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Pathogen Fungal Isolation from Unused and Used Shoes

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ABSTRACT

The purpose of this study is to investigate the level of risk factors in terms of infection source of pathogenic fungi at tanned leather used in shoe making or unused and used shoes. Samples were taken from shoes with swaps. Of the 100 samples taken from the shoe-making leather; pathogenic fungal findings were found in 16(15%) of them, including Penicillium sp.5(5%), Aspergillus versicolor 3(3%), Fusarium sp.2(2%), Aspergillus flavus 2(2%), Aspergillus fumigatus 2(2%), Trichophyton rubrum 1(1%) and Aspergillus niger 1(1%). Besides, in the samples collected from used leather shoes.; 40 pathogenic fungal growth were identified from 100 samples collected in total which are Fusarium sp.7(7%), Rhodotorula sp.7(7%), Candida sp. 6(6%), Penicillium sp. 5(5%), Acremonium sp. 5(5%), Rhizopus sp. 4(4%), Trichophyton rubrum 3(3%), Trichophyton mentagrophytes 1(1%), Epidermophyton floccosum 1(1%) and Chrisosporium keratinophylum 1(%1). As a result of this research, It was observed that there was a significant difference between the samples taken from used shoes (40%) and unused shoes (16%) in terms of the isolation rate of pathogenic and rare pathogenic yeasts and molds($p<0.05$). It has been concluded that the higher fungal isolation in the shoes used may be due to the use of common items and environmental factors, and the isolation of fungus from the samples taken from unused shoes could be due to contamination from the shoemakers and storage in inappropriate conditions.

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Kullanılan ve Kullanılmayan Ayakkabılardan Patojen Fungus İzolasyonu

ÖZET

Bu çalışmanın amacı, kullanılmayan ayakkabı veya ayakkabı yapımında kullanılan tabaklanmış derilerden ve kullanılan ayakkabılardan alınan örneklerin infeksiyon kaynağı olması açısından risk seviyesini araştırmaktır. Örnekler ayakkabılardan steril swaplar ile alınmıştır. Kullanılmayan ayakkabılardan alınan 100 örnekte, 5(%5) *Penicillium sp.* , 3(%3) *Aspergillus versicolor*, 2(%2) *Fusarium sp.*, 2(%2) *Aspergillus flavus*, 2(%2) *Aspergillus fumigatus*, 1(%1) *Trichophyton rubrum* ve 1(%1)*Aspergillus niger* olmak üzere toplam 16(%16) fungus bulunmuştur. Kullanılan ayakkabılardan alınan 100 örnekte, 7(%7) *Fusarium sp.*, 7(%7) *Rhodotorula sp.*, 6(%) *Candida sp.*, 5(%5) *Penicillium sp.*, 5(%5) *Acremonium*, 4(%4) *Rhizopus sp.*, 3(%3) *Trichophyton rubrum*, 1(%1) *Trichophyton mentagrophytes*, 1(%1) *Epidermophyton floccosum* ve 1(%1) *Chrysosporium keratinophylum* olmak üzere 40(%40) fungus bulunmuştur. Sonuç olarak, patojen ve nadir patojen olan maya ve küflerin izole edilme oranı açısından kullanılan ayakkabılardan alınan örneklerde (%40) ve kullanılmayan ayakkabılar (%16) arasında önemli farkın olduğu gözlendi ($p<0.05$). Kullanılan ayakkabılarda fungus izolasyonunun daha yüksek olması kontaminasyon kaynağının ortak eşya kullanımının ve çevresel faktörlerin olduğu kullanılmayan ayakkabılardan alınan örneklerden mantar izole edilmesi ise ayakkabı yapımında çalışanlardan kontaminasyon ve uygunsuz şartlarda depolanmasından dolayı olabileceği sonucuna varılmıştır.

Mikrobiyoloji

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INTRODUCTION

Fungus and fungus spores evolve mostly on animals and plants, on multicellular organisms, in all environments such as soil and water. Most of the pathogenic fungi are contaminated by soil and animals. Fungi which are animal origin have the potential to infect humans through the use of shoes and other products made from animal skins. Leathers that are not well tanned or contaminations after tanning during the manufacturing and storage stages can be a source of contamination of pathogenic fungi (Bauman,2004).

In addition to the visual and aesthetic qualities of the leather sector, at shoe and clothing sector, during the layering operations or after tanning processes, because of various procedures applied, sometimes escalating pathogenic fungus cases are encountered as well as severe consequences.Because fresh leather contains 60-70% water and containing water-soluble easily degradable protein is a suitable environment for microorganisms (Çakmak, 2010).

