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Evaluation of the Effect of Ethyl Alcohol Solutions of Different Concentrations on Bacteria

Farklı Konsantrasyonlardaki Etil Alkol Çözeltilerinin Bakteriler Üzerindeki Etkisinin Değerlendirilmesi

Naile AKINCI[1*], Meriç ÇELİK[2], Hakan Yasin ÇETİNKAYA[3], Gülnaz ÇELİK[4]

[1] Fenerbahce University, Faculty of Health Sciences, Department of Nursing, İstanbul/Turkey.
[2] Gazi University, Faculty of Medicine, Medical Student, Ankara/Turkey.
[3] İzmir Katip Celebi University, Faculty of Medicine, Medical Student, İzmir/Turkey.
[4] Kdz.Eregli Borsa Istanbul Atatürk Anatolian High School, Chemistry Teacher, Zonguldak/Turkey.

Abstract

This randomized controlled experimental study aims to evaluate the effect of different concentrations of ethyl alcohol solutions on bacterial growth. Alcohol solutions with different volüme concentrations were prepared as a preliminary process. In the first step, alcohol solutions of different concentrations by volume were prepared.. Microbiological agar was used as the medium. In the third process, sterile Petri dishes were obtained, and the dishes to be used were labeled with dirty hands, ethyl alcohol solutions of 10%, 30%, 60%, 70%, 80%, and 96%. A laptop (monitor and keyboard), which is one of the devices frequently used in everday life, was chosen as the sample material. As a result, it was found that the number of colonies in the Petri dishes visibly decreased with increasing alcohol concentration. No bacterial colonies were observed at concentrations of ≥70%. The lowest and most effective ethyl alcohol concentration was found to be 70%. We think that observing bacterial growth at different alcohol concentrations with samples from different environments will provide us with more data on the effect of disinfectants and antiseptics in future studies.

Keywords: Ethanol, hand sanitizers, COVID-19, disinfectants

Özet

Bu randomize kontrollü deneysel araştırmada farklı konsantrasyonlardaki etil alkol çözeltilerinin bakteri oluşumu üzerine etkisinin değerlendirilmesi amaçlandı. Ön işlem olarak hacimce farklı konsantrasyonda alkol çözeltileri hazırlandı. Besiyer olarak mikrobiyolojik Agar kullanıldı. Üçüncül işlem olarak steril petri kapları alındı ve kullanılacak petri kapları kirli el, %10, %30, %60, %70, %80 ve %96'lık etil alkol çözeltileri olarak etiketlendi Günlük hayatta sık kullanılan araçlarından biri olan laptop (monitör ve klavye) örnek alınacak materyal olarak seçildi. Araştırma sonucunda alkol konsantrasyonu arttıkça petri kaplarının içerisindeki koloni sayılarının göz ile görülür derecede azaldığı gözlemlendi. En düşük ve etkili etil alkol konsantrasyonu %70 olarak saptandı. İleriki araştırmalarda farklı ortamlardan alınan örneklerle farklı alkol konsantrasyonlarında bakteri üremesinin gözlemlenmesinin dezenfektan ve antiseptiklerin etkisi konusunda daha fazla bilgiye ulaşmamızı sağlayacağı düşünülmektedir.

Anahtar kelimeler: Etanol, el antiseptikleri, COVID-19, dezenfektanlar

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*Corresponding author: naileaksit@gmail.com

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1.Introduction

In our daily lives, we live with microorganisms that we cannot see with the naked eye, but which have positive/negative effects on human and animal health, and which we know exist. It is estimated that about 80% of infectious diseases, which cause 20% of deaths in the world, are caused by contact with contaminated surfaces (Bal&Şanlı, 2020). For this reason, various applications have been developed to remove microorganisms in order to prevent infections. Of these applications, hand washing with soap and water is the most economical and easy-to-use method to prevent the spread of viruses, including SARS-COV-2 (World Health Organization [WHO], 2009; United States Centers for Disease Control & Prevention, 2020a).

