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IN VITRO ANTIMICROBIAL ACTIVITIES OF WIDELY USED CHEMICAL DISINFECTANTS AGAINST VARIOUS MICROORGANISMS

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SUMMARY

Activities of seven different disinfectants that are used commonly against bacteria, fungi and spores have been assessed by using the European Standard EN 1276, EN 1650 and EN 13704 methods, respectively. Disinfectants that contain sodium hypochloride, cationic surfactants and their combinations were found to be effective in five minutes against *Escherichia coli* ATCC 10799, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231 strains. Disinfectants that contain anionic and non-ionic surfactants and organic chloride compounds were found to be effective in 15 minutes against *E.coli* ATCC 10799 and *S.aureus* ATCC 6538, and in 30 minutes against *E.faecalis* ATCC 29212 and *C.albicans* ATCC 10231. Organic chloride compound and cationic surfactant - alcohol ethoxylate combination showed sporicidal activity against *Bacillus subtilis* ATCC 6633 spores in 15 minutes. We found the producer's recommended concentrations were sufficient to achieve an effective disinfection against vegetative bacteria and yeast, but not to bacterial spores.

Key words: disinfection, sodium hypochloride, surfactants, in vitro activity

ÖZET

Sık olarak kullanılan yedi farklı dezenfektanın bakteri, mantar ve sporlara karşı olan aktiviteleri sırasıyla Avrupa standardı EN 1276, EN 1650 ve EN

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13704 yöntemleri kullanılarak araştırılmıştır. Sodyum hipoklorit, katyonik yüzey aktif madde ve bunların kombinasyonlarını içeren dezenfektanlar beş dakikada *Escherichia coli* ATCC 10799, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212 ve *Candida albicans* ATCC 10231 suşlarına karşı etkili bulunmuştur. Anyonik ve non-iyonik yüzey aktif maddeler ve organik klor bileşikleri *E. coli* ATCC 10799 ve *S. aureus* ATCC 6538'e karşı 15, *E. faecalis* ATCC 29212 ve *C. albicans* ATCC 10231'e karşı ise 30 dakikada etkili olabilmişlerdir. *Bacillus subtilis* ATCC 6633'ün spor süspansiyonuna karşı organik klor bileşiği ve katyonik yüzey aktif madde – alkol etoksilat kombinasyonu 15 dakikada sporosidal aktivite göstermiştir. Sonuç olarak üretici tarafından önerilen konsantrasyonlarda vejetatif bakteriler ve mayalar için etkili dezenfeksiyon sağlanırken bu konsantrasyonda sporlara karşı genellikle etki sağlanamadığı belirlenmiştir.

Anahtar kelimeler: Dezenfeksiyon, Sodyum hipoklorit, Yüzey aktif maddeler, İn vitro aktivite

INTRODUCTION

Pathogen and potentially pathogenic microorganisms that can be found widely in nature are encountered very often during our daily lives. These microorganisms can cause serious infections followed by morbidity and mortality with patients whose immune system have been suppressed, who had cell or organ transplants, who have malignant diseases, finally children and old people. Among them *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Candida albicans* are considered as common human environment-originated pathogens (1-3). If bacterial contamination in the environment were important in the transmission of multi-resistant pathogen bacteria, reducing the microbial load in the environment as much as possible would be prudent. Although cleaning is essential to reduce environmental reservoirs of known pathogens, studies have demonstrated that antibiotic-resistant pathogens can persist on room surfaces even after the cleaning (4, 5). As a result, disinfection procedures must be done to keep the pathogen microorganisms away from common areas such as hospitals, drug and food industries, day care centers, schools etc.

Disinfectants are chemical substances that kill or inhibit the growth of microorganisms when they are applied on surfaces, but not directly to tissues

due to their high toxicity. Sodium hypochloride, one of these disinfectants, is an effective antimicrobial agent against most of the microorganisms and is widely used in home, industry and hospital disinfection. Hypochlorous acid molecules, the active metabolite of sodium hypochloride, due to their neutral charge and small size, easily diffuse through the cell walls of bacteria, change the oxidation-reduction potential of the cell, and effectively destroy the microorganisms' functions (6, 7).

