Article

Not peer-reviewed version

Characterization of the Prevalence and Antibiotic Resistance among Staphylococcus species in an Exercise Facility in Central Kentucky, USA

Lilian Jeptoo Kiborus , S. Travis Altheide , Jason W. Marion *

Posted Date: 12 December 2024

doi: 10.20944/preprints202412.1027.v1

Keywords: Methicillin-resistant *Staphylococcus aureus*; antibiotic resistance; antimicrobial resistance; *Staphylococcus saprophyticus*; *Staphylococcus haemolyticus*; contamination; fitness center; multidrug resistance; *E. coli*; oxacillin

 doi:10.20944/preprints202412.1027.v1

Article

Characterization of the Prevalence and Antibiotic Resistance among *Staphylococcus* species in an Exercise Facility in Central Kentucky, USA

Lilian Jeptoo Kiborus [1], S. Travis Altheide [2] and Jason W. Marion [1,*]

[1] Environmental Health Science & Sustainability Program, College of Health Sciences, Eastern Kentucky University, Richmond, Kentucky USA
[2] Medical Laboratory Science Program, College of Health Sciences, Eastern Kentucky University, Richmond, Kentucky USA
* Correspondence: jason.marion@eku.edu; +1 513 317 9508

Abstract: The spread of methicillin-resistant Staphylococcus aureus (MRSA) in community settings, including fitness/exercise centers, remains relevant for public health. MRSA, a cause for severe infections in some, can be transmitted through shared equipment and skin contact. Understanding its prevalence and the frequency of antibiotic resistance in such environments can be useful for informing hygiene and intervention strategies. For investigating, multiple environmental swabs were collected from 14 different sites within a fitness facility, including equipment and locker rooms. Samples were collected for characterizing the prevalence of staphylococci (including MRSA), *E. coli*, and carbapenem-resistant enterococci (CRE). Isolated colonies were identified biochemically and evaluated for antibiotic resistance. Logistic regression was applied to assess risk across different surfaces. Among 42 samples, the highest prevalence of *Staphylococcus* spp. was on locker room surfaces. *S. aureus* was prevalent on locker room floors and benches. Non-*S. aureus* species, such as *S. saprophyticus* and *S. haemolyticus*, were common. Resistance to oxacillin and penicillin was widespread, particularly among non-*S. aureus* species. *E. coli* were detected once, and CRE were not detected. Fitness center surfaces can harbor staphylococci, including MRSA. This facility, and probably others, have notable antibiotic resistance among other staphylococci. Hygiene improvements, including personal hygiene actions, are essential for reducing transmission risks.

Keywords: Methicillin-resistant *Staphylococcus aureus*; antibiotic resistance; antimicrobial resistance; *Staphylococcus saprophyticus*; *Staphylococcus haemolyticus*; contamination; fitness center; multidrug resistance; *E. coli*; oxacillin

1. Introduction

Surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) remains essential for understanding the spread and persistence of these bacteria, particularly in and from community settings. The public health significance of *S. aureus* is considerable. In 2017, there were an estimated 119,247 *S. aureus* bloodstream infections and 19,832 related deaths in the U.S. alone [1]. Surveillance data are crucial for guiding public health interventions, including improvements in hygiene practices and antibiotic stewardship, both of which help reduce infections and enhance the effectiveness of antibiotics. The U.S. has made notable progress in reducing hospital-onset MRSA bloodstream infections from 2005 to 2012, with a 17.1% annual decline, although the rate of decline has slowed in recent years [1]. Of greater concern is the relatively slower decline in community-associated MRSA infections, which decreased by just 6.9% annually from 2005 to 2016, highlighting the importance of community settings in MRSA transmission and colonization, while also illustrating the need for targeted interventions in these environments.

 doi:10.20944/preprints202412.1027.v1

Fitness environments, especially those enabling shared physical contact, such as gyms and athletic facilities, may play a significant role in MRSA transmission. Studies have shown that athletes are at high risk of both *S. aureus* colonization and infection, with MRSA responsible for a third of infectious outbreaks recorded among high school and collegiate athletes [2]. The prevalence of MRSA infections in National Football League (NFL) players is about 400 times higher than in the general population [3]. While many individuals who carry *S. aureus*, including MRSA, remain asymptomatic, this colonization presents a public health risk, as carriers can unknowingly transmit the bacteria to fomite surfaces, such as gym equipment and mats [4]. This is further complicated by the fact that fitness center equipment is shared among multiple users, thereby enabling numerous opportunities for bacterial transmission, especially if proper hygiene practices are not followed.

