

INFECTIOUS DISEASE AGENT SURVEILLANCE IN FITNESS CENTERS IN NORTHERN CYPRUS: IS METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) INFECTION A THREAT?

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Abstract

Fitness centers offer an opportunity for investigating the indirect transmission of pathogens. Many people with varying levels of personal hygiene share sports equipment where direct surface-to-skin contact occurs. This study aimed to investigate the bacterial load and fungal and methicillin-resistant *Staphylococcus aureus* (MRSA) contamination on predetermined sports equipment in fitness centers in Northern Cyprus. Additionally, volunteer personal trainers and gym members were screened to detect whether they were carriers of MRSA. Samples were collected from six fitness centers on sports equipment. MRSA carrier status was investigated for personal trainers (n=10) and gym members (n=100). The study used culture-dependent techniques and used SPSS 20 software for statistical analysis. There was no statistically significant difference between fungal growth on the sports equipment, and *Aspergillus* spp. were predominant. However, one of the fitness centers had a statistically significant difference in fungal growth compared to the others (p<0.005). There was no significant difference in the bacterial load among the sports equipment, but there was a significant difference among the fitness centers (p<0.009). Among all tested individuals, only 2.7% (3/110) were MRSA-positive, all of whom were gym members. No MRSA was detected on any of the equipment. Despite the increasing incidence of community-acquired MRSA infections, the fitness centers in this study did not appear to be significant sources of staphylococcal or fungal infections. However, the detection of MRSA carriers among gym members suggests that the spread of MRSA between individuals in gyms is still possible.

Keywords: Bacterial load. Fitness centers. Fungal contamination. MRSA. Sports equipment.

1. Introduction

The transmission rates and prevalence of antimicrobial-resistant (AMR) strains of bacteria are increasing. This threatens the capacity to treat infectious diseases and leads to a burden on public health. Many studies have revealed the occurrence of AMR and multidrug-resistant (MDR) strains of bacteria in clinical environments, including hospitals. However, the presence of AMR and MDR in sports equipment in fitness centers has yet to be revealed (Fadare and Durojaye 2019).

Interest in exercise is increasing since awareness of the advantages of exercise, such as physical, mental and social benefits, is increasing. Moreover, numerous evidence-based studies have suggested that physical inactivity doubles health risks, such as the development of obesity and hypertension, and shortens life expectancy. Hence, people's attentiveness to exercise has played a significant role in the growth of the health and fitness industry (Kayğusuz et al. 2021).

In fitness centers, equipment is shared between many individuals. This results in an environment of possible indirect fomite transmission of pathogens (Prevost and Simms 2021).

The bacterium *Staphylococcus aureus* (*S. aureus*), which is a gram-positive and nonmotile coccus, is responsible for frequently observed skin and soft tissue infections. Two infectious disease trends are associated with *S. aureus*: community-acquired (CA) infection and hospital-acquired (HA) infection. HA infections are generally caused by the use of intravascular devices in hospitals. In CA infection, the pathogen is acquired from the community.

Methicillin-resistant *S. aureus* (MRSA) is a highly selective antibiotic-resistant variant of *S. aureus* (Bilung et al. 2018). MRSA is resistant to multiple antibiotics, including methicillin and other beta-lactam antibiotics. The presence of *mecA*, a gene that produces a penicillin binding protein (PBP2a) with low affinity for β -lactam antibiotics, is responsible for resistance development. This makes infections caused by MRSA more difficult to treat and can result in treatment failure and poorer clinical outcomes (García-Hidalgo 2022).

CA MRSA infections are common, and many studies have shown that these infections are likely to occur in public fitness centers (Dalman et al. 2019). Additionally, according to a Centers for Disease Control and Prevention (CDC) report on the subject of MRSA spread, it is reported that locker rooms, health clubs, athletic facilities and fitness centers are environments for possible MRSA spread. This is because of the skin-to-skin contact and shared equipment. Thus, keeping surfaces clean is highly recommended to prevent MRSA from spreading in such environments (CDCa 2019).

Multiple body sites can be colonized by *S. aureus* in humans; however, the nasal cavity is the most common carriage site (Wertheim et al. 2005). The nasal carriage of *S. aureus*, including MRSA, refers to the presence of these bacteria in nasal passages without causing any symptoms of infection. Individuals who carry these bacteria in the anterior nares of their nose are referred to as 'carriers'. Carriers can spread bacteria to others through direct contact or through contaminated surfaces. On the other hand, nasal carriage of *S. aureus*, particularly MRSA, is associated with an increased risk of developing infections, both in the carriers themselves and in others who come into contact with them. This is because colonization with these bacteria can lead to infections if they enter the body through breaks in the skin or mucous membranes (Sivaraman et al. 2009; Sharma et al. 2014).

