*J. Fac. Pharm. Istanbul*
**38(1) (2005-2006)**



# A STUDY ON THE MICROBIOLOGICAL ACTIVITIES OF THE WET TOWELS THAT BECAME A PART OF OUR LIVES

**A. S. BİRTEKSÖZ, S. DÖŞLER, B. ÖZBEK, Ç. BOZKURT GÜZEL, A.A. GERÇEKER, G. ÖTÜK**

## SUMMARY

Wet towels which seemed unfamiliar at the begining has begun to become an irresistable part of our daily life as the convenience of its use is seen. They are with us every where; on trips, in the restaurants, in public areas, at the hospitals, in bags, in our pockets. However, how much can these wet towels protect us from the microorganisms that exist widely in nature and can infect us with various ways? Based on this idea, using the European Standard EN 1276 method, the activities of wet towels produced in our country containing antimicrobiological substances that are effective on the hand antisepsis and surface disinfection against several microorganisms have been studied. Suspensions of $10^7$cfu/ml have been used in these experiments and a ratio of at least 99.98 % killing have been observed. We have seen that the wet towels that are used in hand antisepsis and surface disinfection are effective against all tested microorganisms except bacterial spores and it is evident that these towels, with the safety they have and the confindence they give us, will be with us for a long time and will not be out of our life very easily.

## ÖZET

Başlangıçta alışılmışın dışında gelen ıslak mendiller, kullanımdaki kolaylıkları görüldükçe, günlük yaşantımızın vazgeçilmez bir parçası olmaya başladılar. Seyahatte, lokantada, ortak kullanım alanlarında, hastanede, çantada, cebimizde, heryerde bizimle birlikte bulunmaktadırlar. Peki bu ıslak mendiller bizi ne kadar doğada yaygın bir şekilde bulunan ve bize çeşitli yollardan bulaşabilen mikroorganizmalara karşı koruyabilmektedirler. Bu düşünceden yola çıkarak ülkemizde üretilen el antisepsisi ve yüzey dezenfeksiyonunda etkili antimikrobiyal madde içeren ıslak mendillerin çeşitli mikroorganizmalara karşı aktiviteleri European Standard EN 1276 yöntemi ile araştırılmış, $10^7$cfu/ml'lik süspansiyonların kullanıldığı deneylerde en düşük % 99.98

Istanbul University, Faculty of Pharmacy, Dept. of Pharmaceutical Microbiology, 34116 Istanbul-Turkey.

*J. Fac. Pharm. Istanbul*
**38(1) (2005-2006)**

oranında ölüm görülmüştür. El antisepsisinde ve yüzey dezenfeksiyonunda kullanılan ıslak mendillerin bakteri sporları dışında denenen tüm mikroorganizmalara karşı etkili olduklarını gösteren bulgularımız bu mendillerin güven veren etkileri ile hayatımızdan pek de kolay çıkamayacaklarını göstermektedir.

**Key words:** Antimicrobial activity, wet towel, hand antisepsis, surface disinfection

# INTRODUCTION

Do these wet towels we use safely in various areas such as offices, schools, hospitals, restaurants, trips, public vehicles, restrooms and the hygiene of our children due to their practical and easy use really deserve this trust? From this point of view, we aimed to study the antimicrobic effects of the wet towels that are used for two different purposes: for hand antisepsis and for surface disinfection against the microorganisms which are found widely in nature and can threaten our lifes when they find convenient conditions.

Hand antisepsis and surface disinfection is necessary for protection of public health as well as in institutions where public health services are given and industrial branches where products that are directly related to human health such as food and medicine. The success of the effect of the antiseptics and disinfectants on the microorganisms depends on the type of the microorganism, the degree of contamination, the kind of disinfectant, its concentration, its mode of action, the time of contact, and the environmental conditions such as the existing organic substances, pH and temperature. For this reason, we should choose the most active antiseptic and disinfectant against microorganisms for a good antisepsis and disinfection process and we should use them in proper time and concentration (6,7).

