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Assessment of Antimicrobial Activity in Different Sanitizer Products Commonly Used in Food Processing Environment and Home Setting

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Abstract

The presence of microorganisms to work surfaces represents a potential risk to transmit pathogens to food or humans by cross-contaminations. In food processing and in home setting, disinfectants or sanitizer products are used on inanimate objects and surfaces to inactivate all recognized pathogenic microorganisms. Uncorrected disinfection procedures, in term of declared concentration or time of contact, can lead to the survival of different types of microorganisms. In this study, six sanitizers (herein named A, B, C, D, E, F) belonging to different class of chemical disinfectants were considered: acids (products A, E), halogens (B), quaternary ammonium compounds (C), oxidizing agents (F), mixed classes (D). The antimicrobial activity of each sanitizer was evaluated against *Escherichia coli* O157:H7 ATCC 35150, *Staphylococcus aureus* ATCC 43387, *Pseudomonas aeruginosa* ATCC 9027, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 14053 was examined by the quantitative suspension test indicated by EN 1276.

Our investigation confirmed that different factors affect the activity of chemical sanitizers such as formulation, temperature, time of contact, target microorganism, organic load. The last factor is taken in consideration in the suspension test indicated by EN 1276, that required performing experiments in both dirty and clear simulated conditions. Our data showed that, in general, the examined sanitizers are effective in reducing bacterial growth (logarithmic reduction > 5) at the manufacturer recommended concentrations or lower, but in some cases the presence of organic matter interfered with their activity. In this case, it was necessary to use a more high concentration of the chemical product, as reported for the products A, B and F.

In conclusion, this work highlighted the need that, in each sanitizer product label, the exact information regarding concentration and time of contact are better specified to obtain the indicated bactericidal effect and avoid the onset of bacterial resistance. Moreover, it's important that disinfectants are applied on work surfaces after cleaning and removing organic matter that could create a physical barrier protecting microorganisms from the activity of the sanitizers.

Keywords: *Chemical Sanitizers; Food Processing; Home Setting; Antimicrobial Activity; Quantitative Suspension Test*

Introduction

In food processing environment as well as in home setting, the presence and the adhesion of microorganisms to work surfaces represent a potential risk to transmit pathogens to food or humans by cross-contamination from raw products via hands, sponges, cleaning cloths and utensils utilized with foods not subjected to further cooking [1]. In food industry, cleaning and disinfection procedures are essential to limit cross-contamination between work surfaces and food and regular inspections and routine microbiological controls during the production and processing of food are important steps to guarantee food safety. Similarly, in home setting is important that hygienic procedures are correctly carried out [2].

Sanitizers are definite as agents able to reduce, but do not necessarily eliminate, the microorganisms on surfaces they come into contact with [3], while disinfectants are chemical agents applied on inanimate objects and surfaces to inactivate all recognized pathogenic microorganisms [4]. Disinfectant agents are non-specifically active against multiple targets [5] and typically kill bacteria by disruption of the bacterial membrane [6].

Both sanitizers and disinfectants are generally used for maintaining hygiene in stables, abattoirs, food industry, food handling, preparation, service industries and retail shops [7]. There are a variety of chemical commercialized products, commonly used in food processing environment and home setting, such as alcohol-based products, hypochloric solutions including sodium hypochlorite, aldehydes, peracetic acid, hydrogen peroxide, chlorhexidine digluconate, polyhexamethylene biguanides (PHMB) and quaternary ammonium compounds (QACs) [8].

