Original

Evaluation of the efficacy of commercial sanitizers against adhered and planktonic cells of *Listeria monocytogenes* and *Salmonella* spp.

Avaliação da eficiência de desinfetantes comerciais contra células aderidas e planctônicas de Listeria monocytogenes e Salmonella spp.

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Abstract

Antimicrobial activities of two commercial disinfectants, alone or combined with heat, against three *Salmonella* strains and three *Listeria monocytogenes* strains were studied. The efficacy of disinfectants against planktonic bacteria and bacteria attached to three food contact industrial surfaces (stainless steel, polytetraflourethylene, and rubber) was investigated. The tests were conducted using the sanitizer (quaternary ammonium compounds, and alquyldiethylenediamineglycine and di-alquyldiamineethylglycine) concentrations recommended by the manufacturers, and concentrations twice and four times higher than those values. The recommended concentrations were not effective to kill bacteria, especially when they were attached to surfaces. Concentrations of disinfectants twice and four times higher than those recommended were needed to fully eliminate planktonic bacteria. These same sanitizer concentrations were not sufficient to remove attached bacteria. To remove them from the surfaces, a treatment with recommended concentrations in combination with heat was needed. Our results indicate that these two pathogenic bacteria could survive common sanitation programs used in the food industry.

Keywords: *bacterial attachment; food contact surface; disinfection; sanitizer.*

Resumo

Neste estudo, foi avaliada a atividade antimicrobiana de dois desinfetantes comerciais, individualmente e combinados com calor, contra três estirpes de *Salmonella* e três de *Listeria monocytogenes*. A eficácia dos desinfetantes foi investigada contra bactérias em suspensão e contra bactérias aderidas a três superfícies de contacto comuns na indústria de alimentos (aço inoxidável, politetrafluoretileno e borracha). Os ensaios foram realizados com base nas concentrações de desinfetantes recomendadas pelos fabricantes e em concentração dupla e quádrupla. As concentrações recomendadas não foram efetivas contra as bactérias, especialmente quando estas estavam aderidas às superfícies. Foi necessário o dobro ou o quádruplo da concentração recomendada de desinfetante para eliminar as bactérias em suspensão. Essas mesmas concentrações não foram suficientes para eliminar as bactérias aderidas. De forma a erradicá-las da superfície, foi necessário um tratamento com a concentração recomendada em combinação com calor. Os resultados indicaram que essas estirpes patogênicas podem sobreviver a tratamentos de desinfecção habitualmente aplicados na indústria alimentícia.

Palavras-chave: *aderência bacteriana; superfície de contacto com alimentos; desinfecção; desinfectante.*

1 Introduction

Cross contamination of food products due to contact with food processing surfaces might be a major problem in the food industry (KUSUMANINGRUM et al., 2003a). The persistence of bacteria on surfaces has been suggested to relate to their attachment to food contact materials (GRAVESEN; LEKKAS; KNØCHEL, 2005; VALERIANO et al., 2012). Pathogenic bacteria may remain on equipment surfaces increasing the risks of transmission of diseases (SILVA et al., 2008; BELESSI et al., 2011).

Salmonella spp. and *Listeria monocytogenes* are examples of pathogenic bacteria implicated in outbreaks associated with the ingestion of contaminated food (LO FO WONG et al., 2002; GANDHI; CHIKINDAS, 2007). It has been proved that *Salmonella* Typhimurium can survive up to four weeks on dry surfaces in high-enough populations to be transferred to foods immediately on contact (DAWSON et al., 2007). It has also been found that *L. monocytogenes* can be transferred from foods to surfaces (RODRIGUEZ; AUTIO; McLANDSBOROUGH, 2007) and reciprocally (RODRIGUEZ; McLANDSBOROUGH, 2007). Indeed, it has been shown that these bacteria are able to attach to food contact surfaces (SINDE; CARBALLO, 2000; MAI et al., 2006; OLIVEIRA et al., 2006).

Research in this area has indicated that adherent microorganisms may be much more resistant to sanitizing compounds than planktonic cells (JOSEPH et al., 2001; KUSUMANINGRUM et al., 2003b; ALI et al., 2006).

