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SHORT COMMUNICATION

Effectiveness of Different Disinfectants Used in the Food Production Facility on Selected Foodborne Pathogens [1]

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Summary

Staphylococcus aureus, Salmonella typhimurium and *Escherichia coli* are particularly important pathogens for public health and are considered as indicators in the processes used by the food production plants. In this study, four different types of commercial disinfectants (chlorine-based compounds, alcohols, Quarterner Ammonium Compounds (QAC) and nonionic surfactants) used in food production facility at 3 concentrations (0.1%, 0.5%, 2%) were tested at specific periods (1, 3, 5 or 1, 3, 5, 10 and 15 min.) using the TSE EN 1276 Suspension Test Method. QACs were determined to be very effective on the *S. aureus* and *E. coli*. Additionally, chlorine-based compounds is also effective on *S. aureus* and *E. coli* and the alcoholic disinfectants are effective on all microorganisms that have been identified ($P \leq 0.05$).

Keywords: *Food industry, Disinfectant effect, Suspension test, Staphylococcus aureus, Salmonella typhimurium, Escherichia coli*

Gıda Üretim Tesisleri Yüzeylerinde Kullanılan Çeşitli Dezenfektanların Bazı Patojen Mikroorganizmalar Üzerine Etkisi

Özet

Staphylococcus aureus, Salmonella typhimurium ve *Escherichia coli* bilhassa halk sağlığı açısından önemli patojenlerdir ve gıda üretim işletmelerindeki proseslerde indikatör olarak değerlendirilmektedir. Bu çalışmada gıda üretim tesisleri yüzeylerinde kullanılan 4 adet ticari dezenfektan çeşidinin (klorin bazlı komponentler, alkol, Kuarterner Amonyum Bileşikleri (KAB) ve iyonik olmayan surfaktan) belirli süreler (1, 3, 5, ile 1, 3, 5, 10 ve 15 dakika) ile 3 farklı konsantrasyonda (%0.1, %0.5, %2) TSE EN 1276 süspansiyon test yöntemi ile etkinliği incelenmiştir. Buna göre KAB'nin *S. aureus* ve *E. coli* üzerinde çok etkili olduğu tespit edilmiştir. Ayrıca, klorlu bileşiklerin hem *S. aureus* hem de *E. coli* üzerinde, alkollü dezenfektanın ise tüm mikroorganizmalar üzerine etkili olduğu saptanmıştır ($P \leq 0.05$).

Anahtar sözcükler: *Gıda endüstrisi, Dezenfektan etkisi, Süspansiyon testi, Staphylococcus aureus, Salmonella typhimurium, Escherichia coli*

INTRODUCTION

Disinfection is required in the food production and distribution industries where wet surfaces provide favorable conditions for the growth of microbes. The aim in using a disinfectant is to reduce the number of viable micro-organisms left on processing surfaces after cleaning that might contaminate the product [1]. The selection of disinfectants in the food industry depends on the efficacy, safety and rinsability of the agent as well as whether it is corrosive or affects the sensory values of the products manufactured [2]. The effectiveness of disinfectants depends on various factor including chemical composition and concentration of disinfectant, temperature and exposure time, pH, water

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hardness, type, quantity and age of microorganism, and microbial attachment to a solid surface [3,4].

Disinfectants approved for use in the food industry are chlorine-based compounds, alcohols, surfactants, quarternary ammonium compounds (QAC), oxidants, persulphates, and iodophors [2]. Surfactants are classified into cationic, anionic, nonionic, and ampholytic (amphoteric) compounds and use commonly as sanitizers in food industry [5]. Of these, the cationic agents, as exemplified by QAC, are the most useful antiseptics and disinfectants. The greatest effect of QAC is observed against Gram-positive bacteria, whereas Gram-negative microorganisms, many of them significant in the contamination of food, may not be affected [6]. Additionally, nonionic surfactants are compatible with all other classes of surfactants and have been used as detergents, stabilizers, emulsifiers, wetting agents, and dispersants [7]. These compounds are very effective against the common microorganisms, including bacteria, moulds, yeasts, and some viruses [4]. The chloramines e.g. chloramin T, dichloramin T and chloramin B, are much more stable than the hypochlorites in the presence of organic matter. Many alcohol products include low levels of other biocides (in particular glutaraldehyde, chlorhexidine etc.), which remain on the skin following evaporation of the alcohol and decrease the evaporation time of the alcohol and can significantly increase product efficacy [5].

