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Comparison of the Adhesion Ability of Different *Salmonella* Enteritidis Serotypes to Materials Used in Kitchens

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ABSTRACT

Contamination of kitchen surfaces due to bacteria present in foodstuffs is one of the main causes of foodborne outbreaks. *Salmonella* infections are an important cause of foodborne disease, and *Salmonella* Enteritidis is the most common isolate in the past few years. In this study, the adhesion ability of four *Salmonella* Enteritidis isolates to different materials (polyethylene, polypropylene, and granite) used in kitchens was compared. The results indicated that the two plastic materials were generally less prone to colonization than was the granite. As surface properties of both bacteria and materials are a determinant in the adhesion process, surface hydrophobicity was determined through contact angle measurement, and the roughness of the materials was evaluated through the R_a and R_z values by a noncontact laser stylus tracing. The four *Salmonella* strains showed similar degrees of hydrophilicity, while the materials were hydrophobic, with granite having a very low degree of hydrophobicity ($\Delta G_{lwl} = -4.7$ mJ/m^2). However, the different extents of adhesion could not be explained in terms of surface hydrophobicity and roughness of the materials tested. The main conclusion to be drawn is that *Salmonella* adhesion is strongly strain dependent, despite the similar degree of hydrophobicity displayed by all the strains assayed, and this can constitute a factor of virulence among the different serotypes.

During the preparation of naturally contaminated food, potential pathogens are frequently spread to hands and food processing surfaces. Cells that adhere to surfaces of domestic kitchens are not usually removed by normal cleaning procedures *(3).* Therefore, they can be a source of contamination for other foods coming into contact with such surfaces and objects. *Salmonella* is an important pathogenic bacterium of considerable significance for the food processing industry *(4, 13, 19, 22). Salmonella* infections are an important cause of foodborne bacterial diseases *(21).* In many countries, *Salmonella* Enteritidis has been the most common isolate in foodborne diseases in the past few years *(1, 11, 23).*

Several studies have shown that many kitchen sites become contaminated when food that is harboring bacteria is prepared, and this may be an important source of *Salmonella* infections in the home *(6, 15, 34).* The occurrence of *Salmonella* in chicken carcasses can vary from 0.024 to 85.0%, which demonstrates that chicken carcasses are a strong potential source of bacterial contamination of utensils and kitchen surfaces. In this way, the potential of kitchen surfaces to act as chronic sources of microbial contamination can compromise food quality and represent a significant health hazard.

Materials that retain fewer microorganisms after cleaning would be the hygienic choice and present the most minor risk of cross-contamination. Another point to consider is that the wear of surfaces will affect their finish and potentially their hygienic status *(30).* Chopping boards are more prone to cross-contamination, particularly from the juices of raw meat and poultry remaining on the surface, resulting in the transfer of microorganisms to other foods subsequently prepared on the same surface *(12).* In recent years, there has been a steady rise in the use of plastic materials in the food industry *(17),* and some studies have been conducted to evaluate the adhesion of *Salmonella* on plastic surfaces *(7, 19).* Besides roughness, hydrophobicity is one of the most relevant surface properties in the adhesion of *Salmonella (8, 19, 20).*

The aim of this study was to investigate the adhesion ability of four strains of *Salmonella* Enteritidis to different materials (polyethylene, polypropylene, and granite) usually used in kitchens and to evaluate the role of surface hydrophobicity and roughness in the adhesion process.

MATERIALS AND METHODS

Bacterial strains and growth. Four strains of *Salmonella* Enteritidis were selected for this study. Two of these were previously isolated from poultry: *Salmonella* Enteritidis EMB was isolated from the water of packaged chicken, and *Salmonella* Enteritidis MUSC was isolated from chicken breast. The other two *Salmonella* isolates (*Salmonella* Enteritidis PC and *Salmonella* Enteritidis AL) were human isolate outbreak strains.

All bacterial isolates were maintained in Trypticase soy agar. Every strain was subcultured twice in Trypticase soy broth at 37°C in an orbital shaker (130 rpm) overnight. The cells were then harvested by centrifugation at 5,000 × *g* for 10 min and washed

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three times with phosphate-buffered saline (PBS; 0.1 M [pH 7]). The pellets were resuspended in PBS to 10^8 CFU/ml, as determined by optical density at 600 nm.