Disinfectants and antiseptics are chemical agents that have been used for many years to control pathogenic microorganisms. Although hand washing with soap and water is the most recommended method, ethanol-based hand sanitizers are used in some cases because water and soap may not be accessible, these sanitizers are often easily accessible, they provide fast-acting, and they are volatile compounds that do not require rinsing and drying after application (Kampf et al., 2004; Kampf & Hollingsworth, 2008; Czeisler et al., 2020; Sharafi et al., 2020; Lotfinejad et al., 2020). The COVID-19 pandemic that emerged in 2019, has shown us the importance of disinfectants and antiseptics for hygiene in environments where water and soap are not accessible. In this context, the public health guidelines recommended by the WHO emphasize frequent and correct hand washing with commercially available hand sanitizers to prevent transmission and reduce the spread of pandemic diseases (WHO, 2020). Based on these protocols and recommendations, the use of alcohol-based hand rubs is widespread around the world. While it is true that hand hygiene is the most important way to break the chain of infection transmission, the use of an appropriate standard formula solution should definitely be considered. The products sold on the market and in pharmacies as hand antiseptics contain very different concentrations of alcohol (ethyl alcohol). Although it is important for effective disinfection to use the right disinfectant in the right dosage and in the right place, the products sold as hand antiseptics on the market and in pharmacies contain very different proportions of ethyl alcohol. This variety leads to confusion and hesitation among users. In addition, people use these products unconsciously due to panic and fear, and this can cause negative effects on human health and the environment (Rivera et al., 2020; Suen et., 2019; Turkmen et al., 2021). There are not enough studies in the literature on the correct ratio of ethyl alcohol to take the necessary precautions against bacteria on the hands. Given this information, the aim of this research is to investigate the effect of different solutions of ethyl alcohol at different concentrations (10%, 30%, 60%, 70%, 80%, and 96%), which is one of the most commonly used antiseptic substances to keep hands and skin clean during the pandemic process, on bacterial growth and to determine the lowest and most effective concentration of ethyl alcohol to inactivate bacteria. In this way, health problems caused by bacteria are likely to be minimized and prevented.

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2. Method

2.1.Aim

This study was designed and conducted as a randomized controlled trial to investigate the effect of ethyl alcohol solutions of different concentrations on bacterial growth. The study was conducted between September 2021 and February 2022.

2.2.Research Hypothesis

Ethyl alcohol solutions of different concentrations influence bacterial growth on the hands.

2.3.Implementation of the Research

The study was conducted in a laboratory environment. In the first step, ethyl alcohol solutions were prepared at 10%, 30%, 60%, 70%, 80%, and 96% by volume. In the second step, culture media were prepared in which bacteria can grow and multiply. Microbiological agar was used for this purpose. To prepare a microbiological agar solution, 5 grams of agar was weighed and placed in a 600 ml, and 300 ml of pure water was added. The solution was heated and stirred until it became clear. It was then allowed to cool for 30 minutes.

In the third step, sterile Petri dishes were obtained and the dishes to be used were labelled with dirty hand, ethyl alcohol solutions of 10%, 30%, 60%, 70%, 80%, and 96%. Then the liquid agar was carefully poured into the Petri dishes in such a way that it did not form bubbles and covered the bottom of the Petri dish while it was still warm. It was then kept in the refrigerator for 10 minutes to cool and solidify. The laptop (monitor and keyboard), which is frequently used in the participant's daily life during the distance learning conducted due to the COVID-19 pandemic, was selected as a sample and divided into 7 equal parts using a ruler.

From here, samples were randomly taken with 3 fingers and implanted into the medium in the prepared Petri dishes. First, the participants' dirty fingers were touched on one of the surfaces separated by the random method, and then they were touched on the entire surface of the medium, which was labelled as a dirty, hand without any further process following. For other samples, the participant's hands were washed with soap and water for 30 seconds and dried with a napkin, the sample was taken in the same way with 3 fingers, and then the hands were washed with alcohol solutions of 10%, 30%, 60%, 70%, 80%, and 96% (10 ml) respectively. After drying, the fingers were touched on the entire surface of the medium in the Petri dishes. Following the principle of "every living cell forms a colony", the dishes were left for 7 days to allow the microorganisms to form colonies in the solid medium at room temperature. After this period, the bacterial colonies that had formed in the Petri dishes were observed. Bacterial formations were detected during microscopic examination.

The colonies in the culture media, which were determined using the colony counting method, were counted with the naked eye. To minimize the counting error, a piece of square paper was placed under the Petri dish and the contents divided into equal parts. The colonies in each part were then

counted and recorded. It was found that the number of colonies in the Petri dishes visibly decreased with increasing ethyl alcohol concentration.

2.4.Limitations

The limitation of this study is that the research results were obtained with a sample from a single environment.

3. Results

3.1. Results on the Effect of Ethyl Alcohol Solutions of Different Concentrations on Bacterial Growth

The samples taken from the participant's laptop, one of the most used tools in people's daily lives during the distance learning conducted due to the COVID-19 pandemic, were planted in the medium prepared in Petri dishes. They were kept in a dark place at room temperature for 7 days. At the end of the 7th day, the bacterial colonies grown in the Petri dishes were counted with the naked eye using the colony counting method. To minimize the counting error, a piece of square paper was placed under the Petri dish and the contents of the dish was divided into equal parts. The colonies that formed in each part were then counted and recorded. The following findings were obtained as a result of the experiment.

