

## Bacterial Adhesion to Food Contacting Surfaces

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Contamination of kitchen surfaces, during food preparation, due to bacteria present in foodstuff is one of the main causes of foodborne outbreaks. Cells adhered to those surfaces of domestic kitchens are not easily removed by normal cleaning procedures. Therefore, they can be a source of contamination for other foods and objects. Several studies indicate that various pathogenic bacteria like *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter*, *E. coli* and *Staphylococcus aureus* survive in kitchen utensils, hands and in bench cover materials. In this way it is of utmost importance to know the factors responsible for the adhesion of pathogenic bacteria to kitchen materials in order to select the material less prone to bacterial colonization. Accordingly, this chapter will focus on the study of the adhesion of strains of *Listeria monocytogenes* and *Salmonella Enteritidis* to materials commonly used in kitchens (stainless steel 304, marble, granite, glass, polypropylene from a bowl and a cutting board and two kinds of silestone).

**Keywords** adhesion; *Listeria monocytogenes*; *Salmonella* spp.; food contacting surfaces

### 1. Introduction

The main sources of potential harmful microorganisms in the home are contaminated food (foodborne), contaminated water (waterborne), infected persons, air, insects or pets. Under favourable circumstances (temperature, pH, relative humidity), pathogenic microorganisms are able to survive and/or replicate in large scale. In domestic kitchens, foodborne infection can result from incorrect storage of foods, particularly with respect to temperature, contamination of raw or cooked foods before consumption, by contact with other foods or utensils carrying pathogens, and inadequate or poorly controlled cooking which may allow persistence of pathogens in foods. This transfer of microorganisms from people, objects or contaminated food to another food or material is known as cross-contamination. People can be a source of cross-contamination to foods when, for example, handle foods, touch raw meats and prepare other foods without properly washing hands between tasks. Contamination can also be passed from kitchen utensils or equipment when contacting food. This happens when utensils or equipment are not efficiently cleaned and sanitized between each use. Contamination from food to food occurs mainly when raw foods come into contact with cooked or prepared foods. Safe working practices will reduce or even avoid this process.

### 2. Foodborne pathogenic bacteria

#### 2.1 *Salmonella* spp.

*Salmonella* spp. are pathogenic bacteria responsible for one of the most frequent foodborne diseases. 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. Eggs are considered the main source of human salmonellosis in Europe and in many other countries worldwide. There is large inter-country variation: in some countries, *no Salmonella* was

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detected, while other countries had prevalence of up to 80% [1]. *S. Typhimurium* was the predominant *Salmonella* serotype for many decades. However, *S. Enteritidis* has recently emerged as a major serotype in human infections and in chicken contamination [2]. Gillespie et al. [3] considered that most outbreaks are linked to cross-contamination and inadequate heat treatment, strongly associated to the use of raw shell eggs and occurring more likely in spring and summer. Some studies highlight the appearance of new and stronger multidrug-resistant strains [4,5]. The incidence of multidrug-resistant *Salmonella* Typhimurium infections in humans has increased substantially in the past two decades [6], implying that *S. Typhimurium* constitutes an increasing health problem in large parts of the world and which emphasizes the importance of surveillance and control programs. *Salmonella* is a genus of rod-shaped Gram-negative enterobacteria, oxidase negative and catalase positive. The clinically discernable syndromes of Salmonellosis occurring in man are acute onset of fever, abdominal pain, diarrhoea, nausea and sometimes vomiting.

### 2.2 *Listeria monocytogenes*

*Listeria monocytogenes* is a foodborne pathogen of significant concern to the food industry [15]. *L. monocytogenes* is a Gram-positive bacterium, motile via flagella and can be found in all types of food products [16], especially raw products (milk, meat, cheese, flour and others) [17]. *L. monocytogenes* causes listeriosis which manifestations include septicemia, meningitis (or meningoencephalitis), encephalitis, corneal ulcer, pneumonia, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or stillbirth. Listeriosis in humans is rare, with less than ten cases per one million persons [18,19]. Although listeriosis is uncommon it is of concern because of the high mortality rate of about 20% [20]. *L. monocytogenes* is able to grow at refrigeration temperatures (as low as - 1.5 °C) and in environments of reduced water-activity [17], in salt concentrations up to 30% and at pH values below 5.0 [21]. These characteristics contribute to its survival under conditions usually used to control the growth of pathogens in food.