The prevalence of MRSA and *S. aureus* in the general population remains significant. A study from the National Health and Nutrition Examination Survey (NHANES) found that 28.6% of the U.S. population carries *S. aureus*, with 1.5% carrying MRSA [5]. Among MRSA carriers, approximately 20% harbor a community-acquired strain [5]. More recent global data suggest that the prevalence of *S. aureus* and MRSA carriage is high in young children, with carriage rates of 25.1% for *S. aureus* and 3.4% for MRSA [6]. Additionally, healthcare workers remain a high-risk group, with MRSA carriage reported in 6.9% of nursing staff in Europe and the U.S. [7]. These findings underscore the relevance of fitness centers as potential sources of MRSA transmission, given the overlap between populations of gym users, healthcare workers, and athletes, all of whom are more likely to carry the bacteria.

Research on the role of fitness equipment and gym surfaces in harboring *S. aureus* has yielded mixed results. For example, a study conducted by Ryan et al. (2011) in Florida found no MRSA or MSSA on gym surfaces before or after cleaning in three local gyms, suggesting that gym equipment might not always act as a significant reservoir for *S. aureus* [8]. However, the Florida study did not present an enrichment or recovery step, and other studies have indicated that shared gym surfaces, such as weights, bars, and mats, can indeed harbor *S. aureus*, all of which could be contributing to transmission when users engage in skin-to-contaminated surface contact or fail to use best hygienic practices [9].

The increased awareness of the importance of non-aureus staphylococci (NAS) adds another layer of complexity to the surveillance of *S. aureus* in fitness settings. NAS, including *S. epidermidis, S. saprophyticus, S. haemolyticus*, among others, have been observed in healthcare and agricultural environments, where they can acquire and share resistance genes to any staphylococci through horizontal gene transfer [10]. While these species are less clinically significant than *S. aureus*, they are capable of harboring antimicrobial resistance (AMR) genes that may be transferred to more pathogenic species, which could be exacerbating the spread of resistance from fitness environments [11]. The role of NAS in fitness settings is underexplored, but their ability to form biofilms and transfer AMR genes in many environments makes them a growing concern for public health.

In addition to MRSA and MSSA, fitness centers may also harbor other antibiotic-resistant pathogens. A study by Zhang et al. (2023) found that gym equipment surfaces can host multidrug-resistant pathogens, such as *Staphylococcus haemolyticus*, which is capable of harboring resistance genes. Their results also suggested that resistance genes, including sul1 and blaTEM, were abundant on indoor gym equipment surfaces relative to outdoor equipment, indicating that indoor fitness environments may act as hotspots for the dissemination of antimicrobial resistance genes [12].

Given the potential for fitness centers to serve as transmission vectors for MRSA and other resistant pathogens or resistance genes, further surveillance and intervention strategies may be necessary. While some studies have shown that gym surfaces may not always be significant reservoirs for *S. aureus*, the risk of transmission through shared equipment and physical contact remains high. The increasing prevalence of NAS and their role in transferring resistance genes further complicates the issue. Therefore, understanding the diversity of staphylococci species and their resistance profiles in fitness environments is crucial for informing effective hygiene practices and antibiotic stewardship interventions.

In this study, we aimed to investigate the prevalence of MRSA and fecal indicator bacteria in a fitness facility in Central Kentucky, primarily frequented by young adults but open to individuals of

 doi:10.20944/preprints202412.1027.v1

all ages. We hypothesized that skin contact surfaces, locker rooms, and floor areas would yield the highest prevalence of MRSA. By identifying and characterizing the bacterial species present, along with their antibiotic resistance profiles, this study seeks to contribute valuable insights into the microbial risks in fitness centers and guide future interventions to reduce the spread of MRSA, other resistant pathogens, and the transfer of antibiotic resistance genes among bacterial species.

