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Determination and comparison of the antimicrobial efficacy of alcohol based hand hygiene products

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Abstract: Hand hygiene is a general definition for the act of "hand cleaning" which is important for the control and prevention of infectious diseases in society. Hand hygiene is intended to reduce the number of microorganisms on people's hands to a non-infectious level. In this study, we assessed the antimicrobial activities of 9 alcohol-based hand hygiene products that are produced in Turkey.

The antimicrobial activities of 3 each hand rubs, hand washes, and hand wipes were tested against to *Staphylococcus aureus* ATCC6538, *Escherichia coli* ATCC10538, *Pseudomonas aeruginosa* ATCC15442, *Enterococcus hirae* ATCC10541 and *Candida albicans* ATCC10231 according to the European Standard methods (pr)E12054 and EN1275.

70% ethanol+0.3% triclosan and benzyl alcohol+methyl isothiazolinone were the 2 most effective agents within 1 min of treatment, followed by 70% ethanol+0.1% triclosan+benzalkonium chloride and 70% ethanol+0.1% triclosan against *Staphylococcus aureus* ATCC6538, *Escherichia coli* ATCC10538, *Pseudomonas aeruginosa* ATCC15442, *Enterococcus hirae* ATCC10541 and *Candida albicans* ATCC10231.

According to these results, it was observed that the antimicrobial activity of the studied products was dependent on the composition of products, duration of exposure and the type of product.

Keywords: Alcohol, hand hygiene, hand gel, hand wash, hand wipe, in vitro antimicrobial activity

Introduction

Hygienic hands are considered as one of the primary measures to reduce the transmission risks of infections. Semmelweis was the first

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scientist who mentioned and demonstrated the role of hand hygiene in the prevention of transmission of infectious agents (Best at al. 2004). Further, several studies have been performed and they show that hand washing reduces the transmission of microorganisms, from the hands of hospital personnel, which are the source of transmission, to patients through direct physical contact (Mortimer at al., 1966; Mortimer et al. 1965). Thus, several hand hygiene products have been widely used for inhibiting the growth or killing of the microorganisms. In addition, a meta-analysis that was performed to assess the effect of hand hygiene on the risk of infectious diseases in community settings confirmed that hand hygiene interventions prevent gastrointestinal diseases in developed and developing countries (Aiello et al., 2008; Alp et al., 2011). Guidelines for hand hygiene were first published by the Centers for Disease Control and Prevention (CDC) in 1981. Since then, some innovations have been made in hand hygiene products (Loveday et al., 2014; Simmons BP 1981).

One of the high-priority goals of the World Health Organization (WHO) is minimizing nosocomial infections by means of improvement of hand hygiene. Hand hygiene is the most effective and least expensive method for preventing transmission of pathogenic organisms not only in health-care settings but also in pharmaceutical factories, food industry, restaurants, day care centers and schools, and at home. Hand hygiene is a general term that refers to any action of hand cleansing and applies to hand washing by an antiseptic hand wash, an antiseptic hand rub, or surgical hand antisepsis. Antiseptic hand hygiene products are intended to be used with or without water for post-contamination treatment of hands (Boyce, 2013; Kampf, 2003; Mathai et al., 2010; WHO, 2009).

Alcohols are the preferred agents for waterless hand antisepsis, with their good antimicrobial activity and rapid bactericidal action. Such antiseptic formulations may contain one or more types of alcohol and other active ingredients with excipients and humectants. However the hand washing with plain soap was advised for general patient care and removing visible soil, after the directive of the 2002 CDC Guideline for Hand Hygiene in Health-Care Settings, alcohol-based hand rubs became the preferred agent for hand hygiene in situations when hands are not visibly soiled. However, CDC guidelines for hand hygiene state that alcohol-based hand wipes are not as effective as alcohol-based hand rubs, but the former may be

considered as an alternative to wash hands with non-antimicrobial soap and water (Bolon, 2011; Butz et al., 1990; CDC, 2002; Kampf & Kramer, 2004).