Materials. The test surfaces were as follows: polyethylene used in cutting boards, polypropylene from a basin, and granite (Pedras Salgadas, Portugal) commonly used as a bench cover in domestic kitchens. For the adhesion experiments, the materials were cut in coupons of 0.8 by 0.8 cm (polyethylene) and 1.8 by 1.8 cm (polypropylene and granite). For contact angle measurements, materials were cut in slides of 7.0 by 2.5 cm. Each time, the materials were washed in a solution of a commercial detergent (Sonasol Pril, Henkel Ibérica S.A., Portugal) in ultrapure water (Seralpur pro 90 CN, Belgolabo, Overijse, Belgium) for 30 min and then thoroughly rinsed in ultrapure water (to remove any remaining detergent); this procedure was then followed by immersion in 90% ethanol for 30 min for surface disinfection.

Surface tension components and hydrophobicity. Hydrophobicity was evaluated through contact angle measurements and by the approach of van Oss et al. *(26, 28, 29).* In this approach, the degree of hydrophobicity of a given material (*l*) is expressed as the free energy of interaction between two entities of that material when immersed in water (*w*): ΔG_{lwl}. If the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic ($\Delta G_{lwl} < 0$); conversely, for a hydrophilic material, $\Delta G_{lwl} > 0$. ΔG_{lwl} is calculated through the surface tension components of the interacting entities, according to the following formula:

$$\Delta G_{lwl} = -2\left(\sqrt{\gamma_l^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 + 4\left(\sqrt{\gamma_l^+\gamma_w^-} + \sqrt{\gamma_l^-\gamma_w^+} - \sqrt{\gamma_l^+\gamma_l^-} - \sqrt{\gamma_w^+\gamma_w^-}\right) \quad (1)$$

where γ^{LW} accounts for the Lifshitz–van der Waals component of the surface free energy and γ^+ and γ^- are the electron acceptor and electron donor parameters, respectively, of the Lewis acid-base component (γ^{AB}), with $\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-}$. The surface tension components of a solid material are obtained by measuring the contact angles of three pure liquids (one apolar and two polar) with well-known surface tension components *(27),* followed by the simultaneous resolution of three equations of the following form:

$$(1 + \cos\theta)\gamma_l^{TOT} = 2\left(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^+\gamma_l^-} + \sqrt{\gamma_s^-\gamma_l^+}\right) \quad (2)$$

where θ is the contact angle and $\gamma^{TOT} = \gamma^{LW} + \gamma^{AB}$.

Contact angle measurement. Contact angle measurements (at least 25 determinations for each liquid and for each material and microorganism) were performed automatically with the aid of an image analysis system (G2/G40) installed in a standard contact angle apparatus (Kruss-GmbH, Hamburg, Germany). The images were transmitted by a video camera to a 486 DX4 100-MHz personal computer for evaluation. All the measurements were performed by the sessile drop method at room temperature, and three liquids with different polarities were used: water (*W*), formamide (*F*), and α-bromonaphthalene (α-*B*). For bacterial cells, the measurements were performed on a cell lawn according to the method described by Busscher et al. *(5).* Briefly, bacterial cells were deposited on a cellulose acetate membrane filter (pore diameter of 0.45 μm) by filtration under negative pressure. To standardize the moisture content, the filters were then transferred onto petri dishes containing 1% (wt/vol) agar with 10% (vol/vol) glycerol.

Adhesion assays. Each coupon of the tested materials was immersed in a well of a six-well tissue culture plate containing 2 ml of a bacterial cell suspension with a concentration of 10^8 CFU/ml. After 1 h at 25°C with constant shaking at 100 rpm, the coupons were rinsed twice with PBS to remove poorly adhered cells. An aliquot (20 μl/ml) of a 4′,6-diamidino-2-phenylindole solution was added to each coupon and incubated for 30 min in the dark. The coupons were then rinsed with sterile distilled water, and the adherent microorganisms were quantified under epifluorescence microscopy by image analysis software (Image-Pro Plus, Media Cybernetics, Silver Spring, Md.). Thirty fields per coupon were scanned. All experiments were done in triplicate and repeated three times.

Roughness. The surface roughness of the materials studied was evaluated through the R_a and R_z values by a noncontact laser stylus tracing (Perthometer S4P, Perthen GmbH, Gottingen, Germany). The R_a value provides the arithmetical average value of all departures from the mean line throughout the sampling length. The R_z value is the sum of the height of the highest peak plus the lowest valley depth within a sampling length. The default evaluation length consists of five sample lengths.