In general, disinfectants and antiseptics efficacy is determined by standardized tests as indicated by European standards drawn up by control authorities, such as the European Committee for Standardization (CEN), applying standardized tests involving different stages, such as the dilution in water of the product at different concentrations (Phase 1), the presence of inhibitory substances that simulate the organic load (Phase 2/Stage 1) or conditions comparable to the practical use of the product (Phase 2/Stage 2). These standardized tests are fundamental to guarantee food safety and human health. In a normal sanitation cycle process, a cleaning agent is applied and after a certain exposure time (usually minutes) the disinfectant is rinsed off with water. However, disinfection procedures not correctly performed in term of disinfectant concentration or time of contact may results in the survival of different types of microorganisms, including food-borne pathogens and food spoilage bacteria [9,10]. For this reason, the aim of this study was to assess the antimicrobial activity of six sanitizers, belonging to different classes of disinfectants (acids, halogens, QACs, oxidizing agents or mixed classes) and commonly used in food processing and home setting environments, against *Escherichia coli* O157:H7 ATCC 35150, *Staphylococcus aureus* ATCC 43387, *Pseudomonas aeruginosa* ATCC 9027, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 14053 using the suspension test indicated by EN 1276 (Phase 2/Stage 1).

Material and Methods

Sanitizers

Six commercial sanitizer products (herein after referred as A, B, C, D, E, F) based on various active ingredients and kindly furnished by M.D. International s.r.l. (Fermignano, Urbino, Italy) were used in this study (Table 1). For most of the sanitizers, the recommended concentrations as well as higher or lower concentrations were tested. The manufacturer has not revealed the complete composition of the products but only the active ingredients and the fields of application, such as food environments and different types of surfaces in home setting, such as floors, tiles, utensils, washable surfaces. Each product has been diluted in sterile water to obtain the concentration recommended by the manufacturer (stock solutions). All the obtained solutions were then filtered and stored in the dark until use.

Sanitizer	Main ingredients	Recommended concentrations
A	Phosphoric acid 37.5%	5%
B	Sodium hypochlorite 4.5%; potassium hydroxide 10%	5%
C	Benzalkonium chloride 10%	5%
D	Benzalkonium chloride 2.5%; sodium hydroxide 3%	5%
E	Citric acid 30%	5%
F	Hydrogen peroxide 8.75%	5%

Table 1: *Characteristics of the sanitizer products (A, B, C, D, E, F) used in this study as indicated by the manufacturer.*

Bacterial strains and growth conditions

Five reference human pathogens, *E. coli* O157:H7 ATCC 35150, *S. aureus* ATCC 43387, *P. aeruginosa* ATCC 9027, *E. faecalis* ATCC 29212, *C. albicans* ATCC 14053, were used in this study. All the strains were routinely maintained in Tryptic Soy Agar (TSA, Oxoid, Milan, Italy) at 37°C, while stock cultures were keep at -80°C in Nutrient broth (Oxoid) with 15% of glycerol.

Preparation of inoculums

All microbial strains were grown in 15 ml of sterile Tryptic Soy Broth (TSB, Oxoid) overnight at 37°C to obtain an exponential bacterial growth. Before resuspending the microorganisms in sterile physiological solution, the absorbance (OD 610 nm) of each suspension was adjusted to value corresponding to about 10^6 - 10^7 bacteria/ml. A total of 10 ml of each bacterial sample was prepared for the quantitative suspension test.

Assessment of antimicrobial activity by quantitative suspension test

The assessment of antimicrobial activity of the different sanitizers on target bacteria was performed by quantitative suspension test according to the standard procedure EN 1276:2009 "Chemical disinfectants and antiseptics" Technical Committee CEN/TC 216 [11].

First, each sanitizer was distributed in sterile tubes containing 8 ml of sterile water in order to reach the final desired concentration. Then, to simulate dirty and clean conditions, one ml of 0.3% or 0.03% (w/v) bovine albumen serum (BSA, Sigma, Milan, Italy) respectively was added in the tubes. Finally, one ml of each bacterial culture was inoculated and left in contact for 30 sec, 5 and 15 minutes. At each time point, one mL was transferred to new tube containing 9 ml of neutralizing buffer (composed by polysorbate 80 3% v/v, saponin 3% w/v and lecithin 0.3% w/v, Sigma) for 2 minutes; one ml aliquot from each tube was diluted in physiological saline solution, plated in triplicate onto TSA and incubated at 37°C for 24 h. At the end of incubation, the plates were observed and the colony forming units (cfu) were enumerated. Control samples (1 ml of each bacterial culture) were treated with 9 ml of sterile distilled water instead of sanitizer and enumerated as above. Previous experiments were carried out to verify that the neutralizing solution has no antibacterial effect on bacterial strains.