Fungal diseases are transmitted directly or indirectly to people and sources of infection, by touching or by articles and other means contaminated with fungi. With contamination they can even lead to outbreaks. The footwears, with the effect of the perspiration, form suitable microenvironments for the development and reproduction of fungi (Li et al, 2001). In this respect, the persons suffering from pathogenic fungal diseases play a role in infection with their hairs, bristles, scarves, combs, brushes, clothes, and slippers, especially with shoes. Barber suits; floors of washing places;headrests at cinemas, theaters and automobiles; the seats of the alafranga toilets can also be the cause of contamination (Bendeş, 1997; Öztürk, 1999).

The purpose of this study is to investigate the level of risk factors in terms of the sources of contamination of pathogenic fungi of tanned leather used in shoe making, unused shoes and used shoes.

MATERIAL and METHOD

Collection of Leather Samples from Manufacturers

Examples of unused shoe examples, leather shoes parts to be used in shoe making or unused shoes are taken with the permission of the business authorities of shoe producers which are located in Gaziantep's central district Şehitkâmil GATEM. In the manufacturing center, 100 pieces of samples from 10 different shoe companies were cut into pieces of 1 cm in diameter by cutting method and placed in petri dishes. These samples in petri dishes were brought to the Microbiology Laboratory of Kahramanmaras Sutcu Imam University Science and Literature Faculty. 100 samples were placed in test tubes filled with pre-prepared physiological water (45 ml isotonic salt solution). 100 swab samples were taken from the used leather shoes. These samples were taken from the mosques in the central districts of Gaziantep, from the university student dormitories, from shopping centers, from coffee houses and from the leather shoes of the random adults. Samples were placed in sterile isotonic salt solution and brought to Kahramanmaraş Sütçü Imam University Faculty of Science and Literature Microbiology Laboratory.

Culture and Identification of Samples

In the case of used shoes, samples were taken from the public life centers such as university student dormitories, shopping malls, coffee shops, mosques in the central districts of Gaziantep, the samples were taken from the leather shoes of the random volunteer adults by using by swab method. Samples were placed in sterile isotonic salt solution in test tubes. Samples taken to the Microbiology Laboratory were seeded on Sabouraud Dextrose Agar and Potato Dextrose Agar media. Identifications of the fungi that reproduce in culture are done (Öztürk, 1999).

From the samples suspended in the isotonic solution, 1 ml of the cultures were prepared. Specimens and genus levels of the specimens which were reproduced after the incubation were defined. Macroscopically, colony morphology and color formation were microscopically identified by lam cultures and sports and hyphae structures (Larone, 2002). The statistical calculation was made according to the Z test because the number of samples is more than 30.

RESULTS and DISCUSSION

100 samples were taken from 10 different manufacturing companies for the study of pathogen fungal isolation from unused shoe cultivars. After the identification procedures required, 16(16%) of the 100 samples were found to be pathogenic fungus. The results of the described types of pathogenic fungi are shown at Table 1.

According to the identification results of isolated pathogenic fungi species; *Penicillium sp.* 5(5%), *A. versicolor* 3(3%), *A. flavus* 2(2%), *A. fumigatus* 2(2%), *Fusarium sp* 2(2%), *T. rubrum* 1(1%) and *A. niger* 1(1%) have occurred.

From the leathers taken from 10 manufacturing

companies which are located in Şehitkamil, Gaziantep central province where the leather is used in the production of leather shoes, while at 8 companies pathogenic fungi were seen, no pathogenic fungus was detected at the leather samples that are taken from 2 companies.

Table 1. Fungi isolated from unused shoe leathers.
Çizelge 1. Kullanılmayan ayakkabılardan fungus izolasyonu

Isolated Fungi	n (%)
Penicillium sp.	5 (5)
A. versicolor	3 (3)
A. fumigatus	2 (2)
Fusarium sp.	2 (2)
A. flavus	2 (2)
T. rubrum	1 (1)
Aspergillus niger	1 (1)
Total	16 16)

The samples taken from volunteers were recorded. Breeding was observed in 35 cultures from these 100 samples. A total of 40(40%) pathogenic and potential pathogen or opportunistic pathogenic fungi were found to be the result of identification studies of cultured cultures. As a result of identification studies of cultures seen in reproduction, a total of 40(40%) pathogenic and opportunistic pathogenic fungi were found (Table 1,2).