### 2.3 *Campylobacter* spp.

*Campylobacter* spp. is recognized as the most common cause of foodborne bacterial gastroenteritis in humans [7]. *Campylobacter* is a genus of Gram-negative bacteria, motile, with either uni- or bi-polar flagella, the organisms have a somewhat curved, rod-like appearance, and are oxidase-positive. At least a dozen species of *Campylobacter* have been implicated in human disease, with *C. jejuni* and *C. coli* the most common. The former is usually responsible for the majority of enteric *Campylobacter* infections (80-90%). Most of the infections are sporadic and occur at home. Contaminated poultry meat is one of the most important vehicles for infection with *Campylobacter* spp. and studies have shown that carcasses can carry over  $10^8$  *Campylobacter* cells [8]. Cross-contamination is greatly responsible for the widespread of *Campylobacter* contamination.

### 2.4 *Bacillus cereus*

*Bacillus cereus* is a Gram-positive, facultative aerobic, spore-forming rod [9] that is commonly isolated from food. Two types of gastrointestinal diseases are caused by two distinct metabolites: a large molecular weight heat-labile protein (emetic type) and a low molecular weight, heat-stable peptide (diarrhoeal type) [11,12].

### 2.5 *Staphylococcus aureus*

*Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. *S. aureus* is a facultative anaerobic Gram-positive coccus, non-spore-forming, non-motile and catalase and coagulase positive. clusters. *S. aureus* is able to grow in a wide range of

temperatures [10], pH [13] and sodium chloride concentrations (up to 15% NaCl). Foods that are frequently responsible by staphylococcal food poisoning include meat products, poultry and egg products, salads, bakery products, sandwich fillings, and milk and dairy products. A toxin dose of less than 0.1 µg in contaminated food will produce symptoms of staphylococcal intoxication [14]. However, *S. aureus* contamination can be readily avoided by heat treatment of food.

### 2.6 *Escherichia coli*

There are hundreds of strains of *Escherichia coli* (Gram-negative bacterium). Despite most of the strains are harmless, *E. coli* O157:H7 produces a powerful toxin that can cause severe illness. The physiological effects of *E. coli* O157:H7 range from diarrhoea to serious and life-threatening conditions [22]. *E. coli* O157:H7 has been found in the intestines of healthy cattle, deer, goats, and sheep. *E. coli* O157:H7 is a leading cause of foodborne illness [23].

## 3. Presence of foodborne pathogens in kitchens

Kitchens are, without any doubt, the main source of foodborne infection in the domestic environments. Haysom and Sharp [24] investigated changes in levels of bacterial contamination in five key sites in ten domestic kitchens during a period of 24 hours. They observed that contamination levels varied during the day, peaking after meal preparation and generally falling overnight. There was also indirect evidence of cross-contamination, particularly from hands to other surfaces, since sites such as the refrigerator handle, kettle handle and taps, which generally only come into contact with hands, showed highest levels of contamination. The same authors [25] proved that some sites in kitchen as the chopping board, surfaces and handles of taps, kettle and refrigerator can become contaminated during the preparation of a meal from raw chicken. Bacteria can also be readily transferred to chopping boards during preparation of meat and then contaminate vegetables prepared on the same board [26]. De Boer and Hahne [27] showed that *Salmonella* and *Campylobacter* were easily transferred from chicken to a variety of kitchen surfaces, utensils, hands and other foods. During the preparation of a chicken casserole, *Campylobacter* and *Salmonella* were also isolated from several food and hand contact surfaces even after those surfaces have been washed and cleaned [28]. High incidence of cross-contamination in 25 domestic kitchens by potential pathogens (*Salmonella*, *Campylobacter*, *E. coli* and *S. aureus*) was also detected during the preparation of a chicken lunch [29].

It has been also demonstrated that, even after typical and specific hygienic procedures, pathogenic microorganisms can survive in kitchens, often, for hours [30,31].