Sanitizer efficacy calculation

All the experiments were performed in duplicate and the averages of the plate counts were converted from units to log10 cfu/coupon. The decrease of bacteria after sanitizer treatments was calculated from the formula [log (N/N_a)], where N is the count of cfu/coupon prior the treatment and N_a is the cfu/coupon after sanitizer treatment.

Results and Discussion

The resident background microflora is recognized to play an important role in protecting pathogenic strains within food processing and home setting environments. In attempting to improve hygiene measures and to ensure food and human safety, the use of biocides and chemical-based disinfectants to control the microbial ecology has increased [9].

In the present study, the effectiveness of six sanitizers was tested on planktonic cultures as required by EN 1276 using the quantitative suspension test performed in presence of organic matter (BSA) (Table 2 and Table 3). Among the examined sanitizers, the product C was the most effective in reducing the bacterial growth of all the target microorganisms, already at the lowest tested concentration (1%); in fact, after only 30 sec of contact, a complete inactivation of viable cells, with no detectable cfu/ml, was evidenced in the undiluted samples (log reduction > 5) for all the tested bacteria. In addition, the presence of organic matter did not change the efficacy of this sanitizer, showing no difference in log reduction in samples containing 0.3 or 0.03% BSA. For this reason, the higher concentration (5%) was not tested. As regard the product A, it was tested at 3 different concentrations (1, 3 and 5%) and the obtained data showed that the lowest concentration, regardless BSA presence, was ineffective against *E. coli* O157:H7 ATCC 35150 and *E. faecalis* ATCC 29212 at each time of contact, whilst was effective versus *S. aureus* ATCC 43387 and *C. albicans* ATCC 14053 but only after 5 and 15 minutes of exposure; interestingly, against *P. aeruginosa* ATCC 9027, was active also after only 30 sec of contact. The experiments performed with product A at higher concentrations evidenced that 30 sec of contact were still not sufficient to reach a 5 log reduction as required by EN 1276, but after 5 and 15 min, in most cases, no bacterial growth was observed. The main ingredient of product A is represented by phosphoric acid that acts as acidifying and sequestering agent. This dual action helps to remove surface contaminants and kill bacteria by rapidly disrupting the cell membrane. In the present work, the antimicrobial efficacy of product A was evidenced after 15 minutes of contact, and only *P. aeruginosa* was rapidly inactivated. This fact could be explained considering that several factors can affect products containing phosphoric acids, such as the temperature. In this investigation, the suspension test, according to EN 1276, was carried at room temperature, if not differently indicated by the manufacturer, but the work of Lee., *et al*. [12] referred as the increase of temperature shortened the exposure time required to eliminate the test microorganisms in formulation with phosphoric acids. This aspect assumes particularly importance and, in our opinion, the manufacturer must consider to add this information in the product label.