The efficacy of the cleaning and disinfection operations of surfaces in the food industry depends on the design and type of surfaces, as well as on the procedures and products used. The outcome of these operations is limited by the ability of bacteria

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to attach to surfaces and, eventually, to form biofilms (JENSEN; LAMMERT, 2003). In fact, it has been proved that *Salmonella* and *L. monocytogenes* cells are able to attach to different solid surfaces (SINDE; CARBALLO, 2000; BERESFORD; ANDREW; SHARMA, 2001; RAMESH et al., 2002) and they are present in food industrial environments (LO FO WONG et al., 2002; KATHARIOU, 2002).

Sanitizer efficacy is generally determined with microorganisms cultured under ideal conditions (MOORMAN et al., 2005). However, there is a lack of information on sanitizer efficacy against attached microorganisms in conditions resembling those encountered in the food industry (HOLAH et al., 1998; PALMER; FLINT; BROOKS, 2007; CERF; CARPENTIER; SANDERS, 2010).

The objective of this research was to determine the efficacy of two commercial sanitizers, alone or in combination with heat, against strains of Gram-negative (*Salmonella* spp.) and Gram-positive (*Listeria monocytogenes*) pathogenic bacteria attached to food contact surfaces. Gram-negative and Gram-positive bacteria were chosen as models for this study because both types of bacteria can be found in surfaces of the food industry.

2 Materials and methods

2.1 Bacterial strains, growth conditions, and suspensions obtainment

Three strains of *Salmonella* (ES3, ES9 and ES20) and three strains of *L. monocytogenes* (ES15, ES24 and ES25) were used in this study. Bacterial strains were isolated in the course of routine food testing in the Food Control Services (Consellería de Sanidade) of Galicia (Spain). *Salmonella* strains were isolated from chicken liver, fresh sausages, and hamburgers, respectively. *L. monocytogenes* strains were isolated from frozen hake, cheese, and meat, respectively (SINDE; CARBALLO, 2000).

The strains were stored in skim milk (Fluka, Madrid, Spain) at –20 °C. During the experiments, the strains were maintained on Trypticase Soy Agar (TSA, Cultimed, Madrid, Spain) at 4 °C.

Overnight cultures in Trypticase Soy Broth (TSB, Cultimed) (37 °C, 80 rpm) were pelleted by centrifugation (9500 rpm, 4 °C, 10 min). Bacteria washed three times with phosphate buffered saline (PBS, 0.33 M NaCl, 3 mM KCl, 8.4 mM Na_2HPO_4, 1.6 mM KH_2PO_4, pH 7.2), were suspended in PBS. Before the adherence experiments, bacterial cell density was spectrophotometrically adjusted with PBS to 10^8 CFU.mL^{-1} (SINDE; CARBALLO, 2000).

2.2 Materials and their preparation for attachment experiments

Stainless steel type 304 (SS), commonly present in the food processing equipment, was donated by Gamelsa (Santiago de Compostela, Spain) in discs of 100.4 mm^2. Polytetrafluorethylene (PTFE) (Polypenco Engineering Plastics, Barcelona, Spain), utilized for cutting boards, provided in sheets of 1 mm in thickness, was cut in cylinders of 6 mm in diameter, with the result of an area of 75.39 mm^2. Rubber type 7S 15 (a blend of styrene-butadiene copolymer and natural rubber), used for milk tubing, supplied in 2 mm thickness sheets, was cut to obtain pieces of 70.68 mm^2.

Pieces of materials were sequentially washed with 1% (w/v) sodium dodecyl sulphate (SDS, Sigma, Madrid, Spain), distilled water, ethanol (Panreac, Barcelona, Spain), and finally three times with distilled water. Materials were agitated for 10 min in each liquid. Afterwards, they were air dried (SINDE; CARBALLO, 2000).

2.3 Bacterial attachment to materials

Bacterial attachment was determined as described previously by Sinde and Carballo (2000). Three mL of bacterial suspensions, prepared as specified above, were incubated with pieces of materials for 1 hour at room temperature with 90 rpm constant shaking.

2.4 Attached bacterial release and count

After incubation, the materials were rinsed twice with 3 mL of PBS, immersed in 2 mL of TSB, and immediately treated ultrasonically (Branson 250, 30 W, 20 s) in a cooled bath to release the attached bacteria. Ten-fold serial dilutions of TSB in PBS were made and 0.1 mL portions of each dilution were plated on Salmonella-Shigella Agar (Cultimed) or Listeria Oxford Agar (Biolife, Milano, Italy) plates. After 24-48 hours of incubation at 37 °C, the number of Colony Forming Units (CFU) was counted, and the results were expressed as the $\log_{10}$ of the number of released CFU per mm^2. Each adherence experiment was performed three times, and adequate controls (bacterial suspensions without materials) were processed at the same time. This type of experiment was considered our standard of bacterial attachment, so that attached bacteria were treated as indicated in the following sections, and the results obtained in each case were compared with this standard.

