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Inactivation of Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa and Staphylococcus aureus on stainless steel and glass surfaces by neutral electrolysed water

M.A. Deza, M. Araujo and M.J. Garrido
Institute of Food Research and Analysis, University of Santiago de Compostela, Santiago de Compostela, Spain

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ABSTRACT

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Aim: To ascertain the efficacy of neutral electrolysed water (NEW) in reducing *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Listeria monocytogenes* on glass and stainless steel surfaces. Its effectiveness for that purpose is compared with that of a sodium hypochlorite (NaClO) solution with similar pH, oxidation–reduction potential (ORP) and active chlorine content.

Methods and Results: First, the bactericidal activity of NEW was evaluated over pure cultures (8.5 log CFU ml^{-1}) of the abovementioned strains: all of them were reduced by more than 7 log CFU ml^{-1} within 5 min of exposure either to NEW (63 mg l^{-1} active chlorine) or to NaClO solution (62 mg l^{-1} active chlorine). Then, stainless steel and glass surfaces were inoculated with the same strains and rinsed for 1 min in either NEW, NaClO solution or deionized water (control). In the first two cases, the populations of all the strains decreased by more than 6 log CFU 50 cm^{-2}. No significant difference ($P \leq 0.05$) was found between the final populations of each strain with regard to the treatment solutions (NEW or NaClO solution) or to the type of surface.

Conclusions: NEW was revealed to be as effective as NaClO at significantly reducing the presence of pathogenic and spoilage bacteria (in this study, *E. coli, L. monocytogenes, P. aeruginosa and S. aureus*) on stainless steel and glass surfaces.

Significance and Impact of the Study: NEW has the advantage of being safer than NaClO and easier to handle. Hence, it represents an advantageous alternative for the disinfection of surfaces in the food industry.

Keywords: disinfectant, *Escherichia coli*, glass, *Listeria monocytogenes*, neutral electrolysed water, *Pseudomonas aeruginosa, Staphylococcus aureus*, stainless steel, surfaces.

INTRODUCTION

Cross contamination via inanimate surfaces is an important factor in food-borne infections. Pathogens can easily spread from contaminated food or surfaces to cooking utensils, surfaces and other food products (Bradford et al. 1997;

Correspondence to: M.A. Deza, Instituto de Investigacio´n y Ana´lisis Alimentarios,
Universidad de Santiago de Compostela, Campus Sur, E-15782 Santiago de Compostela, Spain (e-mail: madeza@usc.es).

Kusumaningrum et al. 2003). Many pathogenic and spoilage bacteria can attach to surfaces commonly found in food production systems, and become less susceptible to sanitizers and other antimicrobial agents (Frank and Koffi 1990; Lee and Frank 1991). Staphylococcus aureus, Listeria monocytogenes and Escherichia coli can be found within biofilms in food processing plants (Blackman and Frank 1996; Poulsen 1999; Sharma and Anand 2002). Pseudomonas spp. Are spoilage bacteria commonly found in food industries.

Pseudomonas aeruginosa – an opportunistic pathogen – has a high tendency to form biofilms, and a known resistance to disinfectant action (reviewed by Poulsen 1999).

The occurrence of food-borne disease outbreaks can be significantly reduced through frequent cleaning and disinfection of surfaces in contact with food, an effective means to prevent biofilm formation and cross contamination.

Although the spectrum of disinfectants used in food industries is wide – it includes quaternary ammonium compounds, amphoteric products, biguanides, iodophores, peroxo acids, etc. (Taylor et al. 1999; Rossoni and Gaylarde 2000) – chlorine-containing compounds are frequently resorted to as they are effective against bacteria, and require short to moderate contact time (Kim et al. 2000a,b). Sodium hypochlorite (NaClO) being one of the most widely used disinfectants in household sites and food industries, has the disadvantage of being highly unstable as the active chlorine concentration in the solution rapidly decreases during storage (Qin et al. 2002). Besides, the handling of concentrated NaClO is a potential hazard for workers.

In recent years, acidic electrolysed water (AEW) and neutral electrolysed water (NEW) have been introduced for application as sanitizers. These solutions are generated by electrolysis of a dilute NaCl solution passing through the anode of a membrane electrolyser. AEW has a low pH (2–4) and a high oxidation–reduction potential (ORP >1000 mV); it contains active oxidizers like hypochlorous acid (Len et al. 2000) and has a strong bactericidal effect on most known pathogenic bacteria (Venkitanarayanan et al. 1999; Kim et al. 2000a,b). However, it has some disadvantages: it is potentially corrosive for processing equipment and irritating for hands, and has a short storage life because of chlorine loss (Len et al. 2002; Nagamatsu et al. 2002).