In recent years, several commercial alcohol-based hand hygiene products, hand rubs, hand wash products, and hand wipes have been available in the market to improve the compliance with the hand-cleansing guidelines of health authorities in our country. Although the antimicrobial efficacy of these products has been reported in different countries, in Turkey, the studies about antimicrobial profiles of hand hygiene products are very limited. In this study, we assessed the antimicrobial activity of 9 alcohol-based hand hygiene products that are produced in Turkey, against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10538, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541, and *Candida albicans* ATCC 10231, according to the European Standard methods. These 5 pathogenic and opportunistic microorganisms are widely found in water, air, soil, skin, and mucosal membranes or faces of humans and animals are the important causes of hospital- or community-acquired infections (Rosenthal et.al., 2012).

Materials and methods

Microorganisms: The American Type Culture Collection (ATCC) standard strains of *S. aureus* ATCC 6538, *E. coli* ATCC 10538, *P. aeruginosa* ATCC 15442, *E. hirae* ATCC 10541, and as a representative of fungi, the yeast *C. albicans* ATCC 10231 were used in the experiments. Inoculum of bacteria and *C. albicans* were prepared using overnight cultures to attain a concentration of 1×10^8 and 1×10^7 cfu/ml respectively.

Hand hygiene products: Three alcohol-based gels (A–C), 3 hand wash products (D–F), and 3 alcohol-based hand wipes (G–I) were tested. These products, whose composition was listed below, were either provided by their respective manufacturers or purchased commercially. The antimicrobial activity of hand gels and hand wash products were tested both directly and at 50% concentrations, whereas hand wipe solutions were tested directly.

A: 70% w/w ethyl alcohol + 0.1% triclosan

B: 70% w/w ethyl alcohol + 1% triethanolamine

C: 70% w/w ethyl alcohol + 0.3% triclosan

D: 70% w/w ethyl alcohol + 0.1% triclosan

E: 70% w/w ethyl alcohol + colloidal silver

F: 70% w/w ethyl alcohol + 0.3% triclosan

G: 70% w/w ethyl alcohol + 0.1% triclosan + benzalkonium chloride

H: 70% w/w ethyl alcohol + colloidal silver

I: Benzyl alcohol + methyl-isothiazolinone (the concentration was not specified on the tag)

Media: Mueller-Hinton broth (MHB) and tryptic soy agar (TSA; Difco Laboratories, Detroit, Mich., USA) were used for the preparation of the bacterial inoculums and colony counts, respectively; the RPMI 1640 medium (Sigma-Aldrich, Milan, Italy) and sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich., USA) were used for the preparation of *C. albicans* inoculum and for colony counts, respectively.

Reagents: A neutralizer solution containing lecithin 3 g/l, polysorbate-80 30 g/l, sodium thiosulfate 5 g/l, and L-histidine 1 g/l was used in order to inactivate the antimicrobial substances within the test mixtures. A solution of bovine serum albumin and reconstructed milk were diluted to achieve a final concentration of 3 g/l and 0.1%, respectively, and they were used as an interfering substance for mimicking dirty conditions. Hard water that was prepared according to European standards directions was used as a diluent.

Determination of antimicrobial activity: Bactericidal and fungicidal activity of the products were assessed according to the European Standard (pr)EN 12054 and 1275 methods, respectively (EN, 1995; EN, 2005). The solutions of products were incubated at 20°C with interfering substances and the suspensions of the bacteria or fungi at the final concentration of 1×10^7 and 1×10^6 cfu/ml, respectively, for 0.5, 1, and 5 min. After neutralization of the solutions, the surviving microorganisms were quantified using the pour plate viable colony counting technique. Biocidal activity was defined as a ≥ 5 $\log_{10}$ reduction after 1 min for hand rubs and hand wipes, and a ≥ 3 $\log_{10}$ reduction for the hand-washing products. The reduction in viability (R) was calculated according to the following formula (all experiments

were performed in duplicate assays).

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

N: number of total cfu/ml in the inoculum; Na: number of surviving cfu/ml after the test procedure

Validation procedures: In the course of the study, the effects of an interfering substance and the activity and toxic side effects of the neutralizer were evaluated according to European Standard methods to validate the test results.