While ethyl alcohol which is one of the antimicrobic substances that are in the wet towels that we used in our study has a killing effect against the great majority of pathogen bacteria, viruses and fungi it has no effect against the spore forms of bacteria. On the other hand, isopropyl alcohol in the form of 70 % or more concentrated solutions has stronger bactericidal effect, but because of its toxical effects it is only used in disinfection processes. Benzalkonium chloride, which is one of the cationic surface active substances while inhibiting the growth of bacteria and fungi at low concentrations it kills the vegetative forms of bacteria at high concentrations, however it has no effect against spore forms. Because it does not have any serious toxic effect it is a preferable antiseptic and disinfectant. Triclosan is an antimicrobic substance which shows high activity against bacteria but a low one against fungi. It is widely used as a skin antiseptic because its activity is not much interfered by the existence of organic substances and it has a long lasting activity (6,8,9). This is a study which contains different combinations of the substances that have the above mentioned antimicrobic properties, and aims to show the safety of using made in Turkey wet towels for hand antisepsis and surface disinfection against the pathogenic and opportunistic microorganisms.

# RESULTS AND DISCUSSION

The antimicrobic activities of the wet towels, used for hand antisepsis and surface disinfection against several microorganisms have been examined according to the European Standard EN 1276 method and the findings have been summarized in Table 1 and 2. We have determined that the wet towels used for surface disinfection killed some of the bacterial strains we studied at a ratio of 100 %, the once used for hand antisepsis killed all the microorganisms at a ratio of 99.9 %. We found out that none of the wet towels were active against bacterial spores.

**Table 1.** Antimicrobial activities of wet towels used for hand antisepsis

| Microorganisms | Number of microorganisms in suspension (cfu/ml) | | Contact time (minutes) 2.5 | 5 | 7.5 | 10 |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* ATCC 6538 | $2.8 \times 10^7$ | V: | 10 ; 10 | 8 ; 9 | 6 ; 6 | 3 ; 4 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 99.99 % |
| *Staphylococcus epidermidis* ATCC 12228 | $1.1 \times 10^7$ | V: | 20 ; 40 | 15 ; 18 | 10 ; 20 | 7 ; 8 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 99.99 % |
| *Escherichia coli* ATCC 10799 | $4.0 \times 10^7$ | V: | 120 ; 180 | 60 ; 90 | 20 ; 30 | 7 ; 9 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 99.99 % |
| *Pseudomonas aeruginosa* ATCC 27853 | $5.0 \times 10^7$ | V: | 20 ; 40 | 21 ; 19 | 15 ; 16 | 14 ; 15 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 99.99 % |
| *Salmonella typhi* | $7.0 \times 10^7$ | V: | 40 ; 90 | 50 ; 80 | 20 ; 50 | 20 ; 40 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 99.99 % |
| *Enterococcus faecalis* ATCC 29212 | $4.4 \times 10^7$ | V: | 310 ; 390 | 290 ; 300 | 140 ; 170 | 120 ; 190 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 99.99 % |
| *Bacillus subtilis* ATCC 6633 | $1.0 \times 10^7$ | V: | 70 ; 80 | 50 ; 80 | 70 ; 80 | 20 ; 90 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 99.99 % |
| *Candida albicans* ATCC 10231 | $2.5 \times 10^6$ | V: | 90 ; 100 | 60 ; 70 | 50 ; 50 | 20 ; 30 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 99.99 % |

V: Viable count after test procedure (colony)
R: Ratio of killed microorganisms after test procedure (%)

