



# Fungal Contamination of Wrestling and Bodybuilding Clubs in Hamedan Province, Western Iran

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## Abstract

**Background & Aims:** This study first evaluated the fungal contamination of tools and surfaces in wrestling and bodybuilding clubs in Hamedan province, western Iran.

**Materials and Methods:** This cross-sectional study was conducted from September 2018 to May 2019 focusing on nine wrestling and bodybuilding clubs in different areas of Hamedan province. The sterile carpet method (10×10) was used for sampling from mats floor and wall, bath and WC, locker room and parquet surfaces, and environmental air. Common mediums and methods in mycology were employed to culture samples and detect grown fungi.

**Results:** The most frequently isolated fungal genera were *Rhizopus* (24%), *Penicillium* (24%), *Aspergillus flavus* (23%), *Aspergillus niger* (19%), yeast (7%), *Scopulariopsis* (6%) *Mucor*, and *Ulocladium* (1%). Based on the results, no dermatophyte contamination was detected in any of the samples. The most contaminated surfaces (place of sampling) were wrestling mats (95.7%). The highest and lowest prevalence rates of fungal contamination in this study were found in Razan (87.5%) and Malayer (72.7%), respectively.

**Conclusion:** According to our results, the most contaminated surfaces belonged to wrestling mats. Thus, we would suggest that common-sense hygiene measurements should be continued, including showering after every encounter, washing practice clothes daily, and disinfecting mats daily.

**Keywords:** Club, Fungi, Pollution, Hamedan

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## 1. Introduction

Because of socio-economic growth and industrialization, the physical activity of mankind has remarkably decreased, entailing chronic, deteriorating consequences such as obesity, diabetes mellitus, and cardiovascular disorders [1-3]. Hence, nowadays, sports and fitness activities, whether for recreation or professionally, are more emphasized for improving body health status [4]. Wrestling has been a symbol of power and virility for people in a number of countries such as Iran, and many youth Iranians are involved in wrestling throughout the country. Due to the combating nature of this sports activity, profuse body sweating, and persistent contact with the fighting mat, wrestlers are mostly exposed to acquiring and/or transmitting fungal infections [5].

Nevertheless, poor hygienic conditions both for the sportsman himself/herself and/or the sporting environment, would not only endanger the health of the surrounding individuals from a public health perspective but also affect one's ability to compete [6]. Primary preventive measures comprising routine showering and public awareness have been advocated, but still not adequately effective [7]. Thus, the causative agent must be

identified to barricade infectious outbreaks [8]. Evidence-based data demonstrate that wrestling clubs if they are not controlled or examined, are considered suitable places for the growth of pathogenic fungi [9,10]. Accordingly, the surfaces of different devices such as mats, locker rooms, towels, floors, and W.C. walls are also suitable for the growth of dermatophyte species such as *Trichophyton* and *Epidermophyton*, as well as saprophytic fungi, including *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium*, and *Scopulariopsis* [11]. An important aspect of opportunistic fungi is the pathogenic potential for immune deficient and immune-compromised subjects. Furthermore, Saprophytes can cause nail dystrophy. According to previous studies, the most common etiologic agents of onychomycosis are *Aspergillus* spp., *Acremonium* spp., *Scopulariopsis* spp., *Penicillium* spp., and *fusarium* with an incidence rate of 1.43%-17.6% [12]. In sports with skin-to-skin contact, including wrestling ringworm, *Tinea gladiatorum*, has been found to be more common [13-17]. *Trichophyton tonsurans* was also isolated from wrestling mats in Iran [18,19].

Additionally, bodybuilding clubs are among extremely populated places, facilitating the risk of athletes' exposure



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to mycoses. Moreover, such perimeters possess several fomites such as towels, practice clothing and wrestling mats, occlusion, trauma, shower sharing, and sweating with resultant maceration of the epidermis which may carry the sources of infection [5]. Therefore, understanding different transmission routes of fungal contamination is crucial in planning effective interventions to break the chain of transmission. Although extensive surface contamination with fungi has been demonstrated, there is no information available on the health status of the devices, tools, and surfaces of wrestling and bodybuilding clubs in Hamedan. Thus, this study was the first one to report the fungal contamination of tools and surfaces in wrestling and bodybuilding clubs in Hamedan province, western Iran in 2018.