Accordingly, it can be realized that the main sites of the kitchen responsible for cross-contamination are chopping boards, sinks, taps, dishcloths, knives and other working surfaces. On the other hand, it can also be inferred that, frequently, even after typical and other hygienic procedures, contamination still remain on those sites.

## 4. Adhesion of foodborne bacteria to materials used in kitchens

The ability of bacteria to adhere to food contact surfaces compromises the hygiene of those surfaces. Surface physicochemical properties of the bacterial cell as well as of the materials such as hydrophobicity and roughness, are determinant during the initial attachment phase [32-34]. Bacterial adhesion is also affected by the nutrient availability in the surrounding medium and the growth stage of the bacterial cells themselves [35] and by the pH and temperature of the medium, cell structures including EPS (Extracellular Polymeric Substances) and flagella and ionic concentration [36].

In this sense, our studies have been focused on the adhesion ability of foodborne pathogenic bacteria (mainly, *Listeria monocytogenes* and *Salmonella* spp.) to several food contact surfaces. The study of some relevant properties for the adhesion process has been another objective of our work and the idea

behind is to obtain a deeper knowledge on the mechanisms of bacterial adhesion in order to devise strategies for its control. In this sense, two major routes have been followed by several researchers: (i) modification of surface properties of materials to become less prone to bacterial colonization; (ii) development of disinfection products and protocols with higher efficacy against adhered bacteria.

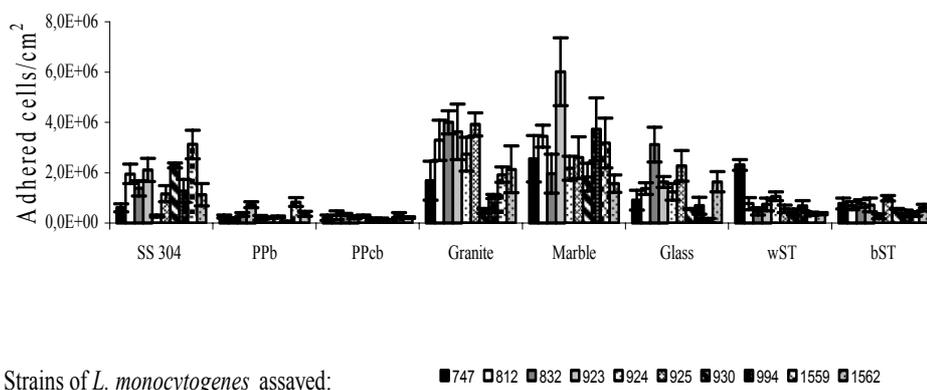
In order to modify the surface characteristics of materials to minimize bacterial adhesion it is important to investigate which properties are determinant in the process. Hydrophobicity and roughness can be quite easily altered and, on account of this, we decided to evaluate the adhesion ability of some foodborne pathogens to different types of materials commonly found in kitchens in terms of the above mentioned surface properties.

#### 4.1 Adhesion of *Listeria monocytogenes* to food contact surfaces

The ability of *L. monocytogenes* to attach to common food contact surfaces such as plastic materials, rubber, stainless steel and glass is well known [37-41]. However, it has been noted that there are differences in both the extent and the rate of adsorption, depending on the selected surfaces [42]. The factors governing the adhesion of *L. monocytogenes* to surfaces are not well understood and have not yet been defined. In fact, several authors have been having difficulty in establishing a relationship between surface properties and the extent of adhesion of *L. monocytogenes* [43,39,44-46].

In order to get more insights on the adhesion process of *L. monocytogenes* we have evaluated the adhesion ability of ten strains (one of clinical source, four of food source and the others of environmental source) to eight materials commonly used in kitchens (stainless steel 304 (SS304), marble, granite, glass, polypropylene from a bowl (PPb) and a cutting board (PPcb) and two kinds of silestone – white (wST) and beige (bST). The latter material is made of quartz and has incorporated a biocide (Microban – containing triclosan). According to the material manufactures', when microbes, such as bacteria, mould and mildew come in contact with the product surface, Microban protection penetrates the cell wall of the microbe and disrupts key cell functions so that the microbe cannot function, grow or reproduce [47].