A number of microbiological tests have been devised to determine the efficacy of disinfectants, such as suspension test, surface test, capacity test, tube dilution test, agar diffusion test [4,5,8]. Suspension test methods are the most widely used methods to determine the efficacy of commercially available disinfectants and antiseptics [3,4].

Salmonella spp., *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* are foodborne pathogens that can be present on food contact surfaces or food equipment and can be hazardous to health. This fact implies the need to control these microorganisms to preserve the microbial quality of food by an adequate cleaning and disinfection program [9]. Therefore, for the detection of effectiveness of disinfectants with suspension tests, there are some test microorganisms such as *S. typhimurium, E. coli, S. aureus, Pseudomonas aeruginosa, B. cereus and Streptococcus faecalis* that are used [10]. Disinfectants must reduce the microbial population at least 5 log units to be considered effective [9].

The purpose of the present study was to investigate the antimicrobial effectiveness of four commercial disinfectants (chlorine-based compounds, alcohols, QAC and nonionic surfactants) used in the food production facilities with the lowest recommended ready-to-use concentration (2%) or less concentration (0.5% and 1%) against important food pathogens *(Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli)*.

MATERIAL and METHODS

Test Microorganisms

Tests microorganisms, *S. aureus* ATCC 25923, *S. typhimurium* ATCC 14028 and *E. coli* ATCC 25922, selected for the disinfection test were obtained from American Type Culture Collection (Manassas, USA). The cultures were propagated on Tryptone soy agar (TSA) [Oxoid CM 131 (Basingstoke, UK)] and incubated at 37°C 24 h. After 24 h, bacterial suspensions were adjusted to 0.5 Mc Farland (~1.5x 10^8 cfu/g). The viable cell concentration was prepared by serial decimal dilutions with 9 ml Tryptone soy broth (Oxoid CM 129) and bacterial count was determined by pour plate method in TSA by adding 1 ml of bacterial suspension and incubation at 37°C 24h. The experiments were carried out in duplicate [10].

Disinfectants

The antimicrobial compounds and the concentrations of four commercial disinfectants (DA-DD) in the ready-to -use solutions tested [DA (pH 11-12): Alkyl dimethyl benzyl ammonium chloride (QAC) Nonionic surfactant, Ethanol, Ethanoldiamine, 20g/L (lowest recommended concentration), 15 min (recommended exposure time); DB (pH 8-9): Non-ionic surfactant, QAC, Nitrilo acetic acid, 20g/L (lowest recommended concentration), 15 min (recommended exposure time); DC (pH 8-10.3): Sodium p-toluenesulfon-chloramide, trihydrate 20 g/L (lowest recommended concentration), 5 min (recommended exposure time); DD (pH 6.8-7.8): Ethanol, Glutaraldehyde, ready to use, until evaporation]. The disinfectants were diluted to the lowest concentration (2%) recommended by the manufacturer and also to lower than the recommended concentration (0.1% and 0.5%), which is of particular interest in the food safety point of view. Additionally, ready-to-use disinfectant DD was directly used.