Identifying individuals who are colonized with *S. aureus* or MRSA, especially in health care settings, is crucial for implementing infection control measures to prevent the spread of these bacteria. This may include measures such as isolation precautions, hand hygiene, and environmental cleaning (Germel et al. 2012).

Apart from *S. aureus*, there are hundreds of other microbial species present in indoor environments. These may be in a dormant state, and when specific conditions are favorable, growth occurs. The main contributors to indoor microbial growth are the availability of nutrients, moisture, and temperature. Another factor influencing microbial growth is the rate of ambient air renewal via ventilation. In the fitness center, microbial growth is promoted due to increased physical activity, resulting in perspiration, water condensation and dust resuspension from the ground. In addition, there are interactions between members and surfaces, which also promotes microbial growth. Moreover, ubiquitous fungi can grow on a variety of environments and surfaces, including wallpaper and wood. This is because they have lower water requirements and are less selective regarding substrates than bacteria are. Moreover, fungal spores in indoor air enhance growth under suitable conditions. Fungi release spores into the air through aerial hyphae, whereas in bacteria, due to their gelatinous colonies, this process is not easily promoted (Ramos et al. 2016).

Therefore, understanding overall fungal and bacterial growth in fitness centers and athletic facilities would shed light on the risk of pathogen propagation in these facilities. Unfortunately, no studies have been conducted on such facilities in Northern Cyprus. This study aimed to evaluate the prevalence of CA MRSA colonization and microbial populations in fitness centers and athletic facilities in Northern Cyprus.

2. Material and Methods

Study Design

Nasal swab samples were obtained from volunteer personal trainers and gym members for MRSA detection. MRSA, bacterial load, and fungal contamination were evaluated on predetermined sports equipment in six fitness centers in Northern Cyprus. For confidentiality, this study coded the fitness centers as fitness center A, fitness center B, fitness center C, fitness center D, fitness center E and fitness center F. Duplicate swab samples were taken from six types of sports equipment: bench presses, barbells, treadmills, dumbbells, bicycles, and lat pull-down equipment. The equipment was chosen based on its frequent utilization and direct skin contact during sporting engagements. One swab sample was taken for the analysis of bacterial load and MRSA detection, and the second was taken for fungal growth detection.

The designed study was approved by the Near East University Ethical Committee in February 2022, NEU/2022/100-1499.

Sample Collection

Sampling was conducted from March to July 2022. Swab samples were collected from bench presses, barbells, treadmills, dumbbells, bicycles, and lat pull-down equipment from each fitness center. Then, the samples were analyzed by following the swab method. The surfaces of the sports equipment were swabbed with sterile cotton swabs and stored in transport media.

Afterward, the samples were transported to the Microbiology Laboratory for microbiological analysis.

Microbiological Analysis of nasal swab samples for MRSA detection

The nasal swab samples were taken to the laboratory within six hours in a cold chain at +4 °C to protect the collected samples from possible external factors. Then, the samples were inoculated in 5% sheep blood agar (Becton and Dickinson) and incubated at 35 °C for 24-48 hours. After incubation, smooth-edged, round, raised and yellow-colored colonies were evaluated as *S. aureus*. Subsequently, catalase–coagulase tests were performed on the abovementioned colonies. Catalase-positive–coagulase-negative strains were identified as coagulase-negative *Staphylococci*, and catalase-positive–coagulase-positive strains were identified as *S. aureus* (Dalman et al. 2019).

Colonies evaluated as *S. aureus* were suspended in brain heart infusion agar according to the standard of McFarland 0.5. Colonies taken from the suspension with a sterile swab were plated on Mueller–Hinton agar. The Kirby–Bauer disc diffusion method was used for MRSA detection. Then, 30 µg of cefoxitin disc (Oxoid, London, UK) was placed on the same Muller–Hinton agar media according to EUCAST standards. The media were incubated at 35 °C for 24-48 hours. Isolates with a zone diameter of <20 mm were identified as MRSA (EUCAST 2019). The isolates were stored in brain heart infusion broth at -80 °C for further processing.