Ithe Petri dish (Figure 1, Figure 2), that we called the dirty hand, too many bacteria were growing to count. It was observed that the agar in the Petri dish could not tolerate the growth of the bacterial colonies, so it melted and turned into a liquid. A small section of the bacterial colony was taken and examined under the microscope. The examination revealed a very high number of bacterial formations (Figure 3). A total of 112 colonies were counted in the Petri dish labelled as 10% (Figure 4). A total of 29 colonies were counted in the Petri dish, which was labelled 30% (Figure 5). A total of 14 colonies were counted in the Petri dish labelled 60% (Figure 6). No colony formation was observed in the Petri dishes labelled 70%, 80%, and 96% (Figure 7; Figure 8; Figure 9).

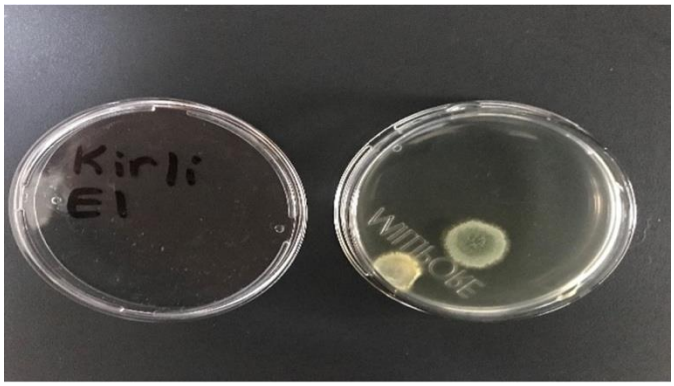

Figure 1: Image of the Petri Dish Labeled as Dirty Hand

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Figure 2: Image of the Petri Dish with 10% Ethyl Alcohol

Figure 3: Microscopic Image of the Dirty Hand Sample

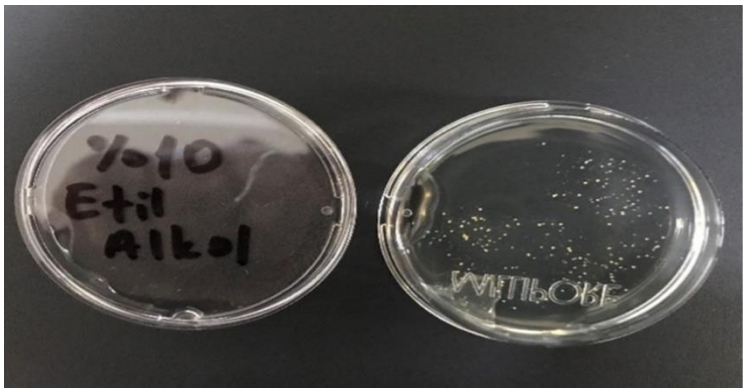

Figure 4: Image of the Petri Dish with 10% Ethyl Alcoho

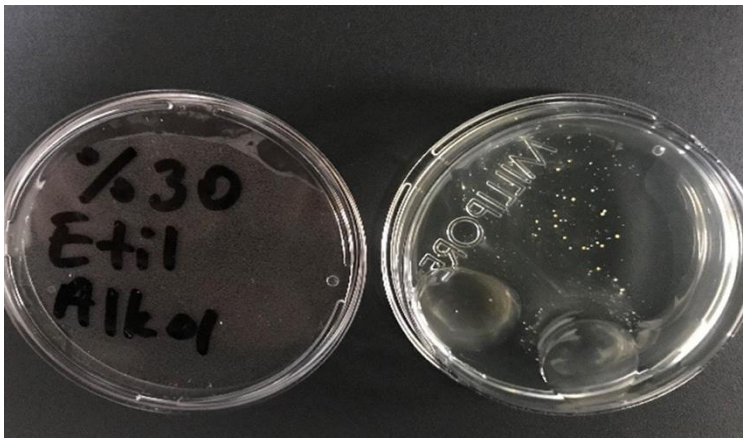

Figure 5: Image of the Petri Dish with 30% Ethyl Alcohol

Figure 6: Image of the Petri Dish with 60% Ethyl Alcohol

Figure 7: Image of the Petri Dish with 70% Ethyl Alcohol

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Figure 8: Image of the Petri Dish with 80% Ethyl Alcohol

Figure 9: Image of the Petri Dish with 96% Ethyl Alcohol

As can be seen in Table 1, a reduction of about 74% was observed in the bacterial colonies between 10% ethyl alcohol and 30% ethyl alcohol. Between 30% ethyl alcohol and 60% ethyl alcohol, a reduction in bacterial colonies of approximately 51% was observed. As can be seen from the table, the number of colonies detected decreased with increasing ethyl alcohol concentration and finally disappeared at 70% ethyl alcohol.