NEW is produced in a similar way as AEW, but then it is partially mixed with OH^- which is transferred through the membrane into the cathode chamber. This produces a neutral solution (pH 8 ± 0.5; ORP >700 mV) in which the main biocidal reagents are **HOCl**, $\mathbf{ClO^-}$, $\mathbf{HO_2}$ and $\mathbf{^*O_2}$. Because of its neutral pH, NEW does not contribute as aggressively to the corrosion of metallic surfaces as AEW does; moreover, NEW is more stable than AEW because chlorine loss is significantly reduced. Taking these facts into account, the use of NEW for the disinfection of contact surfaces in the food industry represents an advantage over that of AEW.

NEW has been proved effective in reducing microbial counts on fresh-cut vegetables (Izumi 1999) and in removing biofilms in dental unit water lines (Marais and Bro¨zel 1998).

Previous studies in our laboratory have demonstrated that rinsing in NEW (89 mg l^{-1}) is an effective method to control the presence of pathogenic bacteria on the surface of fresh tomatoes, without affecting their organoleptic characteristics (Deza et al. 2003).

The objective of this study was to evaluate the efficacy of NEW in reducing E. coli, P. aeruginosa, S. aureus and L. monocytogenes populations on glass and stainless steel surfaces. Its activity was also compared with that of a NaClO solution of the same active chlorine content.

MATERIALS AND METHODS

Bacterial cultures

The strains used for this study were obtained from the Spanish Type Culture Collection (CECT): E. coli CECT 405 (ATCC strain 10536, proposed for testing antibiotics, antimicrobial preservatives and chemotherapeutic agents), P. aeruginosa CECT 116 (ATCC 15442, proposed for testing slimicides and disinfectants), S. aureus CECT 239 (ATCC 6538, proposed for testing surface sanitizers and antimicrobial agents) and L. monocytogenes CECT 4032 (isolated from soft cheese, associated with a case of meningitis). Strains were cultured on TSA plates [tryptone soya broth (Panreac

Quimica SA, Barcelona, Spain) with the addition of 15 gl^{-1} agar Nº 3 (Oxoid, Basingstoke, Hampshire, UK)] at 37ºC for 24 h.
For the inoculation of surfaces, bacteria were harvested from the TSA plates with a sterile glass bent rod and resuspended in 50 ml of tryptone–sodium chloride solution (pH 7.2 ± 0.2), to obtain a suspension of 9–10 log CFU ml^{-1}.
The bacterial population of each inoculum was confirmed by pouring 1 ml of appropriate dilutions of the suspension (using the same solution) on duplicate TSA plates, further incubated at 37ºC for 24 h.

Preparation of treatment solutions

NEW was generated using a Envirolyte el-900 unit (Envirolyte Industries International LTD, Tallinn, Estonia). A 25% NaCl solution and tap water were simultaneously pumped into the generator to obtain amperage of 32 ± 2 A. For this study, NEW (containing approximately 400 mg l^{-1} of active chlorine) was diluted in deionized sterile water, to obtain a final active chlorine concentration of about 60 mg l^{-1}. The NaClO solution was prepared by mixing concentrated NaClO (Panreac Quimica SA) and deionized water to obtain a final active chlorine concentration of about 60 mg l^{-1}. Deionized sterile water was used as control. The properties of the treatment solutions were measured immediately after preparation:
pH and ORP were measured with a pH/ion/conductivity meter (CRISON micro-pH 2001; Crison Instruments, Barcelona, Spain), using respectively a pH electrode (CRISON, 52–11) and an ORP electrode (CRISON platinum Ag/AgCl electrode, 52–61). Active chlorine concentration was measured by an iodometric method (APHA 1998).

Treatment of pure culture

Nine millilitre of either NEW or NaClO solution – containing each approximately 60 mg l^{-1} active chlorine – were added to sterile tubes containing 1 ml of bacterial culture of about 8.5 log CFU ml^{-1}. The tubes were handshaken to mix the resultant suspension, and incubated at room temperature (23 ± 2_C) for 5 min. Deionized water was used as control. Following treatment, 1 ml of each sample was transferred to 9 ml of neutralizing solution (sodium thiosufate 0.5%) and the suspension hand-shaken.
After 5 min of neutralization, 1 ml of the appropriate dilution in tryptone-sodium chloride solution was poured on duplicate TSA plates. The plates were incubated at 37 ± 1ºC for 24 h. The experiment was repeated four times.