	Time of contact	*E. coli* O157:H7	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*C. albicans*
		ATCC 35150	ATCC 43387	ATCC 29212	ATCC 9027	ATCC 14053
Product A :						
Concentration 1%						
BSA 0,3%	30 sec	1.21	3.7	1.16	No growth	2.59
	5 min	1.69	No growth	3.32	No growth	No growth
	15 min	2.51	No growth	3.29	No growth	No growth
BSA 0,03%	30 sec	1.87	4.11	2.06	No growth	4.95
	5 min	2.57	No growth	4.29	No growth	No growth
	15 min	2.94	No growth	4.76	No growth	No growth
Concentration 3%						
BSA 0,3%	30 sec	2.17	3.35	2.71	Not tested	No growth
	5 min	4.37	5.19	No growth	Not tested	Not tested
	15 min	No growth	No growth	No growth	Not tested	Not tested
BSA 0,03%	30 sec	2.68	4.53	3.68	Not tested	No growth
	5 min	5.07	No growth	No growth	Not tested	Not tested
	15 min	No growth	No growth	No growth	Not tested	Not tested
Concentration 5%						
BSA 0,3%	30 sec	1.37	2.80	3.58	Not tested	Not tested
	5 min	5.43	5.15	No growth	Not tested	Not tested
	15 min	No growth	5.15	No growth	Not tested	Not tested
BSA 0,03%	30 sec	2.59	3.67	4.64	Not tested	Not tested
	5 min	5.13	No growth	No growth	Not tested	Not tested
	15 min	No growth	No growth	No growth	Not tested	Not tested
Product B :						
Concentration 2%						
BSA 0,3%	30 sec	1.12	1.32	0.57	No growth	1.20
	5 min	1.18	2.80	0.89	No growth	1.42
	15 min	1.87	4.31	2.24	No growth	2.12
BSA 0,03%	30 sec	No growth	3.90	1.86	No growth	2.90
	5 min	No growth	No growth	3.82	No growth	3.15
	15 min	No growth	No growth	4.99	No growth	5.10
Concentration 5%						
BSA 0,3%	30 sec	1.83	4.11	2.31	Not tested	No growth
	5 min	373	No growth	No growth	Not tested	No growth
	15 min	5.28	No growth	No growth	Not tested	No growth
BSA 0,03%	30 sec	No growth	No growth	No growth	Not tested	No growth
	5 min	No growth	No growth	No growth	Not tested	No growth
	15 min	No growth	No growth	No growth	Not tested	No growth
Product C :						
Concentration 1%						
BSA 0,3%	30 sec	No growth	No growth	No growth	No growth	No growth
	5 min	No growth	No growth	No growth	No growth	No growth
	15 min	No growth	No growth	No growth	No growth	No growth
BSA 0,03%	30 sec	No growth	No growth	No growth	No growth	No growth
	5 min	No growth	No growth	No growth	No growth	No growth
	15 min	No growth	No growth	No growth	No growth	No growth

Table 2: *Efficacy of products A, B, and C against target bacteria assessed by suspension test according to EN 1276. The test is considered as passed if logarithmic reductions of 5 for bacteria and 4 for mycetes were reached in the indicated times of contact.*

Not tested because the product was already active at low concentration (log reduction > 5)

	Time of contact	*E. coli* O157:H7	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*C. albicans*
		ATCC 35150	ATCC 43387	ATCC 29212	ATCC 9027	ATCC 14053
Product D :						
Concentration 1%						
BSA 0,3%	30 sec	No growth	4.7	4.12	4.00	5.44
	5 min	No growth	No growth	No growth	No growth	No growth
	15 min	No growth	No growth	No growth	No growth	No growth
BSA 0,03%	30 sec	No growth	No growth	4,11	No growth	No growth
	5 min	No growth	No growth	No growth	No growth	No growth
	15 min	No growth	No growth	No growth	No growth	No growth
Concentration 2%						
BSA 0,3%	30 sec	not tested	No growth	No growth	No growth	not tested
	5 min	not tested	not tested	No growth	No growth	not tested
	15 min	not tested	not tested	No growth	No growth	not tested
BSA 0,03%	30 sec	not tested	not tested	No growth	No growth	not tested
	5 min	not tested	not tested	No growth	No growth	not tested
	15 min	not tested	not tested	No growth	No growth	not tested
Product E :						
Concentration 5%						
BSA 0,3%	30 sec	1.24	1.82	1.18	No growth	2.48
	5 min	2.32	No growth	3.28	No growth	3.08
	15 min	No growth	No growth	No growth	No growth	4.12
BSA 0,03%	30 sec	3.49	3.25	0.95	No growth	2.35
	5 min	No growth	No growth	2.79	No growth	3.31
	15 min	No growth	No growth	No growth	No growth	4.56
Product F :						
Concentration 1%						
BSA 0,3%	30 sec	0.35	0.45	1.05	0.85	1.25
	5 min	0.92	0.87	1.64	1.99	2.38
	15 min	1.55	1.68	2.57	2.53	3.15
BSA 0,03%	30 sec	0.39	0.59	1.98	1.35	3.33
	5 min	1.08	1.81	2.03	2.27	3.58
	15 min	2.61	2.19	2.98	3.04	3.75
Concentration 5%						
BSA 0,3%	30 sec	2.74	3.05	1.22	2.12	3.10
	5 min	4.76	4.10	2.22	4.34	5.08
	15 min	5.01	No growth	5.45	5.0	No growth
BSA 0,03%	30 sec	3.86	3.38	2.64	2.58	3.26
	5 min	4.98	4.35	3.96	4.97	5.08

