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In vitro Effectiveness of 2-Propanol Based Hand and Skin Disinfectants Against Various Microorganisms

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In this study, in order to define how effective alcohol based hand and skin disinfectant against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 23853), *Candida albicans* (ATCC 10231) strains are reported. To test the final microorganisms at different concentrations of the disinfectant to be tested by being prepared as 2-5 × 10^9 CFU/mL according to the McFarland 5 cloudiness of test disinfectant. After disinfectants were activated with microorganisms at previously experimented 30 s, 1, 5, 15 and 30 min periods, colony counts at 1 mL. Levels were performed by way of cast-cultural plaque method. Consequently, it was determined that 100 % concentration of alcohol based skin and hand disinfectant was effective against *Staphylococcus aureus* in 1 min, while other test microorganisms were effective in 30 s.

Key Words: Alcohol, Disinfectant, Microorganism.

INTRODUCTION

At the present days hospital infections, which are getting important have been among the most important problems in modern medicine. Hospital infection can increase in the 5 and 15 % that sick who lied in the hospital. With the protective measures 30 % infection can be prevent. Hand hygiene and antisepsis of hospital workers the checking is an important factor to prevent spread of hospital infections. Firstly, hand-washing bond are found after epidemiological studies and emphasized how much important on it by Dr. Ignaz Philipp Semmelweis in 1847. It started to neglect by using gloves commonly, which is one of the universal protection method to against human immune deficiency sendrome (HIV) and hepatitis B (HBV) pathogens especially that spread by blood; disinfectant in 1960s and also antibiotics in 1940s[1,2]. In the hospital high virulence and multiple resistant microorganism diffusion among the patients' the most important reason is dirty hands[3].

Biologically, skin that has slough and fresh one is the most important weapon for body defending too. In the skin there are two different groups of bacteria. One of them is permanent flora, the other one is temporary flora. Permanent density of flora bacteria is between 102-103 colony forming unit (cfu)/ cm² in the skin. Generally; it includes gram positive bacteria like coagulase-negative *Staphylococcus*, *Corynebacteriums*, *Micrococcus* and *Streptococcus*. Bacteria level of temporary flora is changeable between 104-107 cfu/mL. Secretion appreciate with the patient; usually microorganism which stay top level of the skin and and contagion to medical personnel by contaminated tools and devices. These kinds of microorganisms can not live in the skin for a long time and they can not accumulate. However they keep their activity to contaminate from patients to patients. Microorganism which cause hospital infections are in this group[4,5].

Hand-washing bond is a community health behaviour pattern. Therefore; we can divided into 4 groups for hand-washing bond like basic social type, hygienic type, hygienic hand disinfection type and type of surgical hand washing. If it applies correctly, it can reduce 80-90 % by using hygienic hand washing, by using surgical type can reach 10^2 cfu/mL [2,6,7]. Hand washing necessity is common especially high risk ambience. Because, patients in there colonize and infective by multiple resistant microorganism and virulence. Patients are getting sensitive to infections depend on invasive applications, injury and reduce on immune functions. Although there are not certain time of hand-washing; in some studies, it is reported that 10-15 s hand-washing is enough time to take away temporary flora. If hands seem to dirty, there will more

time to wash. Ideally features of antiseptic solution should be fast and long term effective, do not be irritant or minimal level of irritancy to keep its stability long, cheap and usability. For this aim there are different types of antiseptics such as emollient and humidifier soap and alcohols (70 % izopropanol, 60 % *n*-propanol or 70 % lik ethanol), chlorhexidine (2-4 %), iodine compounds or alcohol based iodine (1 %), iodoforms, *m*-xylene, hexachlorophene (3 %) and octadene dihydrochloride (0.1 %)[6-8].

One key point about the hand hygiene is using glove. One of the important points about using glove is after the take off glove, hand washing will be need. However important problem is medical workers use gloves to protect themselves and by these gloves cause to carrying microorganisms to other patients and areas[8]. In the intensive care studies show that *Klebsielle* contamined to hospital staff such as unimportant work even after touch a patients hand or taking one's blood pressure[9]. Alcohol has been using since antiquity of history. However a scientific use started at the end of 1800s. Various studies proved a solution that has 50-70 % alcohol was so effective to kill the bacterias and inhibited them[10]. Especially; disinfectants with alcohol based are suggested for the intensive care units. Alcohol and alcohol based antiseptics are really active on viruses and bacteria which have permanent and temporary floras in a short time[11].