2. Materials and Methods

2.1. Study Location and Description

All environmental swabs were collected from within the exercise/fitness facility on 18 March 2022. The large (12,000 m^2) modern facility was constructed within the last 10 years and is in Central Kentucky, USA. The facility was designed purposely for exercise, fitness, and recreation, with features including a large selection of free weights, nautilus equipment, exercise bikes, treadmills, dance studio, swimming pool, whirlpool, several full-size basketball and racquetball courts, game room, locker rooms, saunas, and more.

Users of the facility are mostly young adults (18 to 34); however, the facility also attracts and welcomes older adults, teenagers, and older children. In the week leading up to the testing, there was an estimated 950 unique users, with an estimated 525 (55%) female users and 425 (45%) male users. In the areas of the facility where persons workout (e.g., flat benches, nautilus equipment, free weights, exercise bikes, etc.), there are conspicuously placed bins for dispensing disinfecting (quaternary ammonium) wipes for use before and after using equipment. There are no wipes in the locker room facility and no study was done on the prevalence of wipe use during the study.

2.2. Sample Collection and Culture-Based Methods

For assessing the prevalence of *S. aureus*, MRSA, *E. coli*, and carbapenem-resistant Enterococci (CRE), a cross-sectional study was performed. All samples were collected during the regular hours of the facility just prior to the regular closing time. Permission to collect samples from the facility was granted in advance by the facility management. A total of 42 samples were collected from 14 sites or items in the venue, with a focus on surfaces and crevices of exercise equipment and machines, including a yoga mat, sit-up bench, leg extension nautilus machine, abdominal nautilus machine, leg press, incline press bench, as well as spaces in locker room areas of both men's and women's areas including floors near benches, wooden seating benches, floor tiles of showers, and the men's and women's shower benches within individual shower stalls.

Samples were collected for *Staphylococcus*, including MRSA, as well as CRE using saline-wetted cotton-tipped swabs. *E. coli* samples were obtained using EnviroSwab™ sponges on a stick, premoistened with neutralizing buffer at similar locations. For *Staphylococcus* spp. and CRE testing, at least three saline water-wetted swabs were obtained per location or item before sanitation occurred, and the swabs were then placed into appropriate enrichment media. TSB with 6.5% saline was used for *Staphylococcus aureus* and CRE; whereas Coliglow media [13] was used for *E. coli.* The swabs were placed in the broth and left in the broth during incubation for *Staphylococcus aureus* at 35° C and for thermotolerant *E. coli* at 44° C for 24h. All the tubes were labeled before heading to the facility.

Following incubation in selective enrichment broth, sterile loops were used to inoculate selective-differential plates for the presumptive identification of *S. aureus*, MRSA, CRE, and *E. coli* using Baird-Parker agar, HardyCHROM MRSA/*S. aureus* biPlates, plates, HardyCHROM CRE agar and modified m-TEC agar, respectively. Plates were then incubated for 24h at 35° C. The presence/absence of growth was recorded after incubation on plates, and for plates with growth, the colony color and morphology were documented. For determining growth on Baird-Parker, black to gray colonies were indicative of growth. For HardyCHROM MRSA/SA plates, pink to magenta colonies were presumed MRSA, and other colonies (e.g., blue, white, etc.) were presumed to be other staphylococci or Gram-positive cocci. Pink and magenta colonies on the modified m-TEC agar were deemed to be *E. coli* [14].

 doi:10.20944/preprints202412.1027.v1

2.3. Staphylococci Species Identification and Antibiotic Susceptiblity Testing

Among the colonies growing on HardyCHROM MRSA/SA plates, randomly selected isolated colonies were further evaluated for species identification and antibiotic susceptibility testin (AST) via Positive Combo 44 (PC-44) MicroScan Panels (Beckman-Coulter [15]) with the MicroScan® autoScan-4 system with LabPro v4.42. Specifically, isolates from HardyCHROM MRSA/SA plates were inoculated on blood agar and incubated for 18h. Then, plates were evaluated for hemolysis, and colonies were inoculated into the MicroScan panels using the manufacturer's provided Prompt inoculation system, which standardized the inocula for the necessary microdilutions at 0.5 McFarland turbidity standard for the various biochemical assays and AST analyses occurring with each PC-44 panel. Each panel analyzed by the MicroScan auto-Scan-4 system provides the presumed species identification based from the biochemical results, and the minimum inhibitory concentration to the various antibiotics and antibiotic combinations.