Statistical analysis. The resulting data were analyzed by SPSS software (SPSS [Statistical Package for the Social Sciences], Inc., Chicago, Ill.). A one-way analysis of variance with the Bonferroni test was used to compare the number of adhered cells. All tests were performed with a confidence level of 95%.

RESULTS AND DISCUSSION

Figure 1 presents the number of cells of *Salmonella* Enteritidis (EMB, MUSC, PC, and AL) adhered to the materials tested. The extent of adhesion of the different strains to the materials assayed was statistically different ($P < 0.05$). *Salmonella* EMB adhered to a greater extent to granite, while *Salmonella* MUSC adhered to a greater extent to polypropylene and to a lesser extent to granite. The number of adhered cells of *Salmonella* AL was very similar for all the materials tested, while *Salmonella* PC adhered to a greater extent to granite, followed by polypropylene and, lastly, polyethylene. The source of *Salmonella* isolates does not seem to affect the ability of adhesion. Stepanovic et al. *(22)* also report that the source of *Salmonella* isolates (from humans, animals, or foods) did not affect biofilm formation.

Several studies report different extents of adhesion of *Salmonella,* and it was generally found that *Salmonella* adheres to a greater extent to the more hydrophobic materials *(10, 19).* Joseph et al. *(14)* studied the ability of biofilm formation of two poultry *Salmonella* isolates to plastic, cement, and stainless steel and observed that the biofilm formation of both isolates was very similar, with the highest density being on plastic, followed by cement and stainless steel. The ongoing epidemic of *Salmonella* Enteritidis may be related, in part, to the success of the bacterium in passing down the food chain, with adherence to inanimate surfaces contributing to persistence as well as communicability *(33).* *Salmonella* Enteritidis strains may adhere to surfaces such as eggs, food-processing equipment, animal carcasses, and farmyard implements over a wider range of environmental conditions *(33).* Of the many serotypes of *S. enterica,* Enteritidis is unique in possessing the ability to elaborate SEF14 and SEF17 fimbriae, both of which contribute to adherence although under different environmental conditions *(33).* Stepanovic et al. *(22)* demonstrated that both

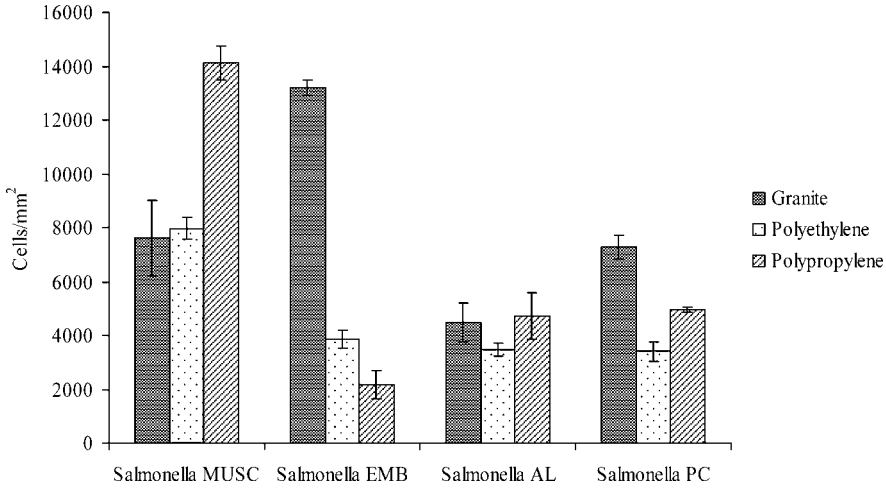

FIGURE 1. *Number of adhered cells per square millimeter of* Salmonella *Enteritidis strains to the different materials studied.*

Salmonella and *Listeria monocytogenes* are better able to form biofilms on plastic surfaces, with *Salmonella* generally producing more biofilms in a nutrient-poor medium. This fact aggravates the phenomenon of cross-contamination in food manipulation.