Microbiological Analysis of Equipment

Duplicate swab samples were obtained from bench presses, barbells, treadmills, dumbbells, bicycles and lat pull-down equipment from six fitness centers. Immediately after the swab samples were collected, one swab sample was inoculated on 5% sheep blood agar for MRSA detection, and the second swab sample was inoculated into Sabouraud dextrose agar (SDA) for fungal growth detection. This process was repeated for each piece of equipment in the fitness centers. Inoculated sheep blood agar was incubated at 35 °C for 24-48 hours. After incubation, colonies were recorded in colony-forming units (CFU/ml) for the prediction of bacterial load in each piece of equipment.

The colonies that formed smooth-edged, round, crusted and yellow-colored colonies on the sheep blood media were evaluated as *S. aureus*, and a catalase–coagulase assay was performed. Colonies

evaluated as *S. aureus* were suspended in brain heart infusion with the standard of McFarland 0.5 (10^8 colonies/ml). Colonies taken from the solution with a sterile swab were inoculated on Mueller–Hinton agar. The Kirby–Bauer disk diffusion method was used for MRSA detection. Then, 30 µg of cefoxitin disk (Oxoid, London, UK) was placed on the same Muller–Hinton agar according to EUCAST specifications. The media were incubated at 35 °C for 24–48 hours. Isolates with a zone diameter of <20 mm were defined as MRSA (EUCAST 2019). The isolates were maintained at -80 °C in brain heart infusion broth for further processing.

In addition, the inoculated SDA media were incubated at 25 °C for 5 days. Fungal colonies were grouped according to macroscopic colony characteristics (e.g., color, shape, and height). For fungal identification, microscopic findings were performed with lactophenol cotton blue procedures.

DNA Extraction

The isolates were grown on blood agar at 35 °C overnight, and genomic DNA was extracted from cultures using the boiling method. A few colonies were diluted in 500 µl of sterile phosphate-buffered saline (PBS) in 1.5 Eppendorf tubes and incubated at 100 °C for 15 minutes using a heat block to lyse the bacterial cells and free the DNA. After 15 minutes of boiling, the tubes were centrifuged at $13\,000 \times g$ for 5 minutes to collect the lysed cells at the bottom of the tube. The supernatant containing genomic DNA was carefully transferred to a new sterile Eppendorf tube and stored at -20 °C until use (Barbosa et al. 2016).

In-House PCR *mecA* detection

A conventional gel-based in-house PCR assay was used for the simultaneous detection of the *mecA* gene to confirm *S. aureus* species. The PCR mixture was prepared in a 25 µl reaction volume, which included 12.5 µl of 2x Taq master mix (Thermo Scientific), 1 µl of each gene-specific primer (*mecA*-F and *mecA*-R) at a 10 µM concentration, and 2 µl of template DNA nuclease-free water. The primer sets used for PCR amplification are shown in Table 1 (Cuny and Witte 2015).

Table 1. Primers used for the amplification of *mecA/nuc* genes and amplicon sizes (Cunny and Witte 2005).

Primer Name	Primer Sequence 5'→3' Amplicon	Size Base Pair (bp)
<i>mecA</i> -Forward	AAA ATC GAT GGT AAA GGT TGG C	533
<i>mecA</i> -Reverse	AGT TCT GCA GTA CCG GAT TTG C	

Statistical Analysis

In this study, the Statistical Package for Social Sciences (SPSS) 20 program was used to perform the appropriate statistical analysis. Continuous variables (quantitative variables) obtained by measurement are presented as the mean ± standard deviation and minimum and maximum values. Categorical variables (qualitative variables) are presented as frequencies and percentages. The chi-square test was used when comparing qualitative variables. The Kruskal–Wallis test was used as a statistical method when comparing group means since parametric assumptions were not provided. In all the statistical analyses, the level of significance was accepted as “ $p \leq 0.05$ ”.

3. Results

Fungal Growth on sports equipment

The comparison of fungal growth found no statistically significant difference between any of the sports equipment. However, a statistically significant difference was found in the fungal growth of the fitness centers ($p < 0.05$). There was a greater percentage of fungal growth (62.5%) in fitness center F than in the other fitness centers. The other fitness centers had similar rates of fungal growth (Table 2).

The distribution of fungal species in the sports equipment was similar ($p>0.05$). Predominantly *Aspergillus* spp. were detected.

Table 2. Fungal Growth in Fitness Centers and on Sports Equipment.