Surfactants are surface-active agents with wide range of properties including the lowering of surface and interfacial tensions of liquids. Surfactants are classified according to the ionic charge of the molecule: anionic, cationic and non-ionic surfactants. Anionic surfactants, such as soap, often have a sodium, potassium, or ammonium group; Cationic surfactants also known as quaternary ammonium compounds, have strong bactericidal properties, often have an ammonium group attached to a halogen; Non-ionic surfactants, such as polyethylene glycol esters (PEG), are used as mild cleansers, or used for adding viscosity to a mixture like shampoo. Surfactants generally cause damage to the cell membrane which leads to loss of function and cell death (8, 9).

In this study, we investigated the activities of seven different disinfectants that are widely used in the home and hospital, against *E. coli*, *S. aureus*, *E. faecalis*, *C. albicans* and *Bacillus subtilis* spore suspension.

EXPERIMENTAL

Microorganisms: The American Type Culture Collection (ATCC) standard strains of *E. coli* ATCC 10799, *S. aureus* ATCC 6538, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633 (spore suspension) and as a representative of fungi, the yeast, *C. albicans* ATCC 10231 were used in the experiments. Inoculums of bacteria and *C. albicans* were prepared with overnight cultures for producing a concentration of $1x10^8$ colony-forming units (cfu/ml) and 1×10^7 cfu/ml, respectively. *B. subtilis* spore suspension was diluted with 0.9 % sterile saline solution from 1×10^{10} stock suspension in order to produce a concentration of $1x10^8$ cfu/ml.

Disinfectants: Sodium hypochloride, sodium hypochloride + non-ionic surfactant, organic chloride compound, cationic surfactant, cationic surfactant

+ alcohol ethoxylate, anionic + non-ionic surfactant and non-ionic surfactant + citric acid were used in this study. These disinfectants were provided by their manufacturers or commercially, and their solutions were prepared on the day of use.

Media: Tryptic soy agar (TSA) and Sabouraud dextrose agar (SDA) (Difco Laboratories, Detroit, Michigan, USA) were used for growth and colony countings for bacteria or *B. subtilis* spore suspension and *C. albicans*, respectively.

Reagents: A solution of physiological sodium chloride with 10 % tyrptone was used for preparation of inoculums. A solution of neutralizer containing lecithin, polysorbate 80, sodium thiosulfate and L-histidine was used in order to inactivate the antimicrobial substances within the test mixtures. A solution of 10 % skim milk was used as an interfering substance to simulate dirty conditions. A hard water solution, containing $MgCl_2$, $CaCl_2$ and $NaHCO_3$ was used for diluting the disinfectants.

Validations: In the course of the study, the effects of interfering substance (skim milk), the activities and the toxic side effects of neutralizer on the experiments were controlled according to the European Standard methods (10-12).

Determination of antimicrobial activity: Bactericidal, fungicidal and sporicidal activities of disinfectants were investigated according to the European Standard EN 1276, 1650 and 13704 methods, respectively (10-12). The solutions of different disinfectants at concentrations of 0.5, 1 and 1.5 times of the producers' recommended concentrations were incubated with the suspensions of the final $1x10^7$ cfu/ml microorganisms and skim milk at room temperature for 5, 15 and 30 minutes. After neutralizing the solutions, the surviving microorganisms were determined using the pour plate viable colony counting technique. Biocidal activity was defined as a $\geq$3-$\log_{10}$ cfu/ml (99.9 %) decrease from the initial inoculum. The reduction in viability (R) has been calculated according to the Formula 1. If Na is equal to or smaller than 15, R is accepted $>10^5$; Na is equal to or greater than 300, R is accepted $< 10^3$ for bacteria and spore suspensions; for fungi if Na is equal to or smaller than 15, R is accepted $>10^4$; Na is equal to or greater than 150, R is accepted $< 10^4$. All experiments were performed in dublicate.