## 2. Materials and Methods

Nine sports centers in Hamedan province having wrestling and bodybuilding clubs were enrolled in this cross-sectional study from September 2018 to May 2019. These clubs were located in different areas such as Hamedan (n=4), Bahar (1), Malayer (1), Razan (1), Asadabad (1), and Maryanaj (1) (Figure 1). Seven samples were taken for each club. Initially, a number of 100 pieces of mats (10×10 cm) were prepared and autoclaved at 121°C for 15 minutes. For each wrestling club, sufficient pieces of mats were placed in direct contact with various surfaces, including the wrestling mat, parquet, wall, and floor, WC bathroom, locker room, and the bodybuilding club. Next, each piece was kept within a clean nylon, tagged, and transferred to the laboratory. For air sampling, the

Sabouraud dextrose agar (SDA) culture medium, which was placed 1 m higher than the earth's surface, was exposed to the environmental air for 30-60 minutes. By tapping the back surface of each contaminated mat piece, the culture mediums were inoculated by the mat components. The media were sealed and incubated at room temperature (SDA) or 37°C (sabouraud dextrose agar (SSD)) for the aim of fungal growth. Macroscopic and microscopic examinations were performed following 2-3 weeks of fungal growth [5]. The slide culture method was implicated in the case of difficulty in diagnosis. Fungi were identified mostly by the close examination of their morphology and characteristics. In slide cultures, the fungi were grown directly on the slide on a thin film of agar. Accordingly, there was no need to remove a portion of the fungus from a culture plate and transfer it to the slide. Briefly, a sterile U-shaped glass rod on the filter paper was placed [8], and enough sterile water was poured (about 4 mL) onto the filter paper to completely moisten it. Then, a sterile slide was placed on the U-shaped rod with forceps. Gently, a scalpel was flamed to sterilize, and a 5-mm square block of the medium was cut from the plate of Sabouraud's agar. The block of agar was picked up by inserting the scalpel, and this block was carefully transferred aseptically to the center of the slide. Four sides of the agar square were inoculated with spores or mycelial fragments of the fungus for examination. Aseptically, a sterile cover glass was placed on the upper surface of the agar cube. Then, the cover was placed on the petri dish and incubated at room temperature for 48 hours. After 48 hours, the slide was examined under low power. If growth



Figure 1. Hamedan Province Geographical Location

had occurred, there would be the growth of hyphae and the production of spores. If growth was inadequate and spores were not evident, the mold was allowed to grow for another 24-48 hours before making the stained slides. Next, a drop of lactophenol cotton blue stain was placed on a clean microscope slide [5,8]. The cover glass was removed from the slide culture, and the block of agar was discarded. A drop of 95% ethanol was added to the hyphae on the cover glass. As soon as most of the alcohol was evaporated, the cover glass was placed, and the mold was placed side down on the drop of lactophenol cotton blue stain on the slide. Finally, given that the microscopic examination can help identify fungal infections using morphological features, the slides were examined under a microscope [20]. Eventually, the chi-square test was used for the investigation of the relevance between variables.

### 3. Results

The results of the present investigation demonstrated that out of 100 examined specimens from nine different sports centers in Hamedan province, 68 specimens were positive for fungal culture. The most frequently isolated fungal genera included *Rhizopus* (24%), *Penicillium* (24%), *Aspergillus flavus* (23%), *Aspergillus niger* (19%), yeast (7%), *Scopulariopsis* (6%) *Mucor*, and *Ulocladium* (1%), the details of which are presented in Table 1. No

dermatophyte contamination was observed in any of the samples. The most contaminated surfaces (place of sampling) were wrestling mats (95.7%), followed by the floor and wall (89.5%), air (86.7%) WC (82.4%), and locker room (50%). The findings revealed that there was a statistically significant association between the total fungal load and different kinds of sampling places ( $P=0.019$ ).

No fungus was detected in the culture of 32 specimens. The highest and lowest prevalence rates of fungal contamination were related to Razan and Malayer (87.5 and 72.7%), respectively. No significant association was found between the study area and fungal genera or species ( $P=0.868$ ). The highest isolated fungi were *Rhizopus* and *Penicillium* (24%) while the lowest ones were yeast, *Mucor*, and *Ulocladium* (1%).

### 4. Discussion

To the best of our knowledge, this is the first study to assess fungal contamination on inanimated objects (i.e., mat, floor and wall, locker room, bath and WC, parquet, and environmental air). This study also provided insight into the epidemiology and distribution of various fungal contamination in nine wrestling and bodybuilding body building clubs in Hamedan province, western Iran. In the present study, 88.8% of the clubs were positive for at