2.5 Sanitizers

Two commercial sanitizers were obtained from commercial suppliers. According to the declared formulation, the antimicrobial activity of each of the sanitizers was based on the presence of quaternary ammonium compounds (QAC) and alquyldiethylenediamineglycine and di-alquyldiamineethylglycine (DETA).

They were used following the instructions of the respective manufacturers to obtain solutions with the recommended and concentrations twice and four times higher than those values.

2.6 Treatment of bacteria with heat

Pieces of materials with attached bacteria, obtained as indicated in the section 2.3, were submerged in 3 mL of PBS and treated at 85 °C for 15 minutes in a thermostatic bath. Then, PBS was removed, and the number of surviving bacteria (CFU.mm^{-2}) was determined as described in section 2.4.

Controls containing 3 mL of bacterial suspension (10^8 CFU.mL^{-1}) of each strain were processed at the same time in order to check the effect of heat on planktonic bacteria. The

eventual survival of bacteria was determined by plating 0.1 mL of the treated suspension onto the specific media indicated in section 2.4.

2.7 Treatment of bacteria with sanitizers

Both planktonic and attached bacteria were treated with each sanitizer at the recommended concentrations and with concentrations twice and four times higher than those values.

Planktonic bacteria were challenged with sanitizers by mixing 1.5 mL of bacterial suspension (containing 10^8 CFU.mL^{-1}) of each strain with 1.5 mL of a solution of each sanitizer with the adequate concentration to obtain the final ones (recommended, twice, or four times higher) in the resulting 3 mL of mixture. After 15 minutes, ten-fold dilutions were made, and 0.1 mL portions were plated onto the media already mentioned to obtain the number of CFU.mL^{-1} surviving the treatment.

Pieces of materials with attached bacteria, obtained as explained previously, were treated with 3 mL of the adequate concentration of each sanitizer for 15 minutes. Then, the materials were washed with PBS (3 mL, twice), and the number or surviving bacteria was detected as already explained.

2.8 Treatment of bacteria with heat and sanitizers

Planktonic and attached bacteria were treated with sanitizers at the recommended concentrations and heat. The procedure used was the same as that indicated in the previous section but heating at 85 °C at the same time.

2.9 Statistical analysis

Statistical analysis was performed with the SPSS package (SPSS 16.0 for Windows, USA). Comparisons were carried out at 95% confidence by using ANOVA and *a posteriori* multiple comparison tests.

3 Results and discussion

3.1 Effect of heat

None of planktonic *Salmonella* or *L. monocytogenes* strains survived the heat treatment, nor did attached *Salmonella* strains. Most of *L. monocytogenes* strains attached to the different materials survived the heat treatment (Table 2), although in all cases of survival there was a significative reduction ($P < 0.05$) of attached bacteria in comparison with the total attached to each material under the standard conditions (Table 1). Our results may confirm previous ones with respect to the survival of attached *L. monocytogenes* to heat. Frank and Koffi (1990) and Lee and Frank (1991) demonstrated the increased heat resistance of *L. monocytogenes* attached to glass and stainless steel. It has also been recognized that *L. monocytogenes* is more resistant to heat when tested in foods than when it is suspended in laboratory media (DOYLE et al., 2001). *Salmonella* resulted more resistant to heat when attached to muscle tissue (HUMPHREY; WILDE; ROWBURY, 1997). Chmielewski and Frank (2004, 2006) developed predictive models for probability of heat inactivation of *L. monocytogenes* biofilms. They proved that high temperatures and times are needed to achieve probabilities of 75% of total inactivation of bacteria on stainless steel (80 °C, 11.7 minutes) (CHMIELEWSKI; FRANK, 2004) and 95% on buna-N rubber (76 °C, 6 minutes) (CHMIELEWSKI; FRANK, 2006).