Formula 1: $R = N \times 10^{-1} / Na$

N: number of cfu/ml in the inoculums
Na: number of surviving cfu/ml after the test procedure.

RESULTS AND DISCUSSION

The efficacy of disinfectants is verified on the basis of the results of test methods and on the conclusions drawn from these. When efficacy testing of disinfectants was first introduced about 60 years ago, the test was done in an exclusively practice-oriented manner. However, these relatively inexact approaches made important contributions to the development of disinfectants, and hence to the prevention of microbial transmission. At this time, the quantitative suspension test was devised after a myriad of experimental studies. The aim of them was to analyze the various influence factors, and the reduction factors with logarithmic units (13). The European quantitative suspension tests used in this study are test procedures that will yield a reproducible and reliable results independent of the test location.

According to European Standard methods, the in vitro activities of seven disinfectants containing chloride, cationic surfactants, anionic and nonionic surfactants against microorganisms are shown in table 1, table 2 and table 3, respectively. The reduction in viability of disinfectants, when they are used at the producer's recommended concentrations, are shown in figure 1.

According to these findings, disinfectants containing sodium hypochloride alone or their combination have good activity against *E. coli* ATCC 10799, *S. aureus* ATCC 6538, *E. faecalis* ATCC 29212 and *C. albicans* ATCC 10231 strains in five minutes when they were used in accordance with the producer's recommended concentrations. Similarly, Radcliffe et al. (14) and Gomes et al. (15) have shown that sodium hypochloride has a rapid bactericidal activity against *C. albicans* and *E. faecalis* strains. The addition of non-ionic surfactant to sodium hypochloride markedly increases sodium hypochloride's antimicrobial activity. Although, sodium hypochloride and its non-ionic surfactant combination have excellent activity against vegetative microorganisms, they are ineffective against spore forms of bacteria.

In this study, organic chloride compound was active against *E. coli* ATCC 10799 and *S. aureus* ATCC 6538 in 15 minutes; *E. faecalis* ATCC 29212, *C. albicans* ATCC 10231 and *B. subtilis* ATCC 6633 spores in 30 minutes

by using the producer's recommended concentrations. Among the chloride containing disinfectants, organic chloride compounds have a weaker activity against all of the vegetative bacteria, but interestingly, they have a sporocidal effect while sodium hypochloride does not. Similarly Fayyad and Al-Sheikh (16) demonstrated that organic N-chloramines have significantly lower disinfectant efficiency than free chlorine for inactivating bacteria.

Among the disinfectants containing surfactants, cationic surfactants and their combinations with alcohol ethoxylate were very effective against *E. coli* ATCC 10799, *S. aureus* ATCC 6538, *E. faecalis* ATCC 29212 and *C. albicans* ATCC 10231 strains, and these activities were comparable with those of sodium hypochloride. Callahan et al. (17) demonstrated that quaternary ammonium compound based disinfectants have a ≥4 log10 reduction against methicillin resistant *S. aureus* and vancimisin resistant *Enterococci* on most hard and soft surfaces tested. In addition, cationic surfactant and alcohol ethoxylate combination has sporocidal activity against *B. subtilis* ATCC 6633 spores in 15 minutes using the producer's recommended concentrations.

Anionic and non-ionic surfactants have weaker activities than cationic ones. In this study, these surfactants were active against *E. coli* ATCC 10799 and *S. aureus* ATCC 6538 in 15 minutes using the producer's recommended concentrations, *E. faecalis* ATCC 29212 in 30 minutes with 1.5 times of producer's recommended concentrations and ineffective against *C. albicans* ATCC 10231 or *B. subtilis* ATCC 6633 spores. Although this agent has weak activity against microorganisms, non-ionic surfactants and citric acid combination was active against *E. coli* ATCC 10799, *S. aureus* ATCC 6538, *E. faecalis* ATCC 29212 in five minutes, *C. albicans* ATCC 10231 in 30 minutes using the producer's recommended concentrations but ineffective against *B. subtilis* ATCC 6633 spores.