**Table 1.** Distribution of isolated fungi species according to the place of sampling (surfaces) and area

	Mat	Bath-WC	Floor-Wall	Lock Room	Parquet	Bodybuilding Tool	Air
Club 1	-	-	<i>Penicillium</i> <i>A. niger</i> <i>A. flavus</i>	<i>Rhizopus</i> <i>Penicillium</i> <i>A. flavus</i> Yeast	<i>Rhizopus</i> <i>A. niger</i> <i>Ulocladium</i>	<i>Rhizopus</i> <i>Penicillium</i>	<i>Rhizopus</i> <i>Penicillium</i> <i>A. flavus</i>
Club 2	<i>Rhizopus</i> <i>A. flavus</i> Yeast	<i>A. niger</i> <i>A. flavus</i> Yeast	<i>Penicillium</i> <i>A. flavus</i>	Yeast	-	-	Yeast
Club 3	<i>Penicillium</i>	-	<i>A. niger</i> <i>A. flavus</i>	-	-	-	<i>A. niger</i> <i>A. flavus</i>
Club 4	<i>Rhizopus</i> Yeast	-	<i>Rhizopus</i>	-	-	-	<i>Rhizopus</i> <i>Scopulariopsis</i>
Club5	<i>Rhizopus</i> <i>A. flavus</i> <i>Scopulariopsis</i>	<i>A. flavus</i>	<i>Penicillium</i> <i>A. flavus</i> <i>Scopulariopsis</i>	-	<i>Penicillium</i> <i>A. flavus</i>	-	<i>Penicillium</i> <i>A. flavus</i> <i>Scopulariopsis</i>
Club 6	<i>Penicillium</i> <i>A. niger</i> <i>Rhizopus</i> <i>Mucor</i>	<i>Penicillium</i> Yeast	-	<i>Penicillium</i>	<i>Penicillium</i> <i>A. niger</i> <i>A. flavus</i> <i>Rhizopus</i>	<i>Penicillium</i> <i>A. niger</i> Yeast	-
Club7	<i>A. niger</i> Yeast <i>Rhizopus</i>	<i>Penicillium</i> Yeast	-	-	<i>Penicillium</i> Yeast <i>A. flavus</i>	<i>A. niger</i> <i>Rhizopus</i>	<i>Rhizopus</i>
Club 8	<i>Penicillium</i> <i>A. niger</i> <i>A. flavus</i> <i>Rhizopus</i>	<i>Penicillium</i> Yeast <i>A. flavus</i>	<i>Penicillium</i> <i>A. niger</i> <i>Rhizopus</i> <i>Scopulariopsis</i>	-	-	Yeast	-
Club 9	<i>Rhizopus</i> <i>A. flavus</i>	<i>Rhizopus</i> <i>Penicillium</i> <i>A. flavus</i>	<i>Rhizopus</i> <i>Penicillium</i>	-	-	-	<i>Penicillium</i>

Note. \*Club 1-4: Hamedan; Club 5: Maryanaj; Club 6: Razan; Club 7: Asadabad; Club 8: Bahar; Club: Malayer; *A. flavus*: *Aspergillus flavus*; *A. niger*: *Aspergillus niger*.

least one saprophytic fungal agent. Saprobes are a group of fungal species with saprophytic nutrition via a process of chemoheterotrophic extracellular digestion, being involved in the decaying organic matter. They may be implicated in wrestlers' mycoses through wrestling mats, WC and bathroom, wall and floor, parquet, bodybuilding clubs, and the environmental air. An important aspect of opportunistic fungi is their pathogenic potential for immune deficient and immune compromised subjects [12].

In sports with skin-to-skin contact such as wrestling, *T. tonsurans* was isolated from wrestling mats in France and Sweden [21,22]. In Iran, previous studies showed that dermatophyte infection may vary among wrestlers ranging from 24 to 47.5% [23,24]. *T. tonsurans* and *Trichophyton rubrum* were also isolated from wrestling mats in different regions of Iran [5,18,19,25,26].

No dermatophyte species were isolated in the current study. Some reasons could illustrate why we were unable to detect dermatophytes from the cultures of different objects. The samples were taken after sterilizing the mats and other objects. All tools and equipment were washed or disinfected after the practice time. The disinfectant used by each club contains an active fungicidal ingredient. These would have not revealed the presence of ringworm fungi. In addition, dermatophytes were probably there, but we did miss our chance by sampling at the end of the day. We may have also missed the opportunity to find any dermatophyte by sampling twice a week. Further, had no access to the specimens of wrestler skin or clothing the wrestlers. Dermatophytes are fungi that require keratin for growth and spread by direct contact from other people. In this regard, our findings are in line with those of previous studies by Kohl et al [27] and Ghasemi et al [12].

## 5. Conclusion

In this study, the most contaminated places of sampling were wrestling mats (95.7%) and the floor and wall (89.5%). We would recommend that common-sense hygiene measurements be continued, including showering after every encounter, washing practice clothes daily, and disinfecting mats daily [28]. Educating wrestlers, coaches, parents, and members of the medical community about skin infections and their prevention, recognition, and treatment is crucial and a part of our continuing effort [29]. The surface should then be cleaned with soap and water or wiped with a clean paper towel lightly wetted with a germicide registered with environmental surfaces. These measures are applicable for most nonabsorbent athletic surfaces. Surfaces should be allowed to dry sufficiently to prevent possible injuries [30].

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## Conflict of Interests

The authors declare that they have no competing interests.

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