Results

The antimicrobial efficacy of 9 alcohol-based hand hygiene products against several microorganisms were determined according to the European Standard methods, and the observations were summarized in Tables 1, 2, and 3. The reduction in viability of products was shown in Figure 1. According to these results, within 1 min, product C (70% ethanol + 0.3% triclosan) and I (benzyl alcohol + methyl isothiazolinone) were the most effective agents followed by products A (70% ethanol + 0.1% triclosan + benzalkonium chloride) and G (70% ethanol + 0.1% triclosan). Validation of the effects of the interfering substance, and activities or toxic adverse effects of the neutralizer was within the margin of error, according to the EN standards.

Discussion

With the changes in social conditions, the time spent outdoors has increased, leading people to search for more effective and convenient products instead of the basic water and soaps for cleaning. Because of their simple, easy, and rapid effects, these new hand hygiene products are extensively used at home, social events, schools, day care centers, hospitals, and in other settings. In this study, we analyzed the in vitro activity of 9 hand hygiene products against 5 pathogenic and opportunistic microorganisms. According to our results, antimicrobial activity of these products depends on the composition of the products. The most active hand hygiene products against all studied microorganisms contain triclosan (especially 0.3%) in addition to ethanol (C and F), and benzyl alcohol +

methyl-isothiazolinone (hand wipe; I). In terms of efficacy, these products are followed by triethylamine- and colloidal silver-containing ones. Similar results were reported by other researchers (Girou et al., 2002; Messina et.al., 2008; Pietsch, 2001).

When considering the effects of contact time, no differences were observed between the antimicrobial activities of products containing triclosan or benzyl alcohol. The less active agents such as triethylamine-containing product B were not active within 30 seconds against *E. coli* and *S. aureus* but were active within 1 min; 70% ethanol + colloidal silver products E and H were not active against *P. aeruginosa* within 1 min but were active within 5 min. These results suggest that the influence of contact time on the antimicrobial activity of hand hygiene products is limited and depends on the effectiveness of the product.

Among the triclosan-containing products, those containing 0.3% triclosan were more effective compared to 0.1% triclosan, even when they were tested at 50% concentrations. Among the 0.1% triclosan-containing products, the one containing benzalkonium chloride (G) was the most effective, as expected. Even though they had similar composition (0.1% triclosan + 70% ethanol), the hand gel (A) was more effective than the hand wash (D). Likewise, the hand gel (H) was more effective than the hand wash product E, although they had the same composition (70% ethanol + silver). These results suggested that the in vitro antimicrobial activity of hand hygiene products depends not only on the composition or contact time but also on the product type. It is possible that physical properties of the products such as pH, viscosity, and frothiness, which are determined by the inactive ingredients, have a negative influence on their antimicrobial activity.

Because alcohols have strong antimicrobial properties and rapid action, they are preferred agents for waterless hand antisepsis (Widmer, 2000). The CDC (2002) recommendation to use alcohol in lieu of traditional hand washing warrants their widespread application. Larson *et al*. (2005) found no significant differences among neonates in terms of health care–associated infections when they compared the effects of antiseptic hand washing with alcohol sanitizers on health care–associated infections in neonatal intensive care units in a 2-year study. According to another

study, it was determined that a liquid soap containing 10% PVP-I and 70% ethyl alcohol is the most effective hand-cleaning agent for removal of methicillin-resistant *S. aureus* from both slightly and heavily contaminated hands (Guilhermetti et.al., 2001). Similarly, there are many studies that have reported various results with different test methods regarding the antimicrobial activity of hand hygiene products. Particularly, in vivo and in vitro methods may yield dissimilar results; for example, a product with an in vitro antimicrobial activity could be ineffective in vivo or vice versa (Rotter et.al., 2009). In the present study, when considering the EN criteria ($\geq$5 $\log_{10}$ reduction within 1 min for a hand rub or hand wipes and $\geq$3 $\log_{10}$ reduction for hand-washing products), there were no significant effects against all of the studied microorganisms without the addition of badly tolerated components such as triclosan or benzyl alcohol in the formulations.