Upon receiving the results of each panel analyzed by the MicroScan auto-Scan-4 system, the minimum inhibitory concentrations (MIC) were evaluated with respect to the MIC breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI). The CLSI-determined MIC breakpoints as viewable in their 2023 MIC breakpoint table [16] provided by the Clinical and Laboratory Standards Institute (CLSI), correspond to their published MIC breakpoints [17]. The focus of our analysis was resistance (>=R) to various antibiotics, which was assessed for *S. aureus* and *Staphylococcus* spp. for all other species.

2.4. Data Analysis

For assessing the potential risk of exposure to *S. aureus* and other bacteria to understand potential hygiene-related interventions, the prevalence of bacterial growth was investigated by location and agar. Specifically, results were analyzed by comparing the overall number of positive results across different agar types and the sample collection locations/items. Categorical assessments were also performed by coding results for enabling larger comparison groups, such as "locker room", which included all results from men's and women's locker rooms, including the floors, locker room changing area benches, shower benches, and shower floors. Based upon the categorical groupings, simple logistic regression analyses were performed in Stata 15 to provide odds ratios and Wald (χ^2) test *p*-values to assist in informing risk assessments relative to the areas sampled.

For characterizing the bacterial isolates with respect to their antibiotic resistance levels, the frequency of resistance was determined. The results were also comparative for enabling the frequencies of resistance between isolate types to be compared. While primarily a quantitative analysis, some qualitative assessment was also performed for informing future studies or research. Specifically, the study describes the species identified and describes the locations in which species were observed.

3. Results

Overall, samples were collected from 14 unique sampling locations within the facility. At each location three swabs were collected and placed in three individual tubes of TSB + 6.5% saline totaling to 42 samples for plating and analysis on Baird-Parker, HardyCHROM MRSA, and HardyCHROM CRE agars. Similarly, 42 EnviroTrans roll swabs were used and placed in ColiGlow media. Negative and positive control swabs were placed in each and achieved appropriate negative and positive growth. The results of the sampling effort are organized around the types of surfaces that were tested.

3.1. Positive Detections for Staphylococcus spp. and E. coli on Sampled Surfaces

Among the locations and surfaces within the fitness center that were evaluated, the results in Table 1 illustrate that growth among different media type was greatest on the Baird-Parker agar indicating a high prevalence a presumably *Staphylococcus* spp., including *S. aureus.* All 24 samples from the locker rooms were positive for growth on Baird-Parker, and nearly all (39 [92%] of 42) samples collected were positive for growth on Baird Parker agar.

 doi:10.20944/preprints202412.1027.v1

Table 1. Frequency of positive swab tests for various categorizations of sampled surfaces organized by culture media type in the exercise center in March 2022.

	Baird-Parker	HardyChrom MRSA Agar		*E. coli* +	CRE+
Types of Surfaces	Media +	Pink +	Blue +		
Locker Room	24/24 (100)	10/24 (42)	20/24 (83)	1/24 (4)	0/0 (0)
Shower Area	12/12 (100)	2/12 (17)	10/12 (83)	0/0 (0)	0/0 (0)
Locker/Shower Floor	12/12 (100)	5/12 (42)	11/12 (92)	0/0 (0)	0/0 (0)
Leg Contact Area	5/6 (83)	1/6 (17)	3/6 (50)	0/0 (0)	0/0 (0)
Flat Surfaces	21/24 (88)	7/24 (29)	17/24 (71)	0/0 (0)	0/0 (0)
Locker Benches	12/12 (100)	3/6 (50)	9/12 (75)	1/12 (8)	0/0 (0)
Men's Locker Room	12/12 (100)	6/12 (50)	10/12 (83)	0/0 (0)	0/0 (0)
Women's Locker Room	12/12 (100)	4/12 (33)	10/12 (83)	1/12 (8)	0/0 (0)
Crevice Surfaces	2/3 (67)	0/3 (0)	1/3 (33)	0/0 (0)	0/0 (0)
Flat Surfaces	21/24 (88)	7/24 (29)	17/24 (71)	1/24 (4)	0/0 (0)
Handle Surfaces	2/3 (67)	1/3 (33)	1/3 (33)	0/0 (0)	0/0 (0)