Table 3: *Efficacy of products D, E and F against target bacteria assessed by suspension test according to EN 1276. The test is considered as passed if logarithmic reductions of 5 for bacteria and 4 for mycetes were reached in the indicated times of contact.*

Not tested because the product was already active at lower concentration (log reduction > 5)

The product B used at low concentration (2%) showed to be always ineffective in presence of 0.3% BSA against the tested bacteria, with the only exception of *P. aeruginosa* ATCC 9027; on the contrary, when the concentration of BSA was lower (0,03%), the product B was able to inhibit bacterial growth up to 5 log reduction after 5 and 15 min of contact and, in the case of *E. coli* O157:H7 ATCC 35150, after only 30 sec. A similar trend was observed with product B used at 5% for 30 sec, which determined the complete inactivation (log reduction > 5) only for *P. aeruginosa* ATCC 9027 and *C. albicans* ATCC 14053, regardless BSA concentration. The presence of 0.3% BSA still interfered with the efficacy of product B against *E. coli* O157:H7 ATCC 35150, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 43387, with a logarithmic reduction less than 5 log. After 5 min of contact time, the complete reduction of growth was evidenced regardless BSA concentration for all the examined strains, with the only exception of *E. coli* O157:H7 ATCC 35150, for which the prolonged time contact of 15 min was necessary (Table 1). The product B is based on a mixture of sodium hypochlorite (NaOCl) and potassium hydroxide (KOH). It is well known that NaOCl is one of the most widely used chlorine containing disinfectants with biocidal activity determined by the amount of the available chlorine of the solution [13]. Our data evidenced that the antimicrobial activity of the tested product at 5% in some cases was affected by the presence of BSA, but the observed reduced activity could be also explained considering that NaOCl quickly loses its stability after being prepared for use or when stored over long periods, especially in the presence of heat or light. To maximize stability and shelf life, products should be stored in a dark, cool location and preferably in stock concentrations, otherwise it may result in the use of a non-efficacious product leading to a false sense of security [13]. In our case, the product B was not immediately used after the delivery in laboratory, and, even if it has been stored in adequate conditions, this may have affected the real efficacy of the sanitizer itself.

In table 2 were summarized the results of the other 3 tested sanitizers (D, E and F). As observed for previously tested sanitizers, the product D determined the reduction of bacterial growth at the tested concentrations, even if, in some cases, the presence of BSA seems to interfere with its activity. As reported, the complete growth reduction was evidenced for *E. coli* O157:H7 ATCC 35150 and *C. albicans* ATCC 14053 (> 5 log) regardless BSA after 30 sec of contact; on the contrary, log reductions less than 5 were observed in presence of 0.3% BSA for the other microorganisms and the complete inhibition was reached only after 5 and 15 minutes of contacts. The products C and D are based on benzalkonium chloride, a quaternary ammonium compounds (QACs), well known for its widespread antimicrobial activity [14,15]. QACs are the major class of cationic surfactants used as the ingredients in fabric softeners, disinfectants, biocides, detergents, phase transfer agent and numerous personal care products. They are effective against a variety of bacteria, fungi and viruses at very low concentration as they induce the release of intracellular components that causes cell membrane damage depending on the concentrations used. In fact, low doses cause loss of cell osmoregulation capacity, intermediate dosages disturb the breathing process, transport of some solutes and wall synthesis, while high concentrations, such as those used in detergents, cause cell death [14]. Our data confirmed the wide antimicrobial activity of QACs as reported by other authors [16,17], but it can be observed that relatively few studies have been conducted to assess their efficacy in practice [14].