Preparation and inoculation of surfaces

The test surfaces used in this study were 50 cm^{-2} portions of glass and stainless steel, respectively. Before inoculation, the surfaces were washed with detergent (Procter and Gamble, Newcastle-upon-Tyne, UK), rinsed in deionized water and autoclaved at 121ºC for 20 min. Then the surfaces were immersed for 20 min in the bacterial suspension, and dried for 15 min at room temperature (23 ± 2ºC) in individual sterile metallic strainers, under sterile air, in a laminar flow cabinet. The initial population on the surface was obtained by swabbing one face (50 cm^{-2}) of an inoculated air-dried surface with a sterile cotton swab.
The swab was washed in 5 ml of sterile tryptone-sodium chloride solution, and 1 ml of appropriate dilutions of this solution was plated onto duplicate TSA plates as described above.

Washing treatment

Inoculated surfaces were placed in individual sterile bags containing 250 ml of electrolysed neutral water, NaClO solution or sterile deionized water (control). The bags were shaken gently by hand for 60 s. After immersion, surfaces were removed with sterile tongs and water was allowed to drain completely. One face of the surface was swabbed with a sterile cotton swab. The swab was washed in 5 ml of neutralizing solution and appropriate dilutions of this solution were plated onto TSA plates.
Immediately after removing the surface from the solution, 1 ml of sodium thiosulfate 20% was added to the washing solutions and bags were hand-shaken for 30 s to allow mixing. After 5 min of neutralization, the number of viable cells in the washing solutions after treatment was determined through serial dilutions in 9 ml of tryptone-sodium chloride solution followed by plating 1 ml on duplicate TSA plates. For enrichment, 5 ml of each treatment solution was transferred to separate bottles containing 50 ml of sterile TSB and incubated at 37ºC for 24 h. All the experiments were conducted at room temperature (23 ± 2ºC).

Data analysis

All trials were repeated at least four times.
Microbial counts were expressed as log CFU ml^{-1} (washing solutions and inocula) or log CFU 50

cm^{-2} (surface). The reported values of plate count or physicochemical properties are the mean values of four individual trials ± standard deviation. Data were subjected to ANOVA and Duncan's multiple range tests using STATGRAPHICS (Statistical Graphics Corporation, Englewood Cliffs, CO, USA). Significant differences in plate count data were established by the least significant difference at the 0.05 level of significance.

RESULTS

The mean pH values for the NEW and NaClO solutions and the deionized water used in this study were respectively 7.79 ± 0.20, 8.10 ± 0.22 and 6.45 ± 0.50. The respective ORP values were 774.0 ± 7.0, 739.0 ± 11.0 and 650.0 ± 10.0. The mean active chlorine concentration of the NEW and NaClO solutions was respectively 63 ± 4.56 and 62 ± 5.60. No active chlorine was detected in deionized water.