When using more selective media, the HardyChrom MRSA agar showed a greater prevalence of blue-colored colonies than pink colonies. Table 1 shows that among both pink and blue *Staphylococcus* colonies, both were commonly observed in the locker room areas. The pink colonies, which were typically *S. aureus* (Table 2), were most abundant in the locker rooms, particularly on the floors and benches, with positive tests being observed from 50% and 33% of enriched swab samples from the men's and women's locker rooms, respectively. Outside the locker rooms, there were areas in the fitness center that were also positive where leg and hand contact occurs, as well as several flat surfaces, which had periodic pink-colored colonies observed. Specifically, among the colonies confirmed as *S. aureus,* they were observed on the back support for the leg press, the seat for the leg press, handles for the leg press, on the floor below locker room benches in men and women's bathrooms, and on a bench in a shower area of the women's locker room (Supplemental Table S1).

Table 2. Species identification by colony color using MicroScan technology among randomly selected isolates from the HardyCHROM™ MRSA/*Staph aureus* Chromogenic Media BiPlate.

Species (Abbreviation)	Total	Color on Chromogenic Media Plate (%)			
		Blue	White	Pink	Black
S. aureus (SA)	11	1 (9.1)	0 (0)	9 (82.8)	1 (9.1)
S. haemolyticus (SH)	8	1 (12.5)	7 (87.5)	0 (0)	0 (0)
S. saprophyticus (SSap)	7	7 (100)	0 (0)	0 (0)	0 (0)
S. epidermidis (SE)	5	2 (40.0)	1 (20.0)	2 (40.)	0 (0)
S. cohnii-cohnii (SCC)	3	1 (33.3)	2 (66.7)	0 (0)	0 (0)
S. hominis-homin (SHH)	3	1 (33.3)	2 (66.7)	0 (0)	0 (0)
Aerococcus viridans (AV)	2	0 (0)	1 (50.0)	1 (50.0)	0 (0)
S. simulans (SSim)	2	0 (0)	1 (50.0)	1 (50.0)	0 (0)
S. warneri (SW)	2	0 (0)	1 (50.0)	1 (50.0)	0 (0)
Very rare biotype	2	1 (50.0)	1 (50.0)	0 (0)	0 (0)
S. cohnii-urea (SCU)	1	1 (100)	0 (0)	0 (0)	0 (0)
S. intermedius (SI)	1	0 (0)	1 (100)	0 (0)	0 (0)
S. sciuri (SSci)	1	1 (100)	0 (0)	0 (0)	0 (0)
Sum of Non-*S. aureus*	37	17 (45.9)	16 (43.2)	3 (8.1)	1 (2.7)
Total (all species)	48	18 (37.5)	16 (33.3)	13 (27.1)	1 (2.1)

In examining the more abundant, blue-colored colonies from the chromogenic agar, there was greater species diversity with *S. saprophyticus* being the most common, representing 7 (41%) of 17 blue colonies. The blue *Staphylococcus* isolates were most commonly observed in the locker room samples, in both male and female locker rooms, which had positive growth from 83% of enriched

 doi:10.20944/preprints202412.1027.v1

media swab samples from both locker rooms. Additionally, throughout the facility, including bench surfaces, arm surfaces, and leg contact surfaces, many were carrying recoverable, blue-colored *Staphylococcus* spp. Specifically, isolates of *S. saprophyticus* were recovered from samples on locker room benches and from the shower benches in both the women's and men's locker rooms. *S. saprophyticus* were also recovered on the incline press, and from the leg extension nautilus machine where leg contact occurs (Supplemental Table S1).