	No growth		Growth Present		Total		p
	n	%	n	%	n	%	
Fitness centers							
Fitness center A	6	21.4%	-	-	6	100.0%	0.005*
Fitness center B	5	17.9%	1	12.5%	6	100.0%	
Fitness center C	6	21.4%	-	-	6	100.0%	
Fitness center D	5	17.9%	1	12.5%	6	100.0%	
Fitness center E	5	17.9%	1	12.5%	6	100.0%	
Fitness center F	1	3.6%	5	62.5%	6	100.0%	
Sports Equipment							
Bench Presses	5	17.9%	1	12.5%	6	100.0%	0.399
Barbells	4	14.3%	2	25.0%	6	100.0%	
Treadmills	5	17.9%	1	12.5%	6	100.0%	
Dumbbells	5	17.9%	1	12.5%	6	100.0%	
Bicycles	3	10.7%	3	37.5%	6	100.0%	
Lat Pulldown	6	21.4%	-	-	6	100.0%	

p: Chi-square test; *: $p \leq 0.05$

Bacterial load results for the swab samples of gym equipment

The bacterial loads among the tested sports equipment are displayed in Table 3. The comparison shows that the sports equipment had similar bacterial loads, with no significant differences ($p>0.05$). However, a statistically significant difference was found in terms of the bacterial load of fitness centers ($p<0.05$). While the bacterial load was lower in Fitness center F than in the other fitness centers, it was the highest in Fitness center E. The other fitness centers showed similar bacterial loads (Table 3).

Table 3. Bacterial Loads of Fitness Centers and Sports Equipment.

Bacterial Load	n	$\bar{X} \pm SD$	Average	Minimum–Maximum	p
Fitness centers					
Fitness center A	6	49166.7±46088.7	40000.00	5000-100000	0.009*
Fitness center B	6	23333.3±15055.5	20000.00	10000-50000	
Fitness center C	6	18666.7±12754.1	20000.00	2000-40000	
Fitness center D	6	35000±25099.8	30000.00	10000-70000	
Fitness center E	6	78333.3±13291.6	80000.00	60000-100000	
Fitness center F	6	10000±5477.2	10000.00	5000-20000	
Sports Equipment					
Bench Presses	6	39166.7±37738.1	25000.00	5000-100000	0.916
Barbells	6	40000±35213.6	30000.00	10000-100000	
Treadmills	6	31166.7±40002.1	10000.00	2000-100000	
Dumbbells	6	36666.7±30767.9	25000.00	10000-80000	
Bicycles	6	39166.7±32002.6	35000.00	5000-80000	
Lat Pulldown	6	28333.3±26394.4	20000.00	10000-80000	

$\bar{X} \pm SD$ average; SD: standard deviation; p: Kruskal–Wallis test; *: $p \leq 0.05$

Results for nasal swab samples

A total of 110 people were included in the study. A total of 32/110 (29.1%) of the participants in the study were female, and 78/110 (70.9%) were male. Nasal swab samples were collected from a total of 100 fitness center members and 10 personal trainers (Table 4).

Table 4. General Information about the Participants.

	n	%
Gender		
Female	32	29.1
Male	78	70.9
Participants		
Personal Trainer (PT)	10	9.1
Gym members	100	90.9

#: Percentage

The distribution of MRSA strains among the participants is shown in Table 5. All of the personal trainers were negative for coagulase-negative *Staphylococcus* spp. and *S. aureus*, and 107/110 (97.3%) of the total participants were negative. There were only 3/107 (2.7%) MRSA carriers. There was no statistically significant difference in the number of MRSA carriers between the trainers and the trainees in this study ($p > 0.05$) (Table 5).

Table 5. Distribution of MRSA among the Participants.

	Personal Trainer		Member		Total		P
	n	%	n	%	n	%	
Coagulase-negative <i>Staphylococcus</i> spp.							
Negative	10	100.0%	97	97.0%	107	97.3%	0.579
Positive	-	-	-	3.0%	-	2.7%	
<i>S. aureus</i>							
Negative	10	100.0%	97	97.0%	107	97.3%	0.579
Positive (MRSA)	-	-	3	3.0%	3	2.7%	

p: Chi-square test; *: $p \leq 0.05$

PCR Results

In-house PCR was used for the simultaneous detection of *mecA* genes in genomic DNA from 3 MRSA-positive samples according to culture methods. Of these 3 positive samples, 66.6% (2/3) were *mecA* positive. PCR revealed that 2 of the isolates that were positive for the *mecA* gene had a 533 bp band detected by agarose gel electrophoresis and were confirmed to be MRSA (Figure 1). The 2 PCR-positive MRSA strains were isolated from 2 gym members, both of whom were male.