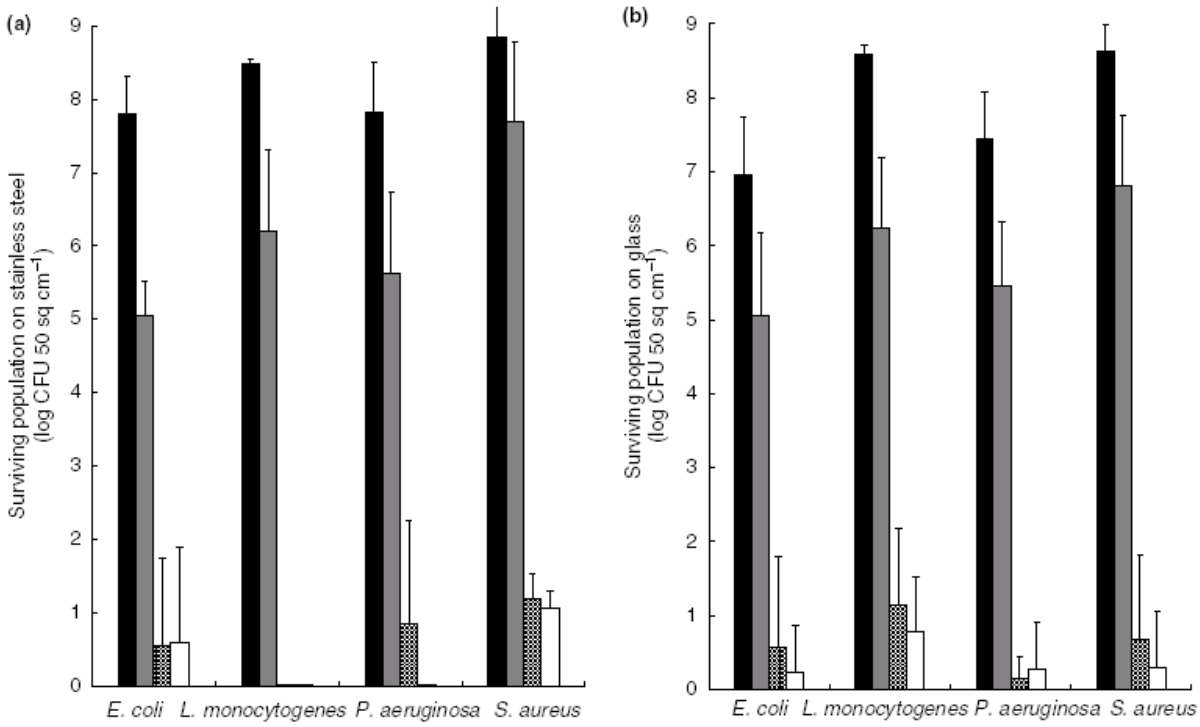
Pure cultures of about 7.5 log CFU ml^{-1} of E. coli, L. monocytogenes, P. aeruginosa and S. aureus, were reduced to undetectable levels – as determined by plating procedures – after 5 min of exposure to NEW (63 mg l^{-1} active chlorine) or NaClO solution (62 mg l^{-1} active chlorine). No significant reduction in bacterial counts was achieved in the control samples.

Figure 1 reports the results of the studies undertaken to determine the efficacy of NEW and NaClO solution in inactivating E. coli, L. monocytogenes, P. aeruginosa and S. aureus on stainless steel and glass surfaces. The initial population of all strains on inoculated surfaces (after drying for 15 min) was between 6.96 and 8.84 log CFU 50 cm^{-2}.

Washing inoculated surfaces for 1 min in either NEW or NaClO solutions decreased the populations of all strains on both materials by more than 6 log CFU 50 cm^{-2}, whereas the control treatment (sterile deionized water) resulted in a reduction of c. 2.1 log CFU 50 cm^{-2} for all strains. For each strain, no significant difference ($P \leq 0.05$) was found between final populations with regard to treatment (NEW or sodium hypochlorite) or surface material.

Table 1 shows the surviving populations of E. coli, L. monocytogenes, P. aeruginosa and S.

Fig. 1 Inactivation of Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, and Staphylococcus aureus on (a) stainless steel and (b) glass surfaces by neutral electrolysed water (NEW; 63 ± 4.56 mg l^{-1} active chlorine) and sodium hypochlorite solution (NaClO; 62 ± 5.6 mg l^{-1} active chlorine) at 23 ± 2ºC. Results are expressed as mean ± SD of at least four repeated measurements. Zero values indicate no detectable survivors by direct plating procedure, ▮ before treatment; ▩ control (water); ▨ NaClO; ◘ NEW



aureus in washing solutions after 1 min treatment of stainless steel and glass surfaces. The surviving populations of all bacteria in NEW washing solution were <1 log CFU ml^{-1} after treating both surfaces and in some cases no survivors could be detected. Instead, an average surviving population of 1.56 log CFU ml^{-1} of S. aureus was still detectable in NaClO washing solutions after treating surfaces of both materials. On the basis of the surviving populations of S. aureus in washing solutions after treating inoculated stainless steel surfaces, NEW efficacy was significantly higher ($P \leq 0.05$) to that of the NaClO solution. In control water, an average of 6 log CFU ml^{-1} of all strains was recovered, after treating both surfaces.