For understanding other *Staphylococcus* spp. observed in the study, white colonies were also evaluated from the HardyChrom agar plates. Among white isolated colonies, 7 (44.5%) of 16 samples were *S. haemolyticus*. In 7 (87.5%) of 8 isolates of *S. haemolyticus*, they were white with one being pink. The isolates of *S. haemolyticus* were recovered from primarily the men's and women's locker rooms, including the changing benches and the women's shower bench. In the exercise area, recovery occurred from the leg extension nautilus equipment, including the leg contact area. Additionally, an isolate was recovered from a yoga mat sample (Supplemental Table S1).

E. coli, however, were not readily detected. A single sample from a women's locker room bench represented the one sample in the study positive for *E. coli* among 42 swabs. The well-known group of carbapenem resistant enterococci (CRE) which are highly resistant to front line antibiotics are uncommon in society and were not observed in this facility. The lack of evidence of *E. coli* and CRE indicate that fecal contamination does not appear to be common in this facility based upon this single sampling effort.

3.2. Associations between Surface Types and Likelihood of Recovering Presumable S. aureus and non-S. aureus

Logistic regression analysis did not reveal any statistically significant associations ($p < 0.05$) between the various location types sampled and the likelihood of recovering a pink-colored colonies from the chromogenic agar (Table 3). Although not significant, the location with the greatest likelihood of having pink colonies (presumable S. aureus) be observed was among samples swabbed from the locker room environment. Specifically, samples from locker rooms had a 3.6 times greater likelihood (OR = 3.6, $p = 0.092$) of having a positive detection of a pink colony than non-locker room samples. Interestingly, the shower areas, which are part of the locker room samples, were estimated to have 60% less pink colonies observed (OR = 0.4), however, due to the sample size, this difference was not significantly ($p = 0.218$). Overall, the results in Table 3 illustrate that pink-colored colonies were observable throughout the facilty with a sufficient prevalence in contrasting areas that resulted in no significant differences being observable.

Table 3. Crude odds ratios indicating the likelihood of observing a pink-colored colony on the MRSA plate, organized by surface type relative to other surface types from this March 2022 recreational/fitness center sampling effort.

Types of Surfaces	cOR	95% CI	*p*
Locker Room	3.6	0.81–15.7	0.092
Shower Area	0.4	0.06–1.87	0.218
Locker/Shower Floor	2.0	0.48–8.00	0.346
Leg Contact Area	0.4	0.04–3.82	0.426
Flat Surfaces	0.8	0.22–3.07	0.773
Locker Benches	2.0	0.48–8.00	0.346
Men's Locker Room	2.0	0.38–10.4	0.410
Flat Surfaces	2.0	0.22–3.07	0.773
Handle Surfaces	1.1	0.09–13.6	0.926

In evaluating blue-colored isolates from the chromogenic agar, like the prior logistic regression analysis on pink isolates, the analysis did not reveal any statistically significant associations ($p < 0.05$) between the various location types sampled and the likelihood of recovering a blue-colored colonies from the chromogenic agar (Table 4). Although not significant, the location with the greatest

 doi:10.20944/preprints202412.1027.v1

likelihood of having blue colonies (presumable non-S. aureus Staphylococci) was the locker room environment. Specifically, samples from locker rooms had a 4.0 times greater likelihood (OR = 4.0, p = 0.056) of having a positive detection of a blue colony than non-locker room samples. These results are similar to the results from whereby pink colonies were also observed. Accordingly, many plates had both pink and blue colonies. The floors, particularly in the locker and shower areas, which are part of the locker room samples, had a 6.4-times greater likelihood of blue colonies than non-locker room floor samples, and was nearly significant (OR = 6.4, p = 0.096). Like the results in Tables 3 and 4 illustrates that blue-colored colonies were observable throughout the facilty with a sufficient prevalence in contrasting areas that resulted in no significant differences being observable.

Table 4. Crude odds ratios indicating the likelihood of observing a blue-colored colony on the MRSA plate, organized by surface type relative to other surface types from this March 2022 recreational/fitness center sampling effort.

