreased the number of viable cells within biofilms.