Suspension Test

Tests were carried out according to TS EN 1276 [10], quantitative suspension test for the evaluation of chemical disinfectants used in food, industrial, domestic, and institutional areas-test method and requirements, phase 2 step 1. Briefly, 1 ml of bacterial suspension (0.5 Mc Farland) of each strain (*S. aureus*, *S. typhimurium*, and *E. coli*) were added to 9 ml appropriate commercial disinfectant solutions (2%, 1%, and 0.5%) at 21°C. After 1, 3, and 5 min for alcohol based disinfectant (DD) and 1, 5, 10, and 15 min for another disinfectants (DA, DB and DC), a 1 ml sample was added to 9 ml neutralizer solution. The neutralizer solution contained phosphate buffered saline [Himedia M1452 (Mumbai, India)] (1.8% w/w), Tween 80 [INC Bromedicals Inc, (Thame, UK)] (3% w/v), and lecithin (0.3% w/v). The neutralizer solutions were sterilized by filtration through a 0.45 µm filter [Milipore Corp. (Billerica, USA)] [3,10]. After 5 min, a 1 ml aliquot of the neutralizer/disinfectant suspension was transferred to 9 ml TSB for serial dilutions (10^{-1} to 10^{-5}). Finally, 1ml of appropriate serial dilutions were plated on TSA (duplicate) and incubated at 37°C for 24 h. After incubation, colony forming units (cfu/ml) were counted on petri dishes. The suspension test was repeated three times.

Statistical Analysis

Statistical analysis of bacterial counts was done based on absolute values. Colony counts were converted into logarithmic values. One-way ANOVA and Duncan's multiple range tests were used to analyze log bacterial counts. Statistical analysis was done using the Statistical Package for the Social Sciences [11].

RESULTS

The results indicate that the majority of the disinfectants showed satisfactory activity against all the test microorganisms. After regular exposure (15 min; DA and DB and 5 min; DC) disinfectants were effective at the lowest concentration (2%) as recommended by the manufacturer against all foodborne pathogens.

S. aureus, S. typhimurium and *E.* coli were treated with several concentrations (0.1%, 0.5% and 2%) of DA for 1, 5, 10 and 15 min at 21°C. Different levels of reduction on microorganisms are shown in *Table 1*. *S. aureus* was not detected after 1 min exposure at all the concentrations of DA. The lowest antibacterial effect of DA (0.1%) was determined after 1 min on the *S. typhimurium* (2.77 log reduction). *E.* coli was not found after 1 min exposure to a 2% concentration of DA. A 2% concentration of DB was effective against *S. aureus* and *E. coli* after 1 min of exposure, but not the *S. typhimurium* strain *(Table 2)*. However, the effectiveness of the 0.1% concentration of DB on the *S. typhimurium* was found to be relatively less as compared to *S. aureus* and *E. coli*. The highest anti-bacterial effect of all concentrations of DB was detected

Table 1. *Effectiveness of 3 different concentrations of commercial disinfectant DA tested against selected foodborne pathogens*
Tablo1. *Üç farklı konsantrasyondaki ticari DA dezenfektanının seçilmiş gıda kaynaklı patojenler üzerine etkisi*

Bacterium	Control $\log_{10}$ cfu/ml	Treatment of DA with Different Concentration and Exposure Time											
		0.1%				0.5%				2%			
		1 min	5 min	10 min	15 min	1 min	5 min	10 min	15 min	1 min	5 min	10 min	15 min
S. aureus	8.38±0.20^{a*}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
S. typhimurim	9.02±0.39^{a}	6.25±0.33^{b}	3.44±0.35^{d}	0.87±0.43^{f}	0^{f}	4.37±0.34^{c}	0^{f}	0^{f}	0^{f}	2.59±0.55^{e}	0^{f}	0^{f}	0^{f}
E. coli	8.01±0.32^{a}	4.99±0.76^{b}	2.42±0.75^{c}	0^{d}	0^{d}	1.83±0.53^{c}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}

** Means in a column with different letters are significantly (P≤0.05) different from one another*

Table 2. *Effectiveness of 3 different concentrations of commercial disinfectant DB tested against selected foodborne pathogens*
Tablo 2. *3 farklı konsantrasyondaki ticari DB dezenfektanının seçilmiş gıda kaynaklı patojenler üzerine etkisi*

Bacterium	Control $\log_{10}$ cfu/ml	Treatment of DB with Different Concentration and Exposure Time											
		0.1%				0.5%				2%			
		1 min	5 min	10 min	15 min	1 min	5 min	10 min	15 min	1 min	5 min	10 min	15 min
S. aureus	8.38±0.20^{a*}	2.68±0.59^{b}	0^{d}	0^{d}	0^{d}	1.73±0.43^{c}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
S. typhimurim	9.02±0.39^{a}	6.01±0.32^{b}	3.71±0.22^{c}	1.69±0.52^{d}	0^{e}	2.63±0.20^{d}	0^{e}	0^{e}	0^{e}	1.48±0.18^{d}	0^{e}	0^{e}	0^{e}
E. coli	8.01±0.32^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}