4. Discussion

This study aimed to explore the microbial loads associated with different equipment surfaces and people from six fitness centers using culture-based methods. The isolates in this study were predominantly *Staphylococcus* spp. The presence of bacteria was detected in all the investigated sports equipment in fitness centers with different bacterial loads. This may be due to frequent contact between equipment and gym members and the presence of bacteria in human flora. However, the importance here is that bacteria are viable on the surfaces of the equipment, and if they are contaminated by any of the disease-causing pathogens, indirect transmission is possible.

One of the most famous antibiotic-resistant strains among staphylococci is MRSA. It is known to be transmitted from person to person through skin contact, fomite contact, or contact with contaminated surfaces (Mukherjee et al. 2014). In the present study, the prevalence of MRSA in fitness centers was zero. Nonetheless, out of 110 nasal swab cultures obtained, 3 (2.7%) samples were positive for MRSA. All MRSA-positive individuals in this study were gym members.

The 3 MRSA carriers identified through conventional methods were verified through an in-house PCR method in which the detection target was *mecA*. However, only 2 patients were PCR-positive. This may be due to several reasons. First, oxacillin resistance mechanisms other than *mecA* resistance are rare. Accurate detection of oxacillin/methicillin resistance can be difficult due to the presence of two subpopulations (one susceptible and the other resistant) that may coexist within a culture of staphylococci. All cells in culture may carry genetic information (*mecA*) for resistance, but only a small number may express resistance *in vitro*. This phenomenon is termed heteroresistance and occurs in staphylococci that are resistant to penicillinase-stable penicillin, such as oxacillin. Second, the in-house PCR method used in the study was able to detect *mecA*, the most common gene mediating oxacillin resistance in staphylococci. However, *mecA* PCR tests cannot detect novel resistance mechanisms, such as *mecC*, or uncommon phenotypes, such as borderline-resistant oxacillin resistance. Therefore, if genes other than *mecA* are present, they will not be detected by the PCR test (CDCb 2019).