3.2 Effect of sanitizers

Table 3 and Table 4 show the effect of different concentrations of sanitizers QAC and DETA, respectively, on planktonic bacteria. As can be observed, *L. monocytogenes* strains were destroyed with the recommended or double concentration of QAC, while to eradicate planktonic *Salmonella* quadruple concentration of QAC was needed (Table 3). The concentration recommended by the manufacturer of DETA was ineffective against both types of bacteria in suspension and, only concentrations four times higher than the recommended ones could guarantee the elimination of these bacteria (Table 4). It is generally recognized that to pass the AOAC International Germicidal and Detergent Sanitizer test, a sanitizer should reduce planktonic microbial populations by five or more log cycles after 30 seconds exposure (LINDSAY; VON HOLY, 1999). Our results indicate that, at the recommended concentration, QAC was more effective against planktonic *L. monocytogenes* cells (which were reduced by at least 5 log cycles) than against planktonic *Salmonella* cells (which were decreased by 1-2 log cycles). This should not be an unexpected finding since it is generally recognized that at low temperatures quaternary ammonium compounds are less effective against Gram-negative bacteria (ADAMS; MOSS, 2008). DETA was much less effective

Table 1. Numbers (log CFU.mm^{-2}) of attached bacteria released from the materials under the standard conditions (media ± standard error).

Strains	Materials		
	SS	PTFE	RUBBER
Salmonella ES3	3.9 ± 3.2	4.9 ± 4.0	4.6 ± 3.5
Salmonella ES9	4.4 ± 3.6	4.1 ± 3.1	4.2 ± 3.0
Salmonella ES20	4.7 ± 3.1	4.7 ± 3.7	4.8 ± 3.3
L. monocytogenes ES15	5.5 ± 4.3	5.8 ± 4.7	5.8 ± 4.6
L. monocytogenes ES24	5.1 ± 4.1	5.6 ± 4.6	5.3 ± 4.0
L. monocytogenes ES25	5.2 ± 3.8	5.5 ± 4.5	5.3 ± 4.7

SS: Stainless steel. PTFE: Polytetrafluorethylene.

Table 2. Survival (log CFU.mm^{-2}) of attached bacteria to heat treatment (85 °C, 15 minutes).

Strains	Materials		
	SS	PTFE	RUBBER
Salmonella ES3	–	–	–
Salmonella ES9	–	–	–
Salmonella ES20	–	–	–
L. monocytogenes ES15	–	1.4	–
L. monocytogenes ES24	2.1	1.1	1.4
L. monocytogenes ES25	2.1	2.4	2.3

–: No survival. SS: Stainless steel. PTFE: Polytetrafluorethylene.

against both types of suspended cells since a reduction range from 2.8 to the total elimination was achieved, but only when double concentration than recommended by the manufacturer was used.

The reduction in the number of surviving attached bacteria caused by the different concentrations of sanitizers QAC and DETA are presented in Tables 5 and 6. These results represent the difference between those shown in Table 1 (which indicates the amount of bacteria attached to each material under the standard conditions, i.e. without heat or sanitizer treatment) and the corresponding surviving bacteria after treatment with each sanitizer.

The treatment of attached bacteria with increasing concentrations of sanitizers resulted in statistically significant ($P < 0.05$) reduction in the numbers of bacteria detected although sanitizers were less effective on attached bacteria than on suspended ones. Since reductions in viable counts of attached bacteria of barely 1 or 2 log cycles were obtained when the recommended concentrations of disinfectants were used, it could be concluded that attached cells of both bacteria were more resistant to disinfection with both sanitizers than planktonic ones. Other authors showed that increases in sanitizer (ozone, chlorine and hydrogen peroxyde) concentrations were required to destroy biofilm cells of *L. monocytogenes* in comparison with the corresponding planktonic cells (ROBBINS et al., 2005).

Disinfectant containing QAC used at double concentration reduced attached bacteria by 2-5 log cycles and most of the strains of attached bacteria did not survive the treatment with the quadruple concentration than the recommended, irrespective of the surface to which bacteria were adhered. It is generally assumed that quaternary ammonium compounds adhere to food processing surfaces even after rinsing, thus maintaining their antibacterial effect for hours after the disinfection operation (ADAMS; MOSS, 2008). In a previous study (SINDE; CARBALLO, 2000), we have demonstrated that the treatment of food contact surfaces with QAC lowers their hydrophobicity and the subsequent attachment of *Salmonella* and *L. monocytogenes* strains. Therefore, the maintained antibacterial effect of quaternary ammonium compounds could be due to the changes they produce in the physicochemical properties of the surfaces on which they are applied. Most strains of attached *Salmonella* or *L. monocytogenes* survived the treatment with a concentration four times higher than that recommended for the sanitizer DETA, regardless the attachment surface.