The aim of this study is effectiveness to search of alcohol based hand and skin disinfectants which are use commonly in the hospitals to different microorganisms in nosocomical hospital infections.

EXPERIMENTAL

The hand and skin disinfectant of A company (63.14 g 2-propanol, 0.12 g 1,3 butandiol 1.00 g lanolin 1.00 g parfüme combination) that is used in this study is obtained from medical stores.

Test microorganisms used in experiment: Test microorganisms that are used in this study such as *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 23853), *Candida albicans* ATCC (10231) strains are obtained from culture collection of our laboratory. When counting colonies of these microorganisms, for *Staphylococcus aureus*, the *Staphylococcus* medium 110 (Oxoid), for *Escherichia coli*, violet red bile agar (oxoid), for *Pseudomonas aeruginosa*, *Pseudomonas* selective medium (oxoid) and for *Candida albicans*, Sabouraud-dextrose agar (oxoid) are used. According to the McFarland 5 blurriness, the final concentration of each strain which are used in this trial will be 2-5X109 CFU/mL and they are prepared correspondent with this concentration[11].

Preparation of neutralizateur used in trial: After the activation of microorganisms of test with disinfectants, for inactivate them, 3 % Tween 80 + 3 % saponin + 0,1 % histidine + 0,1 % sistein combination is used as neutralizer in the study[12-14].

Determination of disinfectants effects: The commercial form of disinfectant with 70 % concentration is accepted 100 % and the solutions are prepared according to this with 50, 25, 10 % concentrations. In order to determine until which concentration the disinfectant is active, the disinfectant material with different concentration (100, 50, 25 and 10 %) is distributed into tubes 9 mL by 9 mL in each. Then by taking 1 mL of beginning microorganism suspension for each tube they are added to test tubes which include disinfectants with different concentrations (1 + 9 mL). Microorganisms are kept waiting in test tubes that includes disinfectant materials, during designed period (1, 5, 15 and 30 min). At the end of these contact periods 1 mL are taken from each test tube and added on to neutralizer materials of 9 mL which are in different test tubes. In 1-5 min 0, 2 mL of sample was taken from each tube and are placed into plaques which includes appropriate medium. After an incubation period of 48 h at 37 ºC, colonies that are reproduced in appropriate mediums are counted and bacteria numbers in 1 mL. are calculated. At the end of the first minute, the concentration of the disinfectant that cause a decline 5 log and above (the reduction factor is 5 log and above) in the number of microorganism according to the number of microorganism that are treated with disinfectant materials is accepted as effective concentration. Besides, it is confirmed that the neutralizer material doesn't have a deterrent effect on the reproduction of microorganisms and don't cause decline in the number of microorganisms. And also is confirmed that it inactivate the effect of disinfectant material by the experiments[9,10].

RESULTS AND DISCUSSION

The results of disinfectant A against test microorganisms are given in Tables 1-5. Alcohol based disinfectant's basic effect mechanism is protein denaturation. It has strong and fast fatal efficiency against to gram-positive and gram-negative microorganism, micro bacteria and many viruses. It makes inactive to many of closed viruses except of rabies virus [for example; herpes simplex virus, HIV, influenza virus, RSV and vaccinia virus]. Although it has less effective on hepatitis B and C viruses, it still active to inactive them. Sporiferous bacteria can live in alcohols for a long time. They are not effective on protozoan oocystes. Based on studied 0,100 % of alcohol based disinfectant hand solutions are affected to test microorganism in 1 min. Nakipoglu and Gürler[15] found alcohol based disinfectant has MIK level is 1/32 for MRSA, MSSA. *E. coli*, *K. pneumonia*, *P. aeruginosa* in a study. The same disinfectant are effective against to MRSA, MSSA. *E.coli*, *K. pneumonia*, *P. aeruginosa* bacteria in 8, 18, 28 min. A study by using *S. aereus* and *P. aeruginosa* septoderm sypray and alcohol based hand antiseptics are more effective then according to prosavon and predex HS 550[15]. Alcohols, when compare the other antiseptics, have perfect activity and fast bactericidal effect. In addition to this they have advantages such as using fast and evaporation. They have optimal antimicrobic spectrum to all bacterias and clinically important virusses and againts to fungus[5,16,17]. Ethanol compared with other disinfectant that has different contents and alcohol disinfectant and raported as it is the best[15]. Using gloves commonly in medical area and it's over trusty reduce the importance of hand and skin antisepsis, caused to lost hand washing habits. This kind of serious problem brings economical charges into medical sector and its institutions, it can not be ignore.