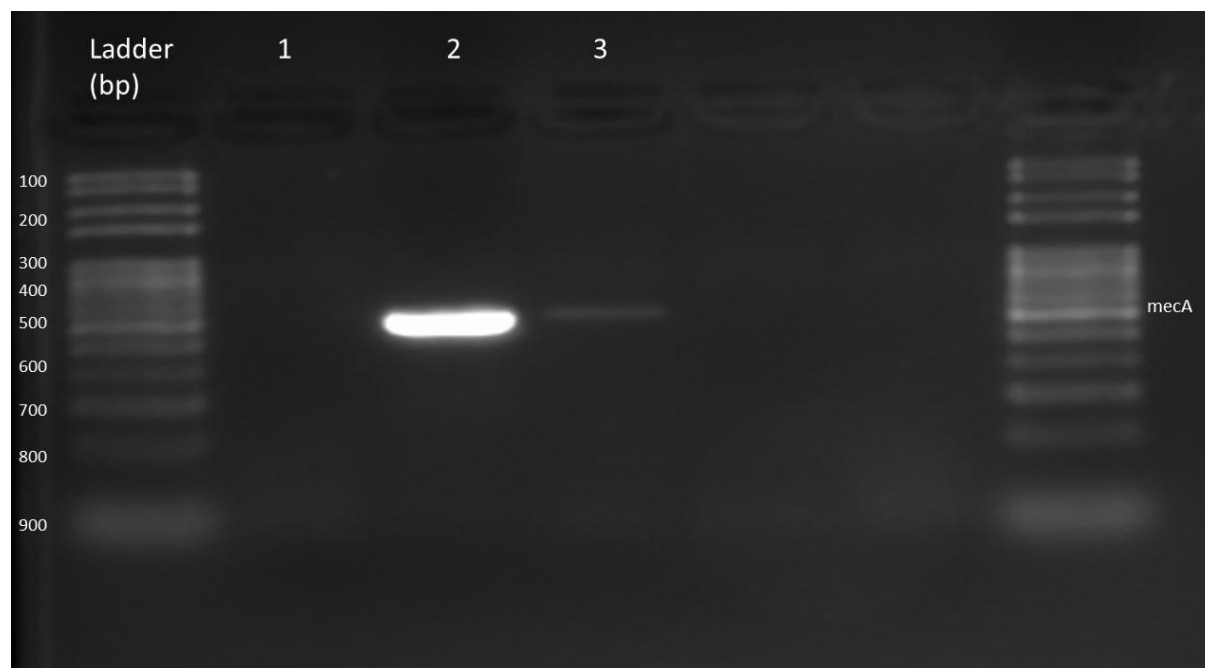


Figure 1. Detection of the *mecA* gene in *Staphylococcus aureus* isolates using an in-house PCR. The ladder in base pairs (bp) is shown on the left. The 3 MRSA samples from MRSA-positive gym members detected via culture techniques were further analyzed via in-house PCR. Among these 3 positive samples, only 2 were *mecA* gene positive according to PCR. Lanes 2 and 3 indicate 2 MRSA-positive samples of approximately 530 bp.

Other studies, such as that of Dalman M et al., reported that the percentages of *S. aureus* and MRSA on environmental surfaces in fitness facilities were 26.7% and 11.5%, respectively (Ramos et al. 2016). Moreover, Ryan et al. reported the absence of *S. aureus* on fitness center facility surfaces (Ryan et al. 2011). The present study found that the prevalence of MRSA in the studied fitness centers was 2.7%. The prevalence rate of *S. aureus* is unique. This may be attributed to the conditions of the fitness centers in this study. Therefore, these findings expand the limited information available on bacterial presence in fitness centers and increase public health awareness about such facilities. There is a clear need for etiquette and proper precautions to be taken when utilizing sports equipment in fitness centers to prevent potential pathogen transmission. Such precautions include taking a shower and washing hands before and after exercise to decrease the risk of transmission of pathogens (Prevost and Simms 2021).

Surprisingly, there was no significant association between bacterial load and fungal growth with different types of equipment. Ibrahim et al. reported that copper alloy weights and grips reduced bacterial colonization by as much as 94% compared to their rubber and stainless-steel counterparts (Ryan 2011). The most prevalent fungal genera found in this study were consistent with those found in other studies. Viegas et al. described *Cladosporium sp.* as the principal isolated genus in a gym, followed by *Penicillium spp.* and *Aspergillus spp.* (Viegas et al. 2010). Additionally, Ramos et al. revealed that in two indoor gyms, fungal

species such as the *Aspergillus* genus, belonging to *A. fumigatus*, are considered to be indicators of moisture-damaged buildings (Ramos et al. 2016). In this study, *Aspergillus* spp. were also predominant.

On the other hand, it is still unclear whether community gym surfaces and equipment support bacterial transmission and colonization. In community gyms, equipment is used frequently; hence, infection transmission through fomite contact is possible (Ryan 2011).

Fitness facilities are advised to establish a hygienic environment for their clients (Fadare and Durojaye 2019). Although the sample size of this study is sufficient and reliable, for future studies, other places in fitness centers, such as doorknobs, gym-provided towels, locker rooms and water foundations, may be included for further analysis.

In this study, the equipment of six fitness centers was evaluated for bacteria and fungi at night, and the cleaning routine of the evaluated fitness centers was performed in the morning. However, information about how cleaning was performed was not gathered; thus, this is one of the limitations of this study. Additionally, cleaning agents used in cleaning processes, such as disinfectant wipes, are key to reducing or eliminating the presence of staphylococci. The second limitation of this study was that only a culture-dependent technique was implemented. With this technique, it is difficult to culture and identify a large number of microorganisms. Therefore, future studies might extend the investigation of microbial populations in fitness centers by using next-generation sequence techniques.

5. Conclusions

In summary, despite the increasing incidence of CA MRSA infections, the fitness centers included in this study did not appear to be significant sources of staphylococcal or fungal infections. However, 3 gym members were identified as MRSA carriers; hence, spread between the members and personal trainers is still possible.

Authors' Contributions: YAZIR, C.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article. Nazife Sultanoglu: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; GUVENIR, M.: conception and design, acquisition of data, analysis and interpretation of data, critical review of important intellectual content; HURDOGAN OGLU, U.: conception and design, analysis and interpretation of data; YAVUZ, H.U.: analysis and interpretation of data, critical review of important intellectual content; SUER, K.: conception and design, analysis and interpretation of data, critical review of important intellectual content.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: The designed study has been approved by the Near East University Ethical Committee in February 2022, YDU/2022/100-1499. The document of the approval has been added as a supplementary file.

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