Our results contrast with those of other authors (KRYSINSKI; BROWN; MARCHISELLO, 1992; RONNER; WONG, 1993; ABRISHAMI et al., 1994; BOURION; CERF, 1996; LINDSAY; VON HOLY, 1999; BREMER; MONK; BUTTLER, 2002), who reported that resistance of bacteria to sanitizers was influenced by the type of surface. These discrepancies could be explained, at least in part, by the fact that they challenged different sanitizers, bacterial species, or attachment surfaces. Moreover, in all of aforementioned investigations, mature bacterial biofilms were used instead of fresh attached bacteria, which was the experimental procedure used in our study. It should be emphasized that if cleaning and disinfection measures are adequately applied in the food industry, the formation of mature

Table 3. Reduction (log CFU.mL^{-1}) of planktonic bacteria (5×10^7 CFU.mL^{-1}) caused by the treatment with different concentrations of sanitizer QAC.

Strains	Concentration of sanitizer QAC		
	R	2R	4R
Salmonella ES3	1.4	2.9	–
Salmonella ES9	1.8	3.9	–
Salmonella ES20	1.8	3.8	–
L. monocytogenes ES15	–	–	–
L. monocytogenes ES24	5	–	–
L. monocytogenes ES25	–	–	–

R: Concentration recommended by the manufacturer. 2R: Double Concentration than recommended by the manufacturer. 4R: Quadruple Concentration than recommended by the manufacturer. –: Total elimination.

Table 4. Reduction (log CFU.mL^{-1}) of planktonic bacteria (5×10^7 CFU.mL^{-1}) caused by the treatment with different concentrations of sanitizer DETA.

Strains	Concentration of sanitizer DETA		
	R	2R	4R
Salmonella ES3	0.3	3.8	–
Salmonella ES9	0.7	–	–
Salmonella ES20	0.4	4.8	–
L. monocytogenes ES15	0.7	3.7	–
L. monocytogenes ES24	0.4	2.9	–
L. monocytogenes ES25	0.4	2.8	–

R: Concentration recommended by the manufacturer. 2R: Double Concentration double than recommended by the manufacturer. 4R: Quadruple Concentration than recommended by the manufacturer. –: Total elimination.

Table 5. Reduction (log CFU.mm^{-2}) of attached bacteria caused by the treatment with different concentrations of sanitizer QAC.

Strains	Materials								
	SS			PTFE			RUBBER		
	R	2R	4R	R	2R	4R	R	2R	4R
Salmonella ES3	0.6	2.4	3.0	2.3	3.3	4.2	2.1	2.7	–
Salmonella ES9	1.4	3.8	–	1.6	–	–	0.8	–	–
Salmonella ES20	1.3	3.9	–	1.1	3.0	–	1.6	4.5	–
L. monocytogenes ES15	2.5	4.3	–	2.8	4.6	–	1.8	3.5	–
L. monocytogenes ES24	1.3	4.8	–	1.7	–	–	1.8	4.7	–
L. monocytogenes ES25	2.0	3.7	–	1.8	3.5	4.9	1.4	2.9	4.5

R: Concentration recommended by the manufacturer. 2R: Double Concentration than recommended by the manufacturer. 4R: Quadruple Concentration than recommended by the manufacturer. –: Total elimination. SS: Stainless steel. PTFE: Polytetrafluorethylene.

Table 6. Reduction (log CFU.mm^{-2}) of attached bacteria caused by the treatment with different concentrations of sanitizer DETA.