** Means in a column with different letters are significantly (P≤0.05) different from one another*

Table 3. *Effectiveness of 3 different concentrations of commercial disinfectant DC tested against selected foodborne pathogens*
Tablo 3. *3 farklı konsantrasyondaki ticari DC dezenfektanının seçilmiş gıda kaynaklı patojenler üzerine etkisi*

Bacterium	Control $\log_{10}$ cfu/ml	Treatment of DC with Different Concentration and Exposure Time								
		0.1%			0.5%			2%		
		1 min	3 min	5 min	1 min	3 min	5 min	1 min	3 min	5 min
S. aureus	8.38±0.20^{a*}	3.31±0.39^{b}	1.16±0.63^{c}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
S. typhimurim	9.02±0.39^{a}	4.15±0.58^{b}	1.56±0.80^{d}	0^{e}	2.75±0.53^{c}	0^{e}	0^{e}	0^{e}	0^{e}	0^{e}
E. coli	8.01±0.32^{a}	2.11±0.10^{b}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}

** Means in a column with different letters are significantly (P≤0.05) different from one another* on *E. coli*. The recommended concentration of DC (2%) was found to be very effective against *S. aureus, S. typhimurium and E.* coli after 1 min of exposure *(Table 3)*. *S. aureus* and *S. typhimurium* were also found after 3 min exposure of 0.1% concentration of DC, 1.16 log_{10} cfu/ml and 4.15 log_{10} cfu/ml, respectively. After 1 min exposure of DD an approximately 7 log_{10} cfu/ml reduction of *S. aureus, S. typhimurium and E.* coli *(Table 4)* was noticed. Additionally, the selected strains were not detected after a 3 min exposure of DD.

over a wide pH range, an increase in alkalinity through formulation with compatible detergents such as nonionic surfactants may enhance their bactericidal activity [6].

There is several chlorine or chlorine-based compounds, which are approved for use in food plants, e.g. cloramines, gaseous chlorine, chlorine dioxide, and sodium and calcium hypochlorites [2]. The range of microorganisms killed or inhibited by chlorine-based compounds is probably broader than any another approved sanitizer [6]. In this study, the disinfectant DC was more effective on *E. coli* and *S. aureus* than against *S. typhimurium (Table 3)*. However, bactericidal activity of DC was also found very effective at the lowest recommended concentration (2%) and exposure time (5 min) on all the test microorganisms. Additionally, *E. coli* was not detected after 1 min exposure to 0.1%, 0.5% and 2% concentrations of DC and the differences were found significant between control groups and treatments (P≤0.05). Conversely, Senel [13] declared less bactericidal activity of chlorine-based compounds on *E. coli*. *E. coli* was not detected (<10 cfu/ml) after 25 min, 20 min, 7 min and 3 min of exposure at 0.5%, 1%, 1.5% and 2% concentrations of chlorine-based compounds, respectively. In this sense, the researchers was detected the bactericidal activity after 3 min 0.5% concentration of chlorine-based compounds on *S. aureus* [13]. In our study *S. aureus* was not determined (<10 cfu/ml) after 1 min exposure of a 0.5% concentration of DC. Similar to our results, Senel [13] did not detect *S. aureus* after 1 min exposure of 1%, 1.5% and 2% concentration of chlorine-based compounds. In this study we also found the lowest bactericidal effect on the *S. typhimurium* test microorganism. According to the results, the exposure time significantly effects the differences between the control groups of *S. aureus*, *S. typhimurium*, and the treated samples (P≤0.05).