The results showed that all strains of *L. monocytogenes* adhered to all the materials assayed even to the two silestones. However, the extent of adhesion was different, depending on the surface and strain (Figure 1). These results are in agreement with other literature reports [44-46].



**Fig. 1** Number of adhered cells per cm<sup>2</sup> for 10 different strains of *Listeria monocytogenes* to SS 304, PPb, PPcb, granite, marble, glass, wST and bST.

It should be pointed out that no correlation between the number of adhered cells and the materials surface hydrophobicity was obtained. In fact, adhesion occurred in great extent to the material with highest hydrophobicity (SS 304), being quite similar to a material moderately hydrophobic (marble) and other hydrophilic (granite). No correlation was also found between the number of cells adhered to the

different materials and their surface topography, since adhesion occurred in great extent to materials with higher values of roughness (SS 304 - average roughness of 30.9 nm and wST – 31.5 nm and bST – 24.6 nm) but is quite similar to the smoothest material (marble – average roughness of 8.5 nm). Moreover, the source of *L. monocytogenes* isolates does not seem to affect the ability of adhesion. Stepanovic *et al.* [38] also reported that the source of *Listeria* isolates (from humans, animals, or foods) did not affect biofilm formation. In conclusion, it was observed that *L. monocytogenes* adhesion to abiotic surfaces is not dependent on the substratum hydrophobicity and roughness being a multi-factorial process strongly dependent on the strain and the kind of surface.

The same conclusions were obtained in another study concerning the ability of adhesion of a culture collection strain (*Listeria monocytogenes* ATCC 15313) to the materials mentioned above [48]. Another interesting point has to do with the behaviour of the two silestones, in fact the lower extent of adhesion was detected on the two polymers being slightly high on the two silestones. 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, which allow to assess bacterial membrane integrity with the LIVE/DEAD Backlight kit (Molecular Probes, Eugene, OR, USA) [49], revealed that the lowest percentage of *L. monocytogenes* survival was found on white silestone (18.5 % cells with intact membrane) while, curiously, in silestone beige the percentage of viable cells was very high (74.0 % cells with intact membrane).

#### 4.2 Adhesion of *Salmonella* spp. to food contact surfaces

*Salmonella* spp. is able to colonize different inert food contact surfaces, however with different extents of adhesion [50-52,37]. Joseph *et al.* [52] 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.

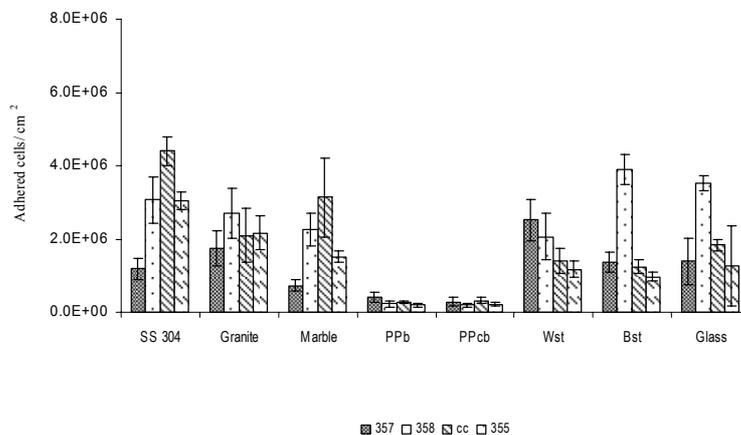
In a similar study to the above mentioned for *L. monocytogenes*, we studied the adhesion ability of four *Salmonella* Enteritidis isolates (two isolated from poultry- one from the water of packaged chicken, and other from chicken breast; the other two were human isolate outbreak strains) to three different materials used in kitchens (polyethylene, polypropylene, and granite) it was concluded that, similarly to *Listeria monocytogenes*, the different extents of adhesion could not be explained in terms of surface hydrophobicity or roughness of the materials tested [53]. In fact, the main conclusion that was 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. This strain variability was confirmed in another study where the adhesion ability of the same isolates was tested on stainless steel 304 [54]. Actually, the extent of adhesion to stainless steel ranged from  $2 \times 10^4$  cells/mm<sup>2</sup> for *S. Enteritidis* isolated from chicken breast to  $4.67 \times 10^3$  cells/mm<sup>2</sup> for *S. Enteritidis* isolated from the water of packaged chicken. Essentially, no change in hydrophobicity or in roughness can be responsible for such variability because the adhesion substratum was the same (SS 304) for all strains assayed.