Table 1 Surviving population of Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, and Staphylococcus aureus in washing solutions (NEW, NaClO and deionized water) after treatment of stainless steel and glass surfaces

Strain	Surface	Surviving population in washing solution (log CFU ml^{-1})		
		NEW	NaClO	Water (control)
E. coli	Stainless steel	0·36 ± 0·54	0*	5·59 ± 0·71
	Glass	0	0·58 ± 1·30	5·94 ± 0·45
L. monocytogenes	Stainless steel	0·18 ± 0·35	0·39 ± 0·78	5·81 ± 0·59
	Glass	0·19 ± 0·43	0·58 ± 1·30	6·14 ± 0·35
P. aeruginosa	Stainless steel	0·56 ± 1·12	0·08 ± 0·15	6·00 ± 0·50
	Glass	0	0	5·87 ± 0·72
S. aureus	Stainless steel	0·27 ± 0·20	1·97 ± 1·00	6·52 ± 0·23
	Glass	0·24 ± 0·39	1·15 ± 1·33	6·17 ± 0·66

Values are the mean ± SD of at least four repeated measurements.
NEW, neutral electrolysed water.
*Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

DISCUSSION

The strains of E. coli, S. aureus and P. aeruginosa used in this study are the ones proposed in the European Standard UNE-EN 1276 (Anonymous 1998) for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Formerly, studies were carried out in our laboratories (data not shown) in order to find the minimum concentration of NEW complying with the European standard UNE–EN 1276 (Anonymous 1998), i.e. producing a population reduction of more than 5 log CFU ml^{-1} in all the evaluated strains in pure culture, under clean conditions. On that basis, a NEW concentration such to contain 63 mg l^{-1} active chlorine was chosen to evaluate its efficacy in reducing E. coli, P. aeruginosa, S. aureus and L. monocytogenes on glass and stainless steel surfaces. In the present work, 5 min of exposure to NEW with such concentration produced a reduction of more than 5 logs in the populations of all the strains in pure culture. These results are similar to those obtained by other authors using AEW (with the same active chlorine content) to inactivate pathogens including L. monocytogenes (Venkitanarayanan et al. 1999; Kim et al. 2000a,b) and S. aureus (Park et al. 2002).

With the chosen NEW concentration (63 mg l^{-1} active chlorine) initial populations of about 8 log CFU 50 cm^{-2} on stainless steel and glass surfaces were reduced to 1 log CFU 50 cm^{-2} or less after treating with NEW for 1 min. In addition, the surviving populations of all bacteria in NEW washing solution were <1 log CFU ml^{-1} after treating both surfaces and in some cases no survivors could be detected.

However, the control treatment (sterile deionized water) resulted in a reduction of a more 2.1 log CFU 50 cm^{-2} for all strains on both surfaces and about 6 log CFU ml^{-1} were still recovered from the wash solution.

The results also show that NEW has a broad spectrum of action over the chosen strains: the reductions underwent by all the populations after 1 min treatment showed no significant difference ($P \leq 0.05$). Moreover, that was so on both materials.

The results obtained in this study using NEW (63 mg l^{-1} active chlorine) during a contact time of 1 min, were similar to those obtained by Park et al. (2002) in inactivating S. aureus and Enterobacter aerogenes on different surfaces (including stainless steel and glass) using AEW containing 53 mg l^{-1} chlorine after an immersion of 5 min. This suggests that NEW might be as effective on other surfaces as well, with the advantage of being less corrosive to metallic surfaces, having a longer storage life and being safer to the environment as well as to operators because at neutral pH no chlorine gas is produced. Sodium hypochlorite is one of the most widely used disinfectants in household sites and food industries. In this

study the effectiveness of NEW was compared with that of NaClO solution with the same active chlorine content, and similar pH and ORP. The obtained results reveal that the bactericidal efficacy of NEW on glass and stainless steel surfaces is similar to that of NaClO. In washing solutions, NEW efficacy was significantly higher ($P \leq 0.05$) to that of NaClO when treating stainless steel surfaces inoculated with S. aureus.compared with NaClO, NEW has several advantages: (i) transport and storage problems are reduced as it is generated on-site; (ii) it is potentially less hazardous for workers, as no concentrated chemicals must be handled; (iii) its potential adverse impact on the environment is much less, as it is produced from pure water (without added chemicals except NaCl); (iv) it is much more stable than concentrated NaClO, where the active chlorine concentration rapidly decreases during storage (Qin et al. 2002). These facts lead to suggest that NEW can be considered as a suitable alternative to NaClO for the disinfection of surfaces in the food industry.

In conclusion, this study revealed that NEW is an effective method to significantly reduce the presence of pathogenic and spoilage bacteria like E. coli, L. monocytogenes, P. aeruginosa and S. aureus on stainless steel and glass surfaces. NEW efficacy in reducing bacterial populations on both surfaces was comparable with that of a NaClO solution of similar pH, ORP and active chlorine concentration, with the advantage of being a safe and easy-to-handle option.

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