When it's necessary to precise disinfection, the reduction in viability of disinfectants should be $\geq 10^3$ (18). In this study, we found that the most of the reduction in viability values of disinfectants by using the producers recommended concentrations, were sufficient to achieve an effective disinfection against vegetative bacteria and yeast, but not to bacterial spores.

In the study, we have found that disinfectants containing sodium hypochloride and cationic surface-active compounds were especially effective against all vegetative microorganisms. In addition, it is very

important to follow the producer's recommended instructions for disinfectant concentrations and the time of contact to achieve successful disinfection. In this respect, producer's recommendations should be considered while selecting disinfectants to eradicate bacteria from the environment.

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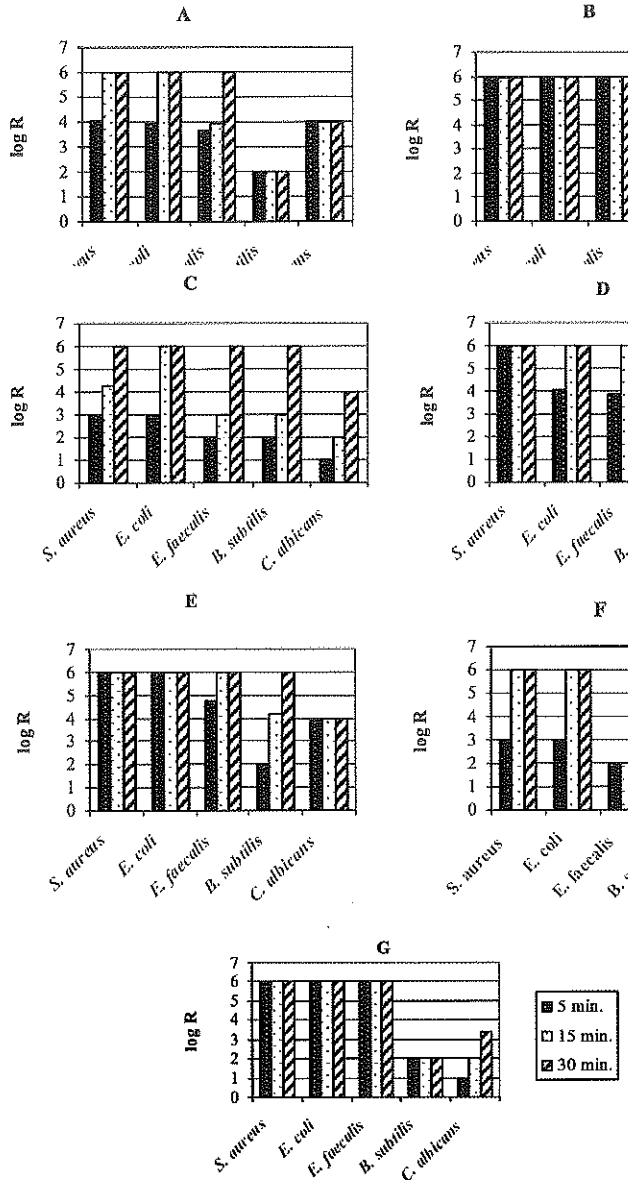

FIGURE 1. The reduction in viability of disinfectants using the producers recommended concentrations; A: sodium hypochloride, B: sodium hypochloride + non-ionic surfactant, C: organic chloride compound, D: cationic surfactant, E: cationic surfactant + alcohol ethoxylate, F: anionic + non-ionic surfactants, G: non-ionic surfactant + citric acid.

Table 1. Mean colony numbers of viable microorganisms after incubation with disinfectants containing chloride.