When the data were analyzed, 40(40%) of the 100 samples taken by swab and slice methods from the shoes of adults, a total of 38 pathogenic fungi were isolated. The most isolated fungi were *Fusarium sp.* 7(7%) and *Rhodotorula sp.* 7(7%), then *Candida sp.* 6(6%), *Penicillium sp.* 5(5%), *Acremonium sp.* 5(5%), *Rhizopus sp.* 4(4%), *T. rubrum* 3(3%), *T. mentagrophytes* 1(1%), *E. floccosum* 1(1%) and *Chrisosporium keratinophylum* (*C. keratinophylum*) 1(1%) were found respectively (Table 2, Figure 1,2).

Table 2. Fungi isolated used shoe leathers.
Çizelge 2. Kullanılan ayakkabılardan fungus izolasyonu

İsolated Fungi	**n (%)**
Rhodotorula sp.	7 (7)
Fusarium sp.	7 (7)
Candida sp.	6 (6)
Penicillium sp.	5 (5)
Acremonium sp	5 (5)
Rhizopus sp.	4 (4)
T. rubrum	3 (3)
T. mentagrophytes	1 (1)
E. floccosum	1 (1)
C. keratinophylum	1 (1)
Total	**40 (40)**

Fungal infections contaminate from soil, animals and sharing of common goods. One of the most important contamination sources are shoes (Öztürk, 1999; Ocak,2010). Fungus can survive on foot and in other parts of the body without normally having a disease. Fungus with tinea pedis effect on the feet multiply rapidly in appropriate environment conditions during the use of the shoes and cause infections. In addition, foot fungal infections can be rapidly passed from person to person with direct contact or with the use of the same footwear (Ocak, 2010.)

Figure 1. Microscopic view of A. fumigatus.
Şekil 1. A. fumigatus un mikroskop görüntüsü

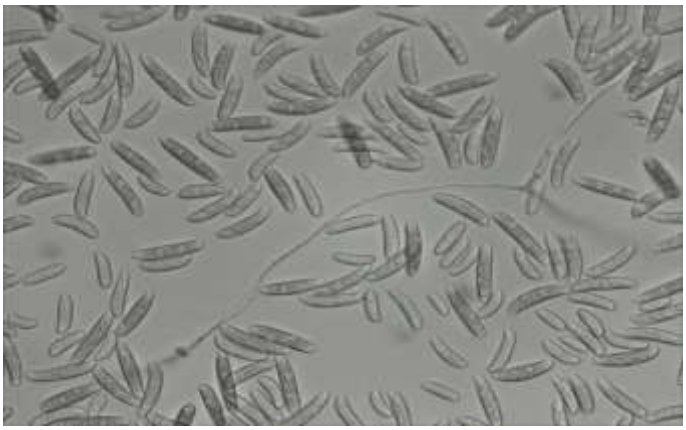

Figure 2. Microscopic view of Fusarium sp. spores.
Şekil 2. Fusrium sp. sporlarının mikroskop görüntüsü

With the effect of hot climatic conditions, some parts of the human body create a more suitable environment for the development and transmission of fungi.The use of common goods and places can lead to the formation of many mycoses in the form of an epidemic. At one of the studies about this; of the 1524 workers in the glass factory, 388 began to complain of redness and pruritus in their groin after they started working, even though they had no complaints before they started working at the factory. Of the samples taken from these workers, 39 were considered positive for hyphe and yeast. As a result of evaluation of the

cultures obtained from the samples, was observed in 7 samples and Candida sp. in 20 samples; in addition, fungi such as Penicillium, Fusarium, Rhizopus and Mucor, which are not pathogenic in most cases in humans have been defined (Soejon et al, 1982).

Köktürk and his colleagues, at their work of isolation of dermatophytes from tinea pedis cases, isolated 77.1% *T. rubrum*, 9.7% *T. mentagrophytes*, 4.8% *E. floccosum* and 1.6% *T. violaceum* (Köktürk et al, 2002).

Bilgili and their colleagues have isolated 22% *T. rubrum*, 22.8% *T. mentagrophtes* and 1.69% *E. floccosum* in tinea pedis cases (Bilgili et al, 2001).

In the study performed by Asci, 15.42% of *T. rubrum*, 5.23% of *T. mentagrophytes*, 7.16% of *Candida sp.*have isolated from tinea pedis lesions (Aşcı, 1992).

In the work done by Doherty, from the chrome-tanned leather, *Rhodotorula mucilagenous, Neurospora sitophila* and *Fusarium sp.* were isolated. In the work of Kallenberger, *Rhodotorula, Cladosporium* and *Torulopsis sp.*have been identified (Birbir et al, 1992).

At the Kallenberger work, *Rhodotorula* and *Cladosporium;* at the Birbir and friends work, *Candida sp.*, *Penicillium sp.*, *Mucor, Fusarium, Rhizopus, Rhodotorula sp.* fungi species and genera isolated from the collected samples resembles the species and genus of the mold and yeast fungus we isolated (Birbir et al, 1992).