The product E showed the greatest efficacy against *P. aeruginosa* ATCC 9027 with log reduction> 5 still after 30 sec of exposure, both with 0.3 and 0.03% of BSA. On the contrary, for the other target species, 15 minutes of contact were necessary to achieve log reduction > 5. The product F showed to be completely ineffective at 1%, while the higher concentration (5%) determined log reductions > 5 but after 15 min of contact, with the exception of *C. albicans* ATCC 14053 that was inhibited also after 5 min of exposure with a log reduction of 5.08. The main ingredients of products E and F are citrate acid and peroxide hydrogen that, in relation to the target microorganism type, generally showed a similar antimicrobial activity against gram-negative and gram-positive species, probably because the cellular targets are present in both the two bacterial groups. In fact, hydrogen peroxide is able to remove electrons from different chemical groups by oxidizing and reducing them [18].

Most of the sanitizers tested in this study comprised specific formulations containing multiple active biocidal agents, targeting the bacterial cell at several levels. However, different factors affect the activity of chemical sanitizers [14,19,20], such as formulation, temperature, time of contact, target microorganism, organic load. This last factor is taken in consideration in the suspension test indicated by EN 1276, that required performing experiments in both dirty and clear simulated conditions. In figure 1 the log reductions reached in both

dirty (0.3% BSA) and clear (0.03% BSA) simulated conditions at each time of contact for all the tested products were compared. As illustrated, the organic matter plays an important role in the effectiveness of sanitizers against bacteria, and this behavior is not related to the contact time, since also after 15 min of exposure the presence of BSA limited the achievement of the required log reduction. In this case, it was necessary to use a more high concentration of the chemical product, as reported for the products A, B (Table 1) and F (Table 2).

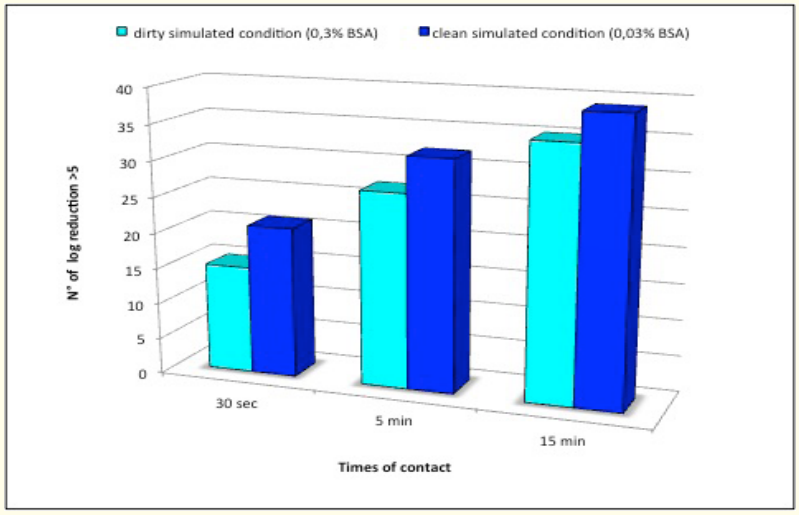

Figure 1: *Comparison of logarithmic reductions (> 5 log for bacteria and > 4 log for mycetes) obtained in the suspension test (EN 1276) in dirty (0.3% BSA) and clean (0.03% BSA) simulated conditions for all the tested sanitizers.*

In conclusion, our data confirmed that most of the examined products were microbiologically active at the recommended manufacturer concentrations or lower, requiring contact time of at least 5 minutes. However, in many cases the organic matter interfered with their activity, and prolonged time of contacts must be applied. From these observations and in according with our previous work [21], we highlight the need that manufacturer, in each product label, specifics the exact information regarding concentration and time of contact in order to obtain the indicated bactericidal effect avoiding the onset of resistance. Finally, it is important to use sanitizers on work surfaces after cleaning and removing organic matter, which could create a physical barrier that protects microorganisms from the activity of the sanitizer products.