*J. Fac. Pharm. Istanbul*
**38(1) (2005-2006)**

**Table 2.** Antimicrobial activities of wet towels used for surface disinfectans

| Microorganisms | Number of microorganisms in suspension (cfu/ml) | | Contact time (minutes) | | | |
|---|---|---|---|---|---|---|
| | | | 2.5 | 5 | 7.5 | 10 |
| *Staphylococcus aureus* ATCC 6538 | $3.0 \times 10^7$ | V: | 10 ; 60 | 10 ; 20 | 0 ; 10 | 0 ; 0 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 100 % |
| *Staphylococcus epidermidis* ATCC 12228 | $7.0 \times 10^7$ | V: | 0 ; 20 | 0 ; 20 | 0 ; 20 | 0 ; 0 |
| | | R: | 99.99 % | 99.99 % | 99.99 % | 100 % |
| *Escherichia coli* ATCC 10799 | $1.3 \times 10^7$ | V: | 0 ; 0 | 0 ; 0 | 0 ; 0 | 0 ; 0 |
| | | R: | 100 % | 100 % | 100 % | 100 % |
| *Klebsiella pneunoniae* ATCC 4352 | $8.0 \times 10^7$ | V: | 0 ; 0 | 0 ; 0 | 0 ; 0 | 0 ; 0 |
| | | R: | 100 % | 100 % | 100 % | 100 % |
| *Pseudomonas aeruginosa* ATCC 27853 | $9.7 \times 10^7$ | V: | 0 ; 0 | 0 ; 0 | 0 ; 0 | 0 ; 0 |
| | | R: | 100 % | 100 % | 100 % | 100 % |
| *Salmonella typhi* | $1.9 \times 10^7$ | V: | 0 ; 0 | 0 ; 0 | 0 ; 0 | 0 ; 0 |
| | | R: | 100 % | 100 % | 100 % | 100 % |
| *Shigella flexneri* | $1.5 \times 10^7$ | V: | 0 ; 0 | 0 ; 0 | 0 ; 0 | 0 ; 0 |
| | | R: | 100 % | 100 % | 100 % | 100 % |
| *Proteus mirabilis* ATCC 14153 | $1.7 \times 10^7$ | V: | 0 ; 0 | 0 ; 0 | 0 ; 0 | 0 ; 0 |
| | | R: | 100 % | 100 % | 100 % | 100 % |
| *Enterococcus faecalis* ATCC 29212 | $7.0 \times 10^7$ | V: | 0 ; 0 | 0 ; 0 | 0 ; 0 | 0 ; 0 |
| | | R: | 100 % | 100 % | 100 % | 100 % |
| *Bacillus subtilis* (spore susp.) ATCC 6633 | $1.3 \times 10^7$ | V: | > 3000 | > 3000 | > 3000 | > 3000 |
| | | R: | < 3 % | < 3 % | < 3 % | < 3 % |
| *Candida albicans* ATCC 10231 | $2.5 \times 10^7$ | V: | 250 ; 320 | 260 ; 280 | 250 ; 270 | 250 ; 280 |
| | | R: | 99.98 % | 99.98 % | 99.98 % | 99.98 % |

V: Viable count after test prodedure (colony)
R: Ratio of killed microorganisms after test procedure (%)

Several chemical substances have been widely used to prevent and control the expansion of infectious diseases, and provide personal hygiene with the aim of inhibiting the growth or killing the microorganisms. The studies have proved the great effect of hand and environmental hygiene on our health protection. 200 000 000 bacteria, viruses and fungi can go away from our hands by washing them even once (8). Eleven different pathogenic and opportunistic microorganisms widely found in water, air, soil, the skin and mucosal membrane of men and animals and faeces have been used in our study. Among them *Escherichia coli*, *Enterococcus faecalis* are important in fecal contamination; *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Candida albicans* in hospital infections they cause; the spores of *Bacillus subtilis* are important in environmental, especially in soil originated contaminations.

With the changes in social conditions the time spent outdoors has increased and this led people to search more practical and available products instead of the basic water and soap cleaning. Wet towels have been produced to fill this gap and the use of them has increased day by day. In order to indicate the antimicrobic activities of wet towels we came to the conclusion that quick and effective cleaning can be supplied against many pathogenic and opportunistic microorganisms by using them. There are several studies on the effects of antimicrobic substances against the microorganisms we have worked on; Güneri et al (5) have found that benzalkonium chloride has killed P. aeruginosa at a concentration of 1/5.000 and *S. aureus* at a concentration of 1/40.000 in 2.5 minutes. In another study it was mentioned that a solution of 1/10 of benzalkonium chloride killed S. aureus ATCC 6538 strain in 5 minutes and *P. aeruginosa* ATCC 15442 strain in 60 minutes (10). In a study where a solution of 70 % of ethyl alcohol was used it was found out that *S. aureus* and *C. albicans* were killed in one minute (1). In another study, it was determined that liquid soap containing 10 % of PVP-I and 70 % of ethyl alcohol was the most effective hand-cleansing agents for removing meticillin resistant *S. aureus* strain from both lightly and heavily contaminated hands (4). In relation to the effect of triclosan, Messager et al (8) found out that this antimicrobic substance killed *S. aureus*, *E. faecalis*, *S. epidermidis*, *P. aeruginosa* and *E. coli* in the ratio of more than 4log10. Gloor et al (3) fond that triclosan cream showed excellent activity against S. aureus. Our study indicates that the wet towels used in hand antisepsis that contain ethyl alcohol of 70 %, benzalkonium chloride and triclosan killed *S. aureus*, *S. epidermidis*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *S. typhi*, *B. subtilis* and *C. albicans* at a ratio of 99.9 % in 2.5, 5, 7.5 and 10 minutes; and the wet towels used for surface disinfection containing benzalkonium chloride, isopropyl alcohol and alcohol dehydrate killed the above mentioned microorganisms, plus *P. mirabilis* and *S. flexneri* during the same periods of time at a ratio of 100 %. As a result, the wet towels we used in our experiments have been quite effective against all tested microorganisms except bacterial spores. This study shows the antimicrobic activities of the wet towels that have become a part of our daily life with the advantages that they provide us with, we believe that wet

*J. Fac. Pharm. Istanbul*
**38(1) (2005-2006)**

towels deserve the trust people feel towards them and will not be out of our lives very easily.