It is well known that bacterial surface hydrophobicity, surface charge, cell density, and the presence of exopolysaccharides are determinant factors in the adhesion process. For example, Sinde and Carballo *(19)* observed that differences found in the degree of attachment of *Salmonella* and *L. monocytogenes* indicate that there must be other factors on the surface of the bacteria, rather than hydrophobicity, contributing to bacterial attachment to food contact surfaces. On the other hand, Walker et al. *(32)* studied the effect of pH, temperature, and contact surface on the elaboration of fimbriae (SEF21, SEF14, and SEF17) and flagella and found differences among the four strains assayed. Hood and Zottola *(13)* observed that growth media and surface conditioning were both significant factors affecting the level of adherence. In the present study, surface hydrophobicity and roughness were determined to find an explanation for the observed differences in the extent of adhesion.

The contact angles on bacterial lawns as well as the surface tension components and hydrophobicity of the strains studied are presented in Table 1. The water contact angle value gives preliminary information about the degree of surface hydrophobicity. The sample is considered hydrophobic or hydrophilic if the angle is higher or lower than 65°, respectively *(31).* According to this criterion, all *Salmonella* strains assayed are hydrophilic, with values of water contact angles ranging from 9.7 to 14.0°, which are somewhat lower than those reported (25.4 to 35.0°) by Sinde and Carballo *(19)* for other *Salmonella* strains. The different serovars studied can explain this fact *(19).* Teixeira et al. *(24)* also observed a great variation of hydrophobicity among strains of the same bacterial species. The changeable complexity of the cellular surface results in hydrophobic or hydrophilic appendices and other macromolecular components that can confer different behaviors according to the method of evaluation. In practice, the nonuniformity of bacterial surface can result in an apparently hydrophilic bacterium in one assay and a hydrophobic bacterium in another *(9).* Affinity techniques, such as microbial adhesion to hydrocarbons *(18),* are more prone to variability, and by such techniques, hydrophobicity is only assessed qualitatively *(16).* By the approach of van Oss *(25),* it is possible to determine the absolute degree of hydrophobicity of any substance (*l*) vis-à-vis water (*w*), which can be precisely expressed in applicable International System of Units. Accordingly, all *Salmonella* strains studied were similarly hydrophilic (Table 1), which is in consonance with the classification obtained through the water contact angles.

The values of the contact angles (in degrees) as well as the values of the surface tension components and the degree of hydrophobicity (ΔG_{lwl}) of the materials assayed are presented in Table 2. Water contact angles of the materials were statistically different ($P < 0.05$) among them. According to the results of the degree of hydrophobicity, all of the materials are hydrophobic ($\Delta G_{lwl} < 0$). The polymers present very similar values for the free energy of self-

TABLE 1. *Contact angle, surface tension components, and degree of hydrophobicity of bacterial cells*[a]

Strain	Contact angle ± σ (°)			Surface tension components					
	θ_W	θ_F	$\theta_{\alpha\text{-}B}$	γ_s^{LW}	γ_s^+	γ_s^-	γ_s^{AB}	γ_s^{TOT}	ΔG_{1w1}
Salmonella MUSC	13.5 ± 1.6	15.9 ± 2.3	27.6 ± 1.7	39.5	1.1	54.4	14.5	54.0	32.2
Salmonella EMB	10.8 ± 2.2	15.6 ± 1.8	26.1 ± 4.2	39.9	1.0	56.0	15.0	54.9	34.1
Salmonella AL	9.7 ± 1.9	14.8 ± 2.6	27.2 ± 2.5	39.5	1.1	55.8	15.7	55.2	33.8
Salmonella PC	14.0 ± 4.4	17.0 ± 3.2	31.7 ± 2.8	38.1	1.2	54.5	16.2	54.3	32.3

[a] Values are expressed in millijoules per square meter. θ_W, contact angle of water; θ_F, contact angle of formamide; $\theta_{\alpha\text{-}B}$, contact angle of α-bromonaphthalene.