In conclusion, we think that in choosing hand disinfectants it is important to select disinfectants which are effective in a little while to vegetative forms of pathogen bacteria and which protect hands when considering the development of resistance of microorganisms.

TABLE-1

S. aureus's NUMBER OF COLONY IN 1 mL AFTER THE TIME LIMIT (CFU/mL) IN DIFFERENT CONCENTRATIONS TREATED WITH A DISINFECTANT'S SOLUTION

	Effect duration (minute)			
Concentration %	30 sn	1 dk	5 dk	15 dk
100	$3.8^{*}10^{4}$	-	-	-
50	$>10^{5}$	-	-	-
25	$>10^{6}$	$>10^{5}$	$>10^{5}$	$>10^{5}$
10	$>10^{6}$	$>10^{6}$	$>10^{6}$	$>10^{6}$

microorganism did not multiply Initial suspension: 4×10^{9} CFU/mL
RF: log reduction factor Final con. in the disinfectant: 3×10^{8} CFU/mL (8, 60 log CFU/mL)

TABLE-2

E.coli's NUMBER OF COLONY IN 1 mL AFTER THE TIME LIMIT (CFU/ML) IN DIFFERENT CONCENTRATIONS TREATED WITH A DISINFECTANT'S SOLUTION

	Effect duration (minute)			
Concentration %	30 sn	1 dk	5 dk	15 dk
100	-	-	-	-
50	-	-	-	-
25	$>10^{5}$	$>10^{5}$	$>10^{5}$	$>10^{5}$
10	$>10^{6}$	$>10^{6}$	$>10^{6}$	$>10^{6}$

Microorganism did not multiply Initial suspension: $2{,}75\times10^{9}$ CFU/mL
RF: log reduction factor Final con. in the disinfectant: $2{,}75\times10^{8}$ CFU /mL (8, 43 log CFU/mL)

TABLE-3

P.aeruginosa's NUMBER OF COLONY IN 1 mL AFTER THE TIME LIMIT (CFU/ML) IN DIFFERENT CONCENTRATIONS TREATED WITH A DISINFECTANT'S SOLUTION

	Effect duration (minute)			
Concentration %	30 sn	1 dk	5 dk	15 dk
100	-	-	-	-
50	-	-	-	-
25	$>10^{5}$	$>10^{5}$	$>10^{5}$	$>10^{5}$
10	$>10^{6}$	$>10^{6}$	$>10^{6}$	$>10^{6}$

Microorganism did not multiply Initial suspension: 5×10^{9} CFU/mL
RF: log reduction factor Final con. in the disinfectant: 5×10^{8} CFU /mL (8, 69 log CFU/mL)

TABLE-4

C. albicans NUMBER OF COLONY IN 1 mL AFTER THE TIME LIMIT (CFU/ML) IN DIFFERENT CONCENTRATIONS TREATED WITH A DISINFECTANT'S SOLUTION

	Effect duration (minute)			
Concentration %	30 sn	1 dk	5 dk	15 dk
100	-	-	-	-
50	-	-	-	-
25	$>10^{4}$	$>10^{4}$	$>10^{4}$	$>10^{4}$
10	$>10^{5}$	$>10^{5}$	$>10^{5}$	$>10^{5}$

Microorganism did not multiply Initial suspension: $2{,}25\times10^{8}$ CFU/mL
RF: log reduction factor Final con. in the disinfectant: $2{,}25\times10^{7}$ CFU /mL (7,35 log CFU/mL)

TABLE-5

MRSA's (ISOLATION IN LABORATUARY) NUMBER OF COLONY IN 1 mL AFTER THE TIME LIMIT (CFU/mL) IN DIFFERENT CONCENTRATIONS TREATED WITH A DISINFECTANT'S SOLUTION

	Effect duration (minute)			
Concentration %	30 sn	1 dk	5 dk	15 dk
100	$4.6^{*}10^{4}$	-	-	-
50	$4.3^{*}10^{3}$	-	-	-
25	$>10^{6}$	$>10^{6}$	$>10^{5}$	$>10^{5}$
10	$>10^{6}$	$>10^{6}$	$>10^{6}$	$>10^{6}$

Microorganism did not multiply Initial suspension: 5×10^{8} CFU/mL
RF: log reduction factor Final con. in the disinfectant: 25×10^{7} CFU /mL (7,35 log CFU/mL)

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