## EXPERIMENTAL

**Microorganisms:** Inoculums producing a final concentration of 1 x $10^7$ colony-forming units (CFU)/ml of *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *E. coli* ATCC 10799, *P. mirabilis* ATCC 14153, *S. flexneri, K. pneumoniae* ATCC 4352, *S. typhi, C. albicans* ATCC 10231, *B. subtilis* ATCC 6633 and *B. subtilis* ATCC 6633 (spor suspension) were used.

**Wet towels:** The wet towels used for hand antisepsis contains ethyl alcohol of 70 %, benzalkonium chloride and triclosan. The one for surface disinfection contains benzalkonium chloride, isopropyl alcohol, alcohol dehydrate.

**Media:** Mueller-Hinton broth (Difco Laboratories, Detroit, Mich., USA) was used for inoculum preparations, pour plates of tryptic soy agar (Difco) were used for colony counts.

**Reagents:** A solution of neutralizer containing lecithin, polysorbate 80, sodium thiosulfate and L-histidine was used in order to inactivate the antimicrobial subtances within the test mixtures. A solution of 10 % of skimmilk was used as an interfering substance.

**Determination of antimicrobial activity:** According to European Standard EN 1276 method the solutions containing the antimicrobic substances absorbed by towels were incubated with the suspensions of the microorganisms at room temperature for 2.5, 5, 7.5 and 10 minutes. After neutralizing the solution, the number of the surviving microorganisms were determined by the pour plate viable colony counting technique (2). The killing ratio of the microorganisms used in the experiment has been calculated according to the following formula:

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

N: number of cfu/ml in the inoculum

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

## REFERENCES

1. Best, M., Springthorpe, S.V., Sattar, A.S., *Am. J. Inf. Control.*,**22**, 152-162 (1994).
2. EN 1276, Chemical disinfectants and antiseptics. Quantative suspension test for the evaluation of bactericidal activity of chemicals, disinfectants and antiseptics used in food, industrial, domestic and institutional araes- Test methods and

requirements (phase 2, step 1). European Committe for Standardization, Brussels (1997).
3. Gloor, M., Becker, A., Wasik, B., Kniehl, *E., Hautarzt.*, **53**, 724-729 (2002).
4. Guilhermetti, M., Hernandes, S.E., Fukushigue, Y., Garcia, L.B., Cardoso, C.L., *Infect. Control. Hosp. Epidemiol.*, **22**, 105-108 (2001).
5. Güneri, S., Kayalı, F., Taşlı, H., Coşar, G., *Turkish Journal of Infection*, **11**, 365-370 (1997).
6. Harvey, S.C. (ed.), Antimicrobial drugs. In: Gennore A.R., Remington's Practice of Pharmacy, 18th ed., 1163, Eoston, Pensilvania, Mack Publishing Company (1990).
7. Marsik, P.J., Denys, G.A., Sterilization, decontamination and disinfection procedures for the microbiology laboratory. In: Murray P.R et. al., (eds.) Manual of Clinical Microbiology, 6th. ed, p. 86-98, Washington DC, American Society for Microbiology (1995).
8. Messager, S., Goddard, P.A., Dettmar, P.W., Maillard, J.Y., Determination of the antibacterial efficacy of several antiseptics tested on skin by an 'ex vivo' test., *J. Med. Microbiol.*, **50**, 284-292 (2001).
9. Scott, E.M, Gorman, S.P., Chemical disinfectants, antiseptics and preservatives. In: Hugo W.B., Russell A.D., (eds.) Pharmaceutical Microbiology, 4th ed. London, Blackwell Scientific Publications (1989).
10. Yüce, A., Sayan M., Songur, M., Yuluğ, N., *Turkish Journal of Infection*, **12** , 65-67 (1998).

Accepted:26 December 2005