***Table 4.** Effectiveness of commercial disinfectant DD tested against selected foodborne pathogens*
***Tablo 4.** Ticari DD dezenfektanının seçilmiş gıda kaynaklı patojenler üzerine etkisi*

Bacterium	Control log_{10} cfu/ml	Treatment of Ready-to-Use Concentration and Exposure Time		
		1 min	3 min	5 min
S. aureus	8.38±0.20^{a*}	1.63±0.20^{b}	0^{c}	0^{c}
S. typhimurim	9.02±0.39^{a}	1.16±0.60^{b}	0^{b}	0^{b}
E. coli	8.01±0.32^{a}	1.20±0.67^{b}	0^{b}	0^{b}

** Means in a column with different letters are significantly (P≤0.05) different from one another*

DISCUSSION

The increasing global incidences of food poisoning cases originating from food contaminated by pathogens has great social and economic costs and causes major concern both to the general public and to the food industry [12]. The effectiveness of disinfectant agent is evaluated by its efficacy in destroying pathogens and removing soil during the cleaning process. During recent years, evaluation of industrial hygiene has become an important factor because of the needs of the industry, the legisletion of autocontrol in food hygiene accepted "Hazard Analysis Critical Control Point" technique in Europe [3].

QAC is widely used in disinfection applications in the food processing industry. In this study, significant differences (P≤0.05) were found between control and 0.1%, 0.5% and 2% concentrations of DA and DB on the microorganisms (*Table 1* and *Table 2*). We found the lowest bactericidal effect at 0.1% and 0.5% concentrations of DA and DB after 1 and 5 min exposure on *S. typhimurium* than the others (*S. aureus* and *E. coli*). In this sense, Senel [13] reported the 1%, 1.5%, and 2% concentration of QAC were effective on *S. aureus* after 1 min of exposure. Additionally, we also determined a high reduction (<10 cfu/ml) with 0.1% and 0.5% concentrations of DA against *S. aureus* after 1 min. Conversely, Kasgar and Cotuk [14] detected *S. aureus* after 30 min exposure of a 0.5% concentration of OAC. Another study, Senel [13] noticed after 7 min exposure of a 0.5% concentration of OAC, *E. coli* was not found in the samples. However, in this study *E. coli* was not detected after a 5 min exposure of a 0.5% concentration of DA. Similarly, Kasgar and Cotuk [14] declared 0.1% and 0.5% concentrations of QAC was effective on *E. coli*. Although QAC retain their activity

Ethanol is volatile and will evaporate rapidly when used on surfaces. Alcohol (ethanol) was diluted 50-70% and used for surface and hands disinfection. Alcohols are preferred in the food industry and for use in the food production facility because of the lack of need of a washing step after it is applied on surfaces [13]. A study researched the bactericidal effect of ethanol on microorganisms such as *S. aureus*, *E. coli* and *S. enteritidis* and found a 60-95% reduction of microorganism counts at room temperature [15]. Kasgar and

Cotuk [14] investigated the bactericidal effect of disinfectants on several test microorganisms *(S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa)* and the highest bactericidal effect was detected with 70% ethanol containing disinfectants. Similar to researchers' results, ready-to use DD showed the highest bactericidal effect on all selected foodborne pathogen microorganisms after 3 min. Additionally, after 1 min exposure to ready-to-use DD, more than 5 log reductions of *S. aureus*, *E. coli* and *S. typhimurium* counts were found as compared to control groups ($P \leq 0.05$). In this study the highest bactericidal effect were determined on Salmonella by ready-to use DD after 3 min exposure *(Table 4)*. However, other disinfectants were not effective especially the lowest concentration (0.1%) and shorter exposure time (1, 3 and 5 min) on the *S. typhimurium*.

The results of this study showed clearly that the choice of disinfectant agent along with the optimum concentration and the action time is very important when destroying food pathogens in the food industry. Increasing the concentration of the disinfectants beyond the recommended user concentration may increase the bactericidial activity, but such concentrations may not be relevant from a practical point of view because of factors such as corrosion, solubility, and costs. It is also important to consider the resistance of microorganisms to different disinfectants, food production hygiene, and also public health. In this study, the antibacteral effect of selected disinfectants was also found effective at the lowest recommended concentration and time. However, using less than recommended concentrations of disfectants and insufficient exposure time could not eliminate all the food pathogens. These possible factors will be included in a working HACCP system for factories.

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