Adhesion to and viability of *Salmonella* Enteritidis on food contact surfaces

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Many cases of food poisoning occur in the domestic environment and can be associated with improper food handling and ineffective cleaning by consumers. These practices could lead to the introduction and spread of bacterial contamination in the kitchen and if not subsequently removed could present an infection risk. When adhered to kitchen surfaces, cells are not easily removed by normal cleaning procedures. Therefore, they can be a source of contamination for other foods and objects. This phenomenon is known as cross-contamination. Many studies have investigated bacterial contamination in domestic kitchens and demonstrated that utensils/sites like sponges, clothes, sinks, taps, chopping boards, and hands of kettle and refrigerator can be contaminated (Gorman et al., 2002; Kusumaningrum et al., 2003; Haysom and Sharp, 2003). Like some other foodborne pathogens, *Salmonella* are able to adhere to inert food contact surfaces and to form biofilms. Nowadays, there are over 2500 known types, or serotypes, of *Salmonella*. However, *Salmonella* enterica serovars Enteritidis and Typhimurium are the *Salmonella* types most frequently associated with human illness. Recently, *S.* Enteritidis has emerged as a major serotype in human infections and in chicken contamination (Burr et al., 2005).
In order to modify the surface characteristics of materials to minimize bacterial adhesion it is important to investigate which properties are determinant in the adhesion process. The aim of this work was to investigate the influence of surface roughness and hydrophobicity in the adhesion ability of four isolates of *Salmonella* Enteritidis. The adhesion was performed on eight types of materials of different surface properties, commonly found in kitchens (stainless steel 304 (SS 304), marble, granite, glass, polypropylene from a basin (PPb), polypropylene from a cutting board (PPcb) and two kinds of silestone (white silestone (wST) and beige silestone (bST)).
Three clinical isolates (355, 357, CC) and one food strain (355) of *Salmonella* Enteritidis were used in this study. Adhesion assays were performed in a static mode, in which 2 cm^2 coupons were immersed in the bacterial suspension for 2 hours. For enumeration of adherent bacterial cells, the coupons were stained with DAPI 0.1 g/l and observed under epifluorescence microscopy. The coupons with adhered cells were also observed by scanning electronic microscopy (SEM). The viability of adhered cells was determined using the LIVE/DEAD Backlight kit (Molecular Probes, Eugene, OR, USA) through which cell membrane integrity is assessed. The hydrophobicity of the materials as well as of the bacterial cells was determined by contact angle measurements through the sessile drop technique. Material surfaces roughness and topography was assessed by atomic force microscopy (AFM).
Figure 1 demonstrates that *Salmonella* strains adhered in greater extent to stainless steel and in the lowest extent to both polymers, while no significant differences on the number of adhered cells were detected among the other materials assayed. It can also be observed that, *S.* Enteritidis 358 is the strain that displayed a higher amount of adhered cells, followed by strain CC, 355 and 357. No correlation was observed between the extent of adhesion and the hydrophobicity of the tested materials. Actually, stainless steel (hydrophobic material) presents the highest number of adhered cells while both polymers (also hydrophobic materials) present the lowest number. In addition, glass and beige silestone are hydrophilic materials and present also a great extent of colonization by the *Salmonella* strains.

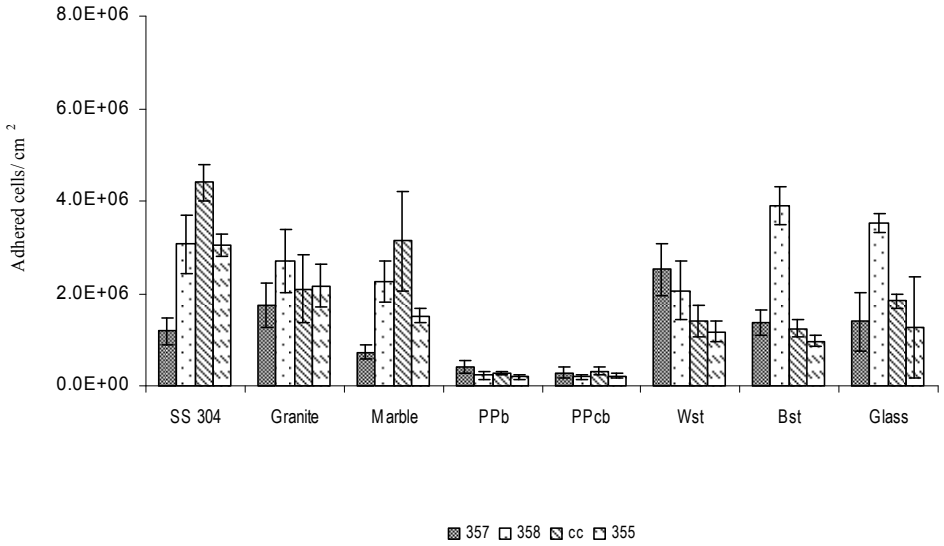

Figure 1. Number of adhered cells per cm^2 of the *Salmonella* strains to the materials.

Considering surface roughness, no correlation was also found between this property and the ability of adhesion of the *Salmonella* strains. In fact, glass is the smoothest material (average roughness of 1.6 nm) and presents a high number of adhered cells of all strains. On the contrary, white silestone is the material with the highest roughness (average roughness of 31.5 nm) and does not display the highest extent of adhesion.

It must be noted that, even though both silestones have incorporated an antimicrobial product (Microban), these materials present an extent of adhesion greater than both polymers. Thus, it seems that the incorporation of Microban is not totally effective against the adhesion of these pathogenic bacteria. On the other hand, this raised the question that adhered cells, especially to silestone, might not be viable. However, cell viability assays, revealed that the lowest percentage of *Salmonella* strains survival was found on white silestone (16 % cells with intact membrane) while, curiously, in silestone beige the percentage of viable cells was high (53.0 % cells with intact membrane). Stainless steel was the material displaying the highest number of viable cells (99.0%).

The results put to evidence that the physico-chemical properties of surface materials are not a determinant factor in the process of adhesion of the *Salmonella* strains studied. In fact, the main conclusion that was drawn is that *Salmonella* adhesion is strongly strain dependent and this can constitute a factor of virulence.

References

Burr, R., Effler, P., Kanenaka, R., Nakata, M., Holland B., Angulo F.J. (2005). Emergence of *Salmonella* serotype Enteritidis phage type 4 in Hawaii traced to locally-produced eggs. *International Journal of Infectious Diseases,* 9, 340-346.

Gorman, R.; Bloomfield, S.; Adley C. C. (2002). A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland. *International Journal of Food Microbiology*, 76, 143-150,

Haysom, I.W., Sharp, A.K. (2003), Cross-contamination from raw chicken during meal preparation, *British Food Journal*, 106, 38-50.

Kusumaningrum, H.D.; Riboldi G.; Hazeleger, W.C.; Beumer, R.R. (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology,* 85, 227-236.