Another study was performed focused on the effect of surface hydrophobicity and roughness on the adhesion ability of four isolates of *Salmonella Enteritidis* (three of clinical source – strain 357, strain 358 and strain CC and strain 355 is of food source) to the eight materials previously referred in the adhesion studies of *L. monocytogenes*. The corresponding results are presented in Figure 2.

Generally, *Salmonella* strains adhered in great 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 adheres in great extent, followed by strain CC, 355 and 357. Besides, 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 assayed.

As far as surface roughness is concerned, it was not possible to establish a direct relation between this property and *Salmonella* adhesion, similarly to what happened with *L. monocytogenes*. 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. Presumably, adhesion is dependent of the number of contact points and not on the value of the average roughness, which measures the average height and depth of peaks and valleys but not the distance between them. Thus, although glass presents a low value of average roughness it can have available a high number of contact points. In fact, if 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  $\text{vvvvv}$  or high  $\text{vvvvvvv}$ ), 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.



**Fig. 2** Number of adhered cells per  $\text{cm}^2$  for four different strains of *Salmonella* Enteritidis to SS 304, PPb, PPcb, granite, marble, glass, wST and bST.

Following the similarities with *L. monocytogenes* adhesion, it was also on white silestone that was detected the lowest number of viable cells (16%) of *Salmonella* Enteritidis, pointing out to some efficacy of this material, but to the need to still improve such materials. Moreover, adhesion of *S. Enteritidis* was also shown to be strongly strain dependent, since it is generally independent on the surface properties of the materials assayed.

Several studies showed a direct, almost linear correlation, between bacterial adhesion and solid surface hydrophobicity. In fact, this was verified in the selection of a suitable carrier for *Alcaligenes denitrificans* in an inverse fluidised bed reactor, among the polymeric materials: high density polyethylene (HDPE), polypropylene (PP), poly(vinyl chloride) (PVC) and polymethylmethacrylate (PMMA) [32]. A similar behaviour of linear correlation between the hydrophobicity of substrata and the number of adhered cells was found for *Staphylococcus epidermidis* ATCC 35984 (RP62A) and four polymeric materials, commonly used in indwelling medical devices: polyethylene, silicone, expanded polytetrafluoroethylene and cellulose diacetate [34]. When assessing the ability of four porous microcarriers, clay, foam glass, pozzolana and sepiolite, to be used as biomass carriers in an anaerobic fluidised bed reactor a linear correlation between support hydrophobicity and biomass retention capacity was also encountered [33]. Many other examples could be presented showing how hydrophobicity is generally a determinant factor in microbial adhesion. Nevertheless, the present results show that the adhesion ability of the most common foodborne pathogens is not dependent on substrata hydrophobicity. Surface roughness is another factor usually considered significant on microbial adhesion. For instance, two strains of *Yersinia ruckeri*, a fish pathogenic bacterium, were characterised according to the ability

to adhere on wood, concrete, polyvinylchloride (PVC) and fibreglass, four materials commonly found in fish farms [55]. The results showed a strong correlation was between roughness *Y. ruckeri* adhesion ability. A similar correlation was obtained by Barnes *et al.* [56] when comparing the adhesion of *Staphylococcus aureus* to polished stainless steel and to rougher stainless steel. Once again, when considering adhesion of foodborne pathogens it is not possible to devise any correlation with solid surface roughness.

## 6. Concluding remarks

The results put to evidence that the physico-chemical properties of surface materials are not a determinant factor in the process of foodborne pathogens ability of adhesion. In fact, foodborne pathogens show very high strain variability in terms of adhesion ability to solid surfaces. Presumably, intrinsic factors to the cell envelope, adhesins, cell wall proteins and extracellular polymers are responsible for such specific response. Accordingly, future studies should be focused on the effect of these cellular characteristics. Special attention should also be given to the development of more efficient disinfections strategies.

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