Li and his colleagues have isolated *Cryptococcus neoformans* (*C. neoformans*), 1 *Candida albicans* (*C. albicans*), 1 *Cryptococcus albidus* (*C. albidus*), 1 *Rhodotorula mucilaqinosa* (*R. mucilaqinosa*), 1 *Candida utilis* (*C. utilis*), 2 *Mucor*, 3 *Aspergillus,* 2 *Penicillium* in the children's shoes (Li et al, 2001).

At the work done by Birbir and his friends, from tanned leather and new shoes, from a total of 63 samples a total of 20 species of fungi, which are 13 *Candida sp.*, 2 *Rhodotorula*, 1 *Saccharomyces cerevisiae* (*S. cerevisiae*), 1 *Phaeococcomyces* species, 1 *Aureobasidium pullulans* (*A. pullulans*), 1 *Geotrichum candidum* (*G. candidum*) and 1 *Wallemia sebi*, have been isolated. From the used shoes leathers; a total of 16 fungi species, which are 8 *Candida sp.*, 4 *Rhodotorula sp,* 3 *S. cerevisiae* and 1 *Phaeococcomyces* species, have been identified. In this work in the samples taken from the feet of the wearers of the shoes in total of 15 fungi, which are 8 *Candida sp.*, 4 *Rhodotorula* sp, 3 *S. cerevisiae* and 1 *Phaeococcomyces* sp, were isolated. As a result in this study, among fungi samples taken from the unused shoes, the used shoes and the feet of the wearers of the shoes, there is a clear correlation among the isolated fungi. According to this correlation, it has been suggested that the shoes that are produced from leather may be a major source of contamination of the infection of yeast fungi in humans (Birbir et al, 1992).

In this work, from 100 samples taken from unused shoe leathers, rarely pathogenic *Penicillium sp.* 5(5%), *A. versicolor* 3(3%), *Fusarium sp.* 2(2%), *A. flavus* 2(2%), *A. fumigatus* 2 (2%), *A. niger* 1(1%), *T. rubrum* 1(1%), which is a direct pathogen, a total of 16(16%) fungi were isolated.

From the 100 swabs taken from the used shoes, 6(6%) *Candida* sp., 3(3%) *T. rubrum*, 1 (1%) *T. mentagrophytes*, 1(1%) *E. floccosum* which are directly pathogenic were identified. Rarely pathogenic, 7(7%) *Fusarium sp.*, 7(6%) *Rhodotorula sp.*, 5(5%) *Penicillium sp.*, 5(5%) *Acremonium sp.*, 4(4%) *Rhizopus sp.*, 1(1%) *C. keratinophylum* , in total 40 (40%) fungi were isolated.

In this work, while the rate of isolation of fungi from unused shoe leathers is 16% the rate at used shoes is 40%.

CONCLUSION

The obtained outcomes for this new footwear (16%) are comparatively lower than the results reported by Birbir and his associates for their new shoes (31.74%). It is noteworthy that in this study, the unused shoes were sampled solely from the shoe manufacturing stage, whereas Birbir and his friends collected samples from both the shoe manufacturing and storage stages. We posit that this divergence in sampling methodologies may account for the observed difference in results.

In this study, it is observed that there is a significant difference between new and used shoes ($P<0.05$). Also, unlike other studies; in addition to the rare pathogenic yeasts and molds, *Penicillium sp.* 5(5%), *A versicolor* 3(3%), *Fusarium sp.*7(7%), *A. flavus* 2(2%), *A. fumigatus* 2 (2%), *A. niger* and direct pathogen *T. rubrum* 3(3%), *T. mentagrophytes* (11%), *E. floccosum* 1(1%) were isolated. Although the tanning process is very well done, there may be contaminations due to heavy workmanship in the shoe making process. During storage process, if the storage conditions are favorable for the reproduction of the fungus, the fungus will be also seen in the shoes that are contaminated. As a result, we think that not taking sufficient measures to minimize the contaminations during shoe manufacturing phases and storage conditions of shoes that are suitable for fungus, are important sources of contaminations in humans.

In conclusion, tanned leather, unused and used shoes made from these leathers, which are tought to be one of the sources of fungal infections, have been investigated in terms of pathogenic fungal infections. İn terms of the isolation rate of yeast and molds, which are pathogenic and rare pathogenic, the rate of isolation with samples taken from new shoes and from the samples taken from the used shoes is found to be significant ($P<0.05$). It is thought that during

the use of contaminated shoes, it may have increased the possibility of transmission in appropriate development conditions.. It is also thought to increase contamination with feet from other contaminated sources. In shoes that are contaminated during production and storage, the use of contaminated shoes is thought to increase human and human-to-human infection.