In the future, it will be interesting to evaluate the efficacy of these products and of others present in the market, also on biofilm-based bacteria, a structure more resistant to antimicrobials than the corresponding planktonic forms, and for this a great concern in terms of food and human safety.

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Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Brown LG., *et al*. "Outbreak characteristics associated with identification of contributing factors to foodborne illness outbreaks". *Epidemiology and Infection* 145.11 (2017): 2254-2262.

2. Barker J., *et al*. "The effects of cleaning and disinfection in reducing Salmonella contamination in a laboratory model kitchen". *Journal of Applied Microbiology* 95.6 (2003): 1351-1360.

3. Richter F and Cords B. "Formulation of sanitizers and disinfectants". In: Seymour B, Block S, editors. Disinfection, sterilization and preservation. 5th edition. Philadelphia, PA: Lippincott Williams and Williams (1999): 473-487.

4. Centers for Disease Control and Prevention, USA. Rutala, W.A., Weber, D.J., & the Healthcare Infection Control Practices Advisory Committee (HICPAC). "Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008.

5. Meyer B and Cookson B. "Does microbial resistance or adaptation to biocides create a hazard in infection prevention and control?" *Journal of Hospital Infection* 76.3 (2010): 200-205.

6. Maillard JY. "Bacterial target sites for biocide action". *Journal of Applied Microbiology* 92.1 (2002): 16S-27S.

7. Cerf O., *et al*. "Tests for determining in-use concentrations of antibiotics and disinfectants are based on entirely different concepts: "resistance" has different meanings". *International Journal of Food Microbiology* 136.3 (2010): 247-254.

8. Coughlan LM., *et al*. "New weapons to fight old enemies: novel strategies for the (bio)control of bacterial biofilms in the food industry". *Frontiers in Microbiology* 7 (2016): 1641.

9. Møretrø T., *et al*. "Bacteria on meat abattoir meat production process surfaces after sanitation: characterization of survival properties of Listeria monocytogenes and the commensal bacterial flora". *Advances in Microbiology* 3.3 (2013): 255-264.

10. Weese JS and Rousseau J. "Survival of Salmonella Copenhagen in food bowls following contamination with experimentally inoculated raw meat: effects of time, cleaning, and disinfection". *Canadian Veterinary Journal* 47.9 (2006): 887-889.

11. EN 1276. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas. Test method and requirement (phase 2) (2009).

12. Lee J., *et al*. "Efficacy of two acidic sanitizers for microbial reduction on metal cans and low-density polyethylene film surfaces". *Journal of Food Science* 72.8 (2007): M335-M339.

13. Dvorak G. "Disinfection". Center for Food Security and Public Health, Iowa State University (2008).

14. Gerba C. "Quaternary ammonium biocides: efficacy in application". *Applied and Environmental Microbiology* 81.2 (2015): 464-469.

15. Tezel U and Pavlostathis SG. "Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology". *Current Opinion in Biotechnology* 33 (2015): 296-304.

16. Merianos JJ. "Surface-active agents". In Block SS (ed), Disinfection, sterilization, and preservation. Lippincott Williams & Wilkins, Philadelphia, PA (2001): 63-320.

17. Iñiguez-Moreno M., *et al*. "Antimicrobial activity of disinfectants commonly used in the food industry in Mexico". *Journal of Global Antimicrobial Resistance* 10 (2017): 143-147.

18. Finnegan M., *et al*. "Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms". *Journal of Antimicrobial Chemotherapy* 65.10 (2010): 2108-2115.

19. Hong Y., *et al*. "Effects of contact time and concentration on bactericidal efficacy of 3 disinfectants on hard nonporous surfaces". *American Journal of Infection Control* (2017).

20. Ortega Morente E., *et al*. "Biocide tolerance in bacteria". *International Journal of Food Microbiology* 162.1 (2013): 13-25.

21. Vittoria E., *et al*. "Evaluation of the antimicrobial activity of products used for disinfection of work surfaces in the food industry". *Igiene e Sanità Pubblica* 71.6 (2015): 589-599.

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