Triclosan, i.e., 5-chloro-2-(2,4-dichlorophenoxy)-phenol, which was present in the 5 studied products, prevents microorganisms from synthesizing fatty acids, one of the important constituents of the cell membrane (Ramos et al., 2009). Several studies have pointed out that triclosan or similar products might promote the emergence of bacterial resistance or tolerance to antibiotics and development of multi-drug resistant strains (Davin-Regli et al., 2012; Hermandez, A; Yazdankhah et al., 2006). Although triclosan is mentioned as a non-toxic antimicrobial agent for humans, there have been several reports of contact dermatitis, skin irritation, blocking the metabolism of thyroid hormones, accumulation in adipose tissues, and presence in human milk (Allmyr et al., Bhutani & Jacob, 2009; Heath et al., 2001; Koeppe, 2013)

Because it prevents transmission of pathogens via contact or the fecal-oral route, hand hygiene is the most important factor in the prevention of infectious diseases. The CDC has recommended hand washing as the gold standard of hand hygiene for decades and states that “plain soap should be used for hand washing unless otherwise indicated” (WHO, 2012). Antimicrobial hand rubs or hand-washing products are type of drugs like any other currently known anti-infective agents available in the market. They are necessary for health care workers, immunocompromised patients, pregnant or nursing women, and older people as long as these products are used appropriately.

Table 1: Numbers and reduction in viability of surviving microorganisms after incubation with alcohol-based hand gels

Microorganisms	T	A				B				C			
		direct		50%		direct		50%		direct		50%	
		Na	R	Nb	R	Na	R	Nb	R	Na	R	Nb	R
S. aureus ATCC 6538 N: 5.10^7	30''	1	$> 10^5$	26	$> 10^5$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1'	0	$> 10^5$	0	$> 10^5$	71	$7,1.10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5'	0	$> 10^5$	0	$> 10^5$	20	$4,1.10^4$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
E. coli ATCC 10538 N: $1,1.10^8$	30''	0	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1'	0	$> 10^5$	0	$> 10^5$	92	$1,1.10^4$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5'	0	$> 10^5$	0	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
P. aeruginosa ATCC 15442 N: $1,1.10^8$	30''	9	$> 10^5$	20	$> 10^5$	132	…….	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1'	3	$> 10^5$	0	$> 10^5$	24	$4,5.10^4$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5'	0	$> 10^5$	0	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
E. hirae ATCC 10541 N: $7,9.10^7$	30''	10	$> 10^5$	65	…….	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1'	6	$> 10^5$	8	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5'	0	$> 10^5$	0	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
C. albicans ATCC 10231 N: $4,3.10^7$	30''	3	$> 10^5$	0	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1'	0	$> 10^5$	0	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5'	0	$> 10^5$	0	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$

T: time of contact, N: cfu/mL in the inocula, Na: mean colony numbers of surviving microorganisms after treatment with undiluted products, Nb: mean colony numbers of surviving microorganisms after treatment with 50% diluted products R: reduction in viability, A: 70% ethanol + 0.1% triclosan, B: 70% ethanol+1% triethylamine, and C: 70% ethanol + triclosan.

Table 2: Numbers and reduction in viability of surviving microorganisms after incubation with alcohol-based hand washes

		D				E				F			
		direct		50%		direct		50%		direct		50%	
Microorganisms	T	Na	R	Nb	R	Na	R	Nb R		Na	R	Nb	R
S. aureus ATCC 6538 N: 5.10^7	30”	0	$> 10^5$	17	$> 10^5$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1’	0	$> 10^5$	3	$> 10^5$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5’	0	$> 10^5$	2	$> 10^5$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
E. coli ATCC 10538 N: $1,1.10^8$	30”	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1’	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5’	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
P.aeruginosa ATCC 15442 N: $1,1.10^8$	30”	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1’	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5’	>300	$< 10^3$	>300	$< 10^3$	115	……	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
E. hirae ATCC 10541 N: $7,9.10^7$	30”	3	$> 10^5$	19	$> 10^5$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1’	0	$> 10^5$	8	$> 10^5$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5’	0	$> 10^5$	0	$> 10^5$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
C. albicans ATCC 10231 N: $4,3.10^7$	30”	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	1’	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$
	5’	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	>300	$< 10^3$	0	$> 10^5$	0	$> 10^5$