		Sodium hypochloride			Sodium hypochloride + non-ionic surfactant			Organic chloride compound		
Microorganism	T	0.5 x K	1 x K	1.5 x K	0.5 x K	1 x K	1.5 x K	0.5 x K	1 x K	1.5 x K
S. aureus	5'	>300	175	49	3	0	0	>300	>300	>300
ATCC 6538	15'	17	10	0	0	0	0	>300	97	9
N: 1.9 x 10^8	30'	0	0	0	0	0	0	14	0	0
E. coli	5'	182	99	17	0	0	0	>300	>300	>300
ATCC 10799	15'	6	0	0	0	0	0	>300	5	2
N: 9 x 10^7	30'	0	0	0	0	0	0	3	0	0
E. faecalis	5'	>300	233	158	0	0	0	>300	>300	>300
ATCC 29212	15'	195	124	116	0	0	0	>300	>300	47
N : 1.1 x 10^8	30'	0	0	0	0	0	0	>300	12	0
B. subtilis (spore)	5'	>300	>300	>300	>300	>300	>300	>300	>300	250
ATCC 6633	15'	>300	>300	>300	>300	>300	>300	>300	>300	103
N: 2.3 x 10^8	30'	>300	>300	>300	>300	>300	>300	>300	1	0
C. albicans	5'	4	2	0	0	0	0	>300	>300	>300
ATCC 10231	15'	0	0	0	0	0	0	>300	>300	124
N: 5.4 x 10^6	30'	0	0	0	0	0	0	250	8	4

T: Time of contact, K: Producer's recommended concentration, N: number of cfu/ml in the inoculum.

Table 2. Mean colony numbers of viable microorganisms after incubation with disinfectants containing cationic surfactants.

		Cationic surfactant			Cationic surfactant + alcohol ethoxylate		
Microorganisms	T	0.5 x K	1 x K	1.5 x K	0.5 x K	1 x K	1.5 x K
S. aureus	5'	116	1	0	17	10	0
ATCC 6538	15'	18	0	0	2	0	0
N: 1.9×10^8	30'	7	0	0	0	0	0
E. coli	5'	>300	76	1	>300	0	0
ATCC 10799	15'	>300	8	0	>300	0	0
N: 9×10^7	30'	68	0	0	197	0	0
E. faecalis	5'	>300	149	26	95	20	0
ATCC 29212	15'	48	2	0	7	0	0
N : 1.1×10^8	30'	0	0	0	2	0	0
B. subtilis (spore)	5'	>300	>300	>300	>300	>300	250
ATCC 6633	15'	>300	>300	>300	>300	138	103
N: 2.3×10^8	30'	>300	>300	>300	>300	10	0
C. albicans	5'	300	130	26	1	0	0
ATCC 10231	15'	3	0	0	1	0	0
N: 5.4×10^6	30'	0	0	0	0	0	0

T: Time of contact, K: Producer's recommended concentration, N: number of cfu/ml in the inoculum.

Table 3. Mean colony numbers of viable microorganisms after incubation with disinfectants containing anionic and nonionic surfactants.

		Anionic + non-ionic surfactants			Non-ionic surfactant + citric acid		
Microorganisms	T	0.5 x K	1 x K	1.5 x K	0.5 x K	1 x K	1.5 x K
S. aureus	5'	>300	>300	43	4	0	0
ATCC 6538	15'	>300	3	0	2	0	0
N: 1.9×10^8	30'	87	2	0	0	0	0
E. coli	5'	>300	>300	104	>300	2	0
ATCC 10799	15'	156	11	0	63	0	0
N: 9×10^7	30'	5	0	0	0	0	0
E. faecalis	5'	>300	>300	>300	55	1	0
ATCC 29212	15'	>300	>300	34	4	0	0
N : 1.1×10^8	30'	>300	>300	27	1	0	0
B. subtilis (spore)	5'	>300	>300	>300	>300	>300	>300
ATCC 6633	15'	>300	>300	>300	>300	>300	>300
N: 2.3×10^8	30'	>300	>300	>300	>300	>300	>300
C. albicans	5'	>300	>300	>300	>300	>300	>300
ATCC 10231	15'	>300	>300	>300	>300	>300	52
N: 5.4×10^6	30'	>300	>300	>300	>300	26	2

T: Time of contact, K: Producer's recommended concentration, N: number of cfu/ml in the inoculum.