Since 16% from samples taken from unused shoes Isolation of pathogenic fungi is an important risk factor for the source of transmission.It can be said that it created.

Therefore, determination of these infectious agents of pathogenic fungi sources and the identification of these factors are extremely important in terms of preventive public health.

Isolation and identification studies of pathogenic fungi are usually carried out in clinical studies. Unlike clinical studies it will present important data to the public health and clinical studies since it is related to the the prevalence of pathogenic fungi in terms of source of infection.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Statement of Conflict Interest

The authors of the article declare that there is no conflict of interest between them.

REFERENCES

Aşçı, Z. (1992). Elazığ Yöresinde İzole Edilen Dermatofit Etkenleri ve İnvitro Duyarlılıklarının Araştırılması. [Doktora Tezi.Fırat Üniversitesi Sağlık Bilimleri Enstitüsü, Mikrobiyoloji Anabilim Dalı]. Yüksek Öğretim Kurulu Ulusal Tez Merkezi.75 p

Bauman, R.W. (2004). Microbiology, Contributions by Elizabeth Machunis-Masuoka, Phd. University of Virginia, Ian Tizard, Phd. Texas A.& M. University USA, p: 624-62.

Bendeş, A.O. (1997). Ege Bölgesinden İzole Edilen *Trichophyton rubrum* Şuşlarının Griseofulvine in Vitro Duyarlılığı. [*Doktora Tezi*. Ege Üniversitesi Fen Fakültesi Biyoloji Anabilimdalı.] Yüksek Öğretim Kurulu Ulusal Tez Merkezi. 78 p.

Bilgili, M. E., Sabuncu, İ., Saraçoğlu, Z. N., Ürer, S. M., Kiraz, N., & Akgün, Y. (2001). Kliniğimize Başvuran Dermatofitozlu Olgulardan İzole Edilen Dermatofitler. *Türk Klinik Dermatoloji*, *11,* 185-190.

Birbir, M., Özyaral, O., Johansson, C., & Ilgaz, A. (1992). Detection of yeast strains in chrome-tanned leather and shoes. *The Journal of The American Leather Chemists Association, 87*(12), 466-472.

Çakmak, G. (2010). Astarlık ve Yüzlük Ayakkabı Derilerinin Ağır Metal Toksisitesinin Enzim Aktiviteleri ile Belirlenmesi. [*Yüksek Lisans Tezi*. Ege Üniversitesi Fen Bilimleri Enstitüsü.]. Yüksek Öğretim Kurulu Ulusal Tez Merkezi 93 p.

Köktürk, A., Delğaloğlu, N., Kaya, T.İ., Baz, K., İkizoğlu, Demirseren, et al. (2002). Mersin İlinin Dermatofit Florası. Türk Klinik Dermatoloji, 12, 135-139.

Köktürk, A., Delğaloğlu, N., Kaya, T.İ., Baz, K., İkizoğlu, G., Demirseren, DD, Kanık, A (2002). Mersin İlinin Dermatofit Florası. *T. Klinik Dermatoloji 12*(3), 135-139

Larone, D.H. (2002). Medically Important Fungi, Aguide to identifation. 4th ed., Printed in the United States of America:USA p11–180.

Li, H., Zhou, J., Shi, R., and Wuyong, C. W. (2011). Identification of fungi from children's shoes and application of a novel antimicrobial agent on shoe insole. *African Journal of Biotechnology*, *10*(65), 14493-14497

Ocak, B. (2010). Antifungal Ayakkabı Üretiminde Mikrokapsülasyon Yönteminin Kullanımı Üzerinde Araştırmalar.[*Doktora Tezi*. Ege Üniversitesi Fen Bilimleri Enstitüsü], Yüksek Öğretim Kurulu Ulusal Tez Merkezi 133 p.

Özkütük, A.A. (1999). Dermatomikoz Olgulardan İzole Edilen Dermatofitlerin Tiplendirilmesi ve Antifungal Ajanlara Duyarlılıkları. [*Uzmanlık Tezi*. Dokuz Eylül Üniversitesi Tıp Fakültesi Mikrobiyoloji Anabilim Dalı.] Yüksek Öğretim Kurulu Ulusal Tez Merkezi 67 p.

Soejon, R., Gunawan, M.S., Zein, N., and Pradona, J. (1982). Proceedings *of the Tenth Asian Conference on Occupational Health.* 2, p 703.