TABLE 2. *Contact angle, surface tension components, and degree of hydrophobicity of the materials assayed*[a]

Surface	Contact angle ± σ (°)			Surface tension components					
	θ_W	θ_F	$\theta_{\alpha\text{-}B}$	γ_s^{LW}	γ_s^{+}	γ_s^{-}	γ_s^{AB}	γ_s^{TOT}	ΔG_{1w1}
Granite	53.4 ± 3.6	41.7 ± 2.3	22.4 ± 3.6	41.1	0.3	26.3	5.6	46.7	−4.7
Polyethylene	74.3 ± 8.3	54.1 ± 7.1	19.3 ± 2.9	41.9	0.0	9.1	0.0	41.9	−46.4
Polypropylene	87.8 ± 3.4	70.8 ± 2.7	26.4 ± 3.1	39.9	0.5	5.6	3.3	43.3	−52.2

[a] Values are expressed in millijoules per square meter. θ_W, contact angle of water; θ_F, contact angle of formamide; $\theta_{\alpha\text{-}B}$, contact angle of α-bromonaphthalene.

interaction in water (−52.12 and −49.96 mJ/m^2, respectively), while granite presents a lower value (−4.7 mJ/m^2), displaying a less hydrophobic character. Considering the surface tension parameters, granite is a surface predominantly electron donor (higher values of γ^-), with a low electron acceptor parameter (γ^+). Its γ^- is much higher than the γ^- of the other surfaces being studied. This is likely due to the granite polar groups formed by O and N, which are electron donors, while polymer surfaces are formed only by carbon and hydrogen atoms without polar groups, as can be observed by the γ^{AB} parameter that corresponds to the polar component. However, in the present situation, it is not possible to hypothesize about a specific role of Lewis acid-base interactions in the adhesion process. At least, it is not possible to establish any correlation between the electron donor and electron acceptor capabilities of the interacting surfaces.

The values of surface roughness (R_a and R_z) are shown in Table 3. Polyethylene was the roughest material (with a higher value of R_a [longitudinal = 36.0 μm and transversal = 30.9 μm] and of R_z [longitudinal = 196.0 μm and transversal = 145.3 μm]), but it was the material displaying the lesser extent of bacterial colonization. Flint et al. *(10)* reported that the adhesion of thermoresistant streptococci to stainless steel with surface roughness (R_a) values ranging from 0.5 to 3.3 μm was largely independent of the substrate topography, although bacterial entrapment may occur at an R_a value of 0.9 μm. Barnes et al. *(2)* compared the adhesion of *Staphylococcus aureus* to polished stainless steel and to rougher stainless steel and observed that a greater number of *S. aureus* adhered to the rougher surface. According to the same authors, scanning electron micrographs showed that organisms did not orient themselves exclusively along polishing lines. In fact, it has been widely suggested that surface roughness plays an important role in the adhesion of microorganisms by protecting them from shear forces and increasing the available surface area. However, for a microbial cell to be entrapped because of surface roughness, it is necessary to have enough space available between two consecutive peaks of surface topography for the cell to sit there. It has to be noted that the same value of R_a can correspond to different surface topographies. Actually, R_a measures the average height and depth of peaks and valleys but not the distance between them. Because adhesion is dependent on the number of contact points between the interacting surfaces, it might be the distance between peaks that also determines the peak density (i.e., low VVVVVV or high WWWW), which is responsible for the extent of contact between the microbial cell and the surface. This means that a higher number of peaks close together will promote more contact points between the surface and the cell sitting on it. Along this same reasoning, the most common parameter used to express surface roughness is not the most appropriate to assess the effect of roughness on microbial adhesion.

Because the adhesion process is multifactorial (i.e., involving several physicochemical and microbiological factors), for a better understanding, it would be necessary to investigate the role of cell wall proteins as well as fimbriae and flagella. Furthermore, other structures such as pili, polysaccharides, capsules, and ''slime layers'' have been related to the adhesion process.

Considering all the tentative explanations based on the physicochemical properties of bacterial cells and surfaces, it is not possible to establish any direct correlation to elicit the hypothesis of a reasonable model of adhesion. The main conclusion to be drawn is that *Salmonella* adhesion is strongly strain-dependent, despite the similar degree of hydrophobicity displayed by all the strains assayed, and this can constitute a factor of virulence among the different serotypes.

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TABLE 3. *Roughness of the surfaces studied*[a]

Surface	R_a		R_z	
	Longitudinal	Transversal	Longitudinal	Transversal
Granite	32.4 ± 9.2	24.9 ± 2.0	155.2 ± 42.3	114.4 ± 12.1
Polyethylene	36.0 ± 18.9	30.9 ± 8.9	196.0 ± 88.0	145.3 ± 32.7
Polypropylene	6.2 ± 0.3	0.2 ± 0.04	39.6 ± 8.2	4.8 ± 2.9

[a] Values are expressed in micrometers.

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