Table 1. Findings on the Effect of Ethyl Alcohol Solutions of Different Concentrations on Bacterial Growth

The concentration of the ethyl alcohol used	**Dirty hand**	**%10 ETHYL ALCOHOL**	**%30 ETHYL ALCOHOL**	**%60 ETHYL ALCOHOL**	**%70 ETHYL ALCOHOL**	**%80 ETHYL ALCOHOL**	**%96 ETHYL ALCOHOL**
Count of colonies detected	Too many to count	112	29	14	0	0	0

4.Discussion

Ethyl alcohol is the most popular active ingredient among alcohol-based disinfectants and is used as an antiseptic and surface disinfectant. It is effective against vegetative forms of bacteria, enveloped viruses, some non-enveloped viruses, fungi and yeasts. The effectiveness of an alcohol-based disinfectant varies depending on the type, concentration, and amount of alcohol used (Yusuf, 2021; Cruz et al., 2022). The careless use of disinfectants can cause many problems, such as resistance to antibacterial effects, allergic reactions, poisoning, and skin problems. In addition, unnecessary consumption causes an economic burden. However, it is widely recognised that the amount of alcohol-based hand sanitizer recommended by manufacturers should be sufficient to pass the standard in-vivo efficacy test and typically the EN-1500 (WHO, 2009; Rodrigues et al., 2016; Wilkinson et al., 2017; Jing et al., 2020).

In this study, it was observed that the number of colonies in the Petri dishes decreased significantly with increasing alcohol concentration. At concentrations of 70% and above, no bacterial colonies were observed (Figure 7; Figure 8; Figure 9). This result can be explained by the decreasing ethyl alcohol concentration when the alcohol concentration drops below 70%. The results of the study are consistent with the literature. In the literature, 70% and higher concentrations of ethyl alcohol were found to be effective against vegetative bacteria, mycobacteria, fungi, and lipid-containing viruses (Avcı et al., 2017; Lotfinejad et al., 2020; Yusuf, 2021; Cruz et al., 2022; Sauerbrei, 2020). In a study on the effects of ethyl alcohol at concentrations of 95%, 70% and 50% on the standard strain of E. Coli ATCC25922 growth was only observed at a 50% concentration of 50, no growth was found at the other concentrations (Avcı, 2017). In another study, the effect of ethanol on Staphylococcus spp. was stronger at higher concentrations (100%, 90%, and 80%), and lower at lower concentrations (70%, 60%, and 50%) with shorter exposure times (Elzain et al., 2019). The use of products based on 70% ethyl alcohol in liquid or gel form has also been generally advocated by the WHO since the declaration of the COVID-19 pandemic (spread by the SARS-CoV virus-2) on March 11, 2020 (WHO,2020).

Hand washing with soaps and the use of hand sanitizers have increased during the COVID-19 pandemic. The global hand sanitizer market is expected to grow further in the coming days. The outcome of this study is that the efficacy levels and effects of products sold as hand sanitizers that contain different concentrations of alcohol (ethyl alcohol) and informs society, healthcare professionals, manufacturers and vendors on what percentage of alcohol they should have. This result can help reduce or eliminate confusion, misuse and negative effects of alcohol-based hand sanitizers. It also highlights the importance of conducting studies to raise public awareness about the proper use of hand sanitizers.

5.Conclusion

In summary, the lowest and most effective ethyl alcohol concentration was 70%. Further studies on which types of bacteria ethyl alcohol is more effective on, on the effect of different concentrations of ethyl alcohol solutions on bacteria depending on the contact time and on the efficacy levels of alcohols used with different alcohol mixtures could provide more information on the effect of disinfectants and antiseptics. It is becoming increasingly important to carry out studies to raise public awareness of the correct use of hand sanitizers.

*Corresponding author: naileaksit@gmail.com

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Yazarların Katkısı/Authors Contributions

Conceptualization: NA; Data curation: NA, MÇ, HYÇ, GÇ; Formal analysis: NA, MÇ, HYÇ, GÇ Investigation: NA, MÇ Methodology: NA,GÇ Project administration: NA Resources: HYÇ, GÇ; Software: NA, HYÇ; Supervision: NA ; Validation: NA, MÇ ; Visualization: NA, MÇ; Writing–original draft: NA, MÇ; Writing–review & editing: NA, MÇ, HYÇ,GÇ

Çıkar Çatışması / Conflict of Interest

All authors report no conflict of interest.

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*Corresponding author: naileaksit@gmail.com

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