Strains	Materials								
	SS			PTFE			RUBBER		
	R	2R	4R	R	2R	4R	R	2R	4R
Salmonella ES3	0.8	1.9	2.6	1.6	2.8	3.3	1.7	2.6	3.2
Salmonella ES9	1.7	3.4	–	1.0	3.3	–	1.2	3.2	–
Salmonella ES20	1.2	2.6	2.9	1.2	2.4	2.8	1.4	2.9	3.7
L. monocytogenes ES15	1.3	1.8	4.1	1.7	2.2	3.6	1.5	2.1	3.9
L. monocytogenes ES24	0.9	2.9	3.4	1.2	3.0	3.9	1.0	2.5	3.3
L. monocytogenes ES25	1.4	2.0	2.9	1.4	2.1	3.7	1.2	2.0	3.8

R: Concentration recommended by the manufacturer. 2R: Double Concentration than recommended by the manufacturer. 4R: Quadruple Concentration than recommended by the manufacturer. –: Total elimination. SS: Stainless steel. PTFE: Polytetrafluorethylene.

biofilms should not occur, but the attachment of bacteria would take place anyhow. That is the reason why our experimental design would be more relevant for the food industry.

The overall results indicate that cells of these pathogenic bacteria attached to food processing surfaces would survive the treatment with the recommended concentrations of these commercial sanitizers, thereby these products would be unlikely to avoid the cross-contamination. Goeres et al. (2004) reported the survival of bacteria in other environments, such as a swimming pool laboratory model operating at recommended ranges. On the other hand, our results would corroborate the current idea that standard tests for the evaluation of sanitizers' efficacy should include both planktonic bacteria and their sessile counterparts (SPRINGTHORPE; SATTAR, 2005). Those tests should also be carried out in the presence of food debris and with consortia of bacteria (DEVERE; PURCHASE, 2007).

3.3 Effect of sanitizers and heat

Planktonic or attached bacteria could not survive heating at 85 °C combined with the treatment with the recommended concentrations of sanitizer QAC or DETA. Hence, combining heat treatment with chemical treatment would be useful to improve the disinfection of food industry surfaces without increasing the concentrations of sanitizers. Consistent with the data reported here, Moorman et al. (2005) found that heat stressed *Listeria innocua* were more sensitive to a quaternary ammonium sanitizer. Laboratory experiments have shown that bacterial exposure to sublethal concentrations of disinfectants may result in acquisition of resistance (LANGSRUD et al., 2003). Given that *Salmonella* (MARIN; HERNANDIZ; LAINEZ, 2009) as well as *L. monocytogenes* (GANDHI; CHIKINDAS, 2007) seemed to be able to develop resistance against disinfectants, our approach would also be useful to reduce the opportunities of resistant bacterial strains selection in the food industry. Oh and Marshall (1995) demonstrated the usefulness of combining chemical and physical (monolaurin and heat) treatments to control *L. monocytogenes* biofilm problems in the food industry. Biofilm extracellular polymeric substances produced by *Pseudomonas putida* were removed with a hot alkali cleaner (ANTONIOU; FRANK, 2005). The use of ultrasonication together with chlorine or quaternary ammonium-based sanitizers improved the performance of sanitizers on *L. monocytogenes* biofilms (BERRANG; FRANK; MEINERSMANN, 2008). These and other researchers (JENSEN; LAMMERT, 2003; AMMOR et al., 2004; RYU; BEUCHAT, 2005) studied biofilms of bacteria. However, the formation of a biofilm starts with the attachment of bacteria to the surface. Sharma, Ryu and Beuchat (2005) found differences in sensibility to disinfectants of *Escherichia coli* O157:H7 attached to stainless steel or embedded in a biofilm. Sagripanti and Bonifacino (2000) showed that the increased resistance of attached *Pseudomonas aeruginosa* to treatment with disinfectants precedes biofilm formation. The present study focuses on early attachment rather than on established biofilm. Actually, we have shown that the combination of sanitizers and heat is effective to remove attached bacteria from the surfaces prior to the formation of a biofilm, thus proving the usefulness of this approach for the prevention of the formation of biofilms. In addition, minimizing the attachment of bacteria and formation of biofilm could be advantageous in reducing the early stages of biofouling (BARRIOS et al., 2005). The combination of heat and chemicals for the decontamination of surfaces could provide an additional security in the food industry.

4 Conclusions

The results obtained contribute to the awareness of attachment as mechanism of survival of bacteria in the food industry.

They also indicate that the combination of physical and chemical disinfection would be useful to avoid set up of resistant strains and the development of biofilms in the food industry.

The findings of this study should also warn sanitizer manufacturers about the decreased efficacy of sanitizers against attached bacteria. We suggest that instructions for use should be elaborated after testing sanitizers' effectiveness against attached bacteria.

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