T: time of contact, N: cfu/ml in the inocula, Na: mean colony numbers of surviving microorganisms after treatment with undiluted products, Nb: mean colony numbers of surviving microorganisms after treatment with 50% diluted products, R:reduction in viability, D:70% ethanol+0.1% trichlosan, E:70% ethanol+colloidal silver, F:70% ethanol+0.3% triclosan

Table 3: Numbers and reduction in viability of surviving microorganisms after incubation with alcohol-based hand wipes

Microorganisms	T	G		H		I	
		Na	R	Na	R	Na	R
S. aureus ATCC 6538 N: 5.10^7	30''	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
	1'	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
	5'	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
E. coli ATCC 10538 N: $1,1.10^8$	30''	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
	1'	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
	5'	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
P.aeruginosa ATCC 15442 N: $1,1.10^8$	30''	3	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
	1'	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
	5'	3	$> 10^5$	146	$7,5.10^3$	0	$> 10^5$
E. hirae ATCC 10541 N: $7,9.10^7$	30''	10	$> 10^5$	0	$> 10^5$	0	$> 10^5$
	1'	3	$> 10^5$	0	$> 10^5$	0	$> 10^5$
	5'	0	$> 10^5$	0	$> 10^5$	0	$> 10^5$
C. albicans ATCC 10231 N: $4,3.10^7$	30''	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
	1'	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$
	5'	0	$> 10^5$	>300	$< 10^3$	0	$> 10^5$

T: time of contact, N: cfu/ml in the inocula, Na: mean colony numbers of surviving microorganisms after treatment with products, R: reduction in viability,
G: 70% ethanol + 0.1% triclosan+benzalkonium chloride, H: 70% ethanol + colloidal silver, I: benzylalcohol + methylisothiazoli

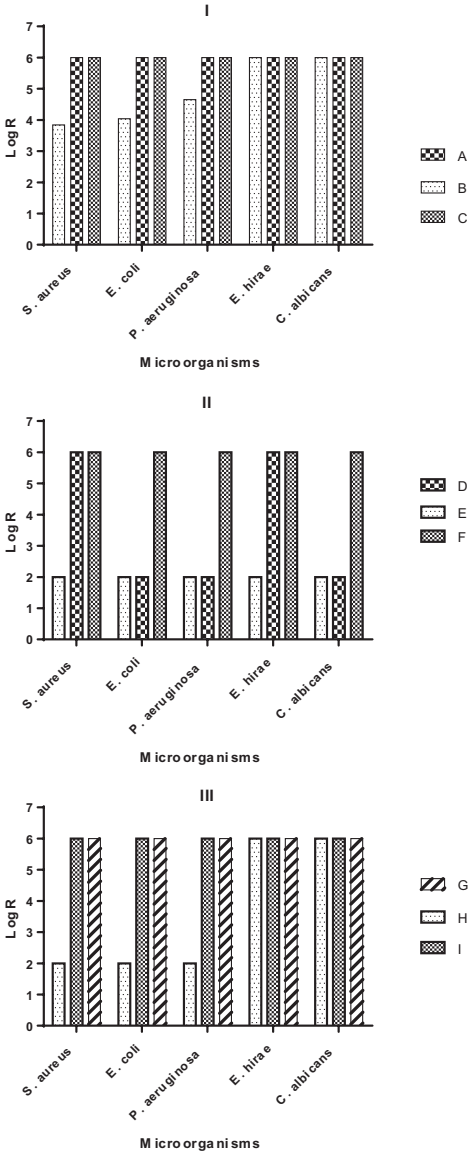

Figure 1: Reduction in viability of microorganisms after 1 min treatment with I: hand gels, II: hand wash products, or III: hand wipes. A: 70% ethanol + 0.1% triclosan, B: 70% ethanol + 1% triethanolamine, C: 70% ethanol + 0.3% triclosan, D: 70% ethanol + 0.1% triclosan, E: 70% ethanol + colloidal silver, F: 70% ethanol + 0.3% triclosan, G: 70% ethanol + 0.1% triclosan + benzalkonium chloride, H: 70% ethanol + colloidal silver, and I: benzyl alcohol + methyl isothiazolinone

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