Types of Surfaces	cOR	95% CI	P
Locker Room	4.0	0.97–16.6	0.056
Shower Area	2.5	0.46–13.6	0.290
Locker/Shower Floor	6.4	0.72–56.2	0.096
Leg Contact Area	0.3	0.06–2.00	0.224
Flat Surfaces	0.9	0.24–3.62	0.921
Locker Benches	1.3	0.28–5.89	0.746
Men's Locker Room	0.8	0.12–8.56	1.000
Crevice Surfaces	0.2	0.01–2.11	0.169
Flat Surfaces	1.3	0.28–5 89	0.746
Handle Surfaces	0.2	0.01–2.11	0.169

3.3. Antibiotic Resistance among Isolated Colonies Recovered from HardyChrom Chromogenic MRSA Agar

Among all isolates evaluated, inclusive of all species successfully identified, oxacillin and penicillin resistance was most common with 27 (59%) of 46 isolates being resistant (Table 5). When specifically focusing on the S. aureus (SA) isolates, resistance was not commonly observed despite coming from agar designed to select for methicillin-resistant isolates. For the comparable oxacillin, only one (9%) of nine S. aureus isolates was resistant, and 5 (56%) were resistant to penicillin (Table 5).

Table 5. The prevalence of resistance to various antibiotics among the most frequently isolated species and total isolates studied.

Antibiotic	Species Abbreviation (% resistant)						Among All n = 46
	SA n = 11	SH n = 8	SSap n = 7	SE n = 5	SCC n = 3	SHH n = 3	
Ampicillin	4 (36)	5 (63)	1 (14)	5 (100)	0 (0)	0 (0)	17 (37)
Azithromycin	1 (9)	5 (63)	4 (57)	1 (20)	2 (67)	2 (67)	17 (37)
Cefoxitin	1 (9)	4 (50)	2 (29)	1 (20)	0 (0)	1 (33)	9 (20)
Cefazolin	0 (0)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	1 (2)
Ceftaroline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Clindamycin	0 (0)	0 (0)	3 (38)	0 (0)	1 (33)	0 (0)	4 (8)
Daptomycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Doxycycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Erythromycin	2 (18)	5 (63)	4 (57)	1 (20)	2 (67)	2 (67)	18 (39)
Gentamicin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Levofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

 doi:10.20944/preprints202412.1027.v1

Linezolid	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Minocycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Moxifloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nitrofurantoin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)
Oxacillin	1 (9)	4 (50)	7 (100)	2 (40)	2 (67)	1 (33)	27 (59)
Penicillin	5 (45)	6 (75)	5 (71)	5 (100)	1 (33)	2 (67)	27 (59)
Rifampin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Synercid	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tetracycline	0 (0)	2 (25)	0 (0)	0 (0)	1 (33)	0 (0)	3 (7)
Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*Cell colors represent the prevelance of antibiotic resistance observed. Green < 20%, Yellow 20-39%, and Red ≥ 40%.

Upon examaning non-S. aureus species, S. haemolyticus (SH) and S. saprophyticus (SSap), had elevated levels (>40%) of resistance to four or more antibiotics. Specifically, the S. haemolyticus isolated from the chromogenic agar had elevated resistance to ampicillin (63%), azithromycin (63%), cefoxitin (50%), erythromycin (63%), oxacillin (50%), and penicillin (75%). Among the S. saprophyticus isolates, there was an elevated frequency of resistance observed for azithromycin (57%), erythromycin (57%), oxacillin (100%), and penicillin (71%). Among the five S. epidermidis isolates, all five (100%) were resistant to ampicillin and penicllin (Table 5).

Among the less common isolates that were obtained, among the two isolates of Aerococcus viridans, which were obtained from the men's shower floor and women's shower bench (Supplementary Table 1), both (100%) were resistant to oxaillin and nitrofurantoin (Table 6). No other bacteria were observed to be resistant to nitrofurantoin. The majority of the less commonly isolated species observed in this study are illustrated as having susceptibilty to most isolates; however, for several species only one or two isolates were detected and evaluated for their response to the various antibiotics.

Table 6. The prevalence of antibiotic resistance to various antibiotics among the less common isolated species observed.

Antibiotic	AV n = 2	Ssim n = 2	SW n = 2	SCU n = 1	SI n = 1	SSci n = 1
Ampicillin	0 (0)	0 (0)	1 (50)	0 (0)	1 (100)	0 (0)
Azithromycin	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)
Cefoxitin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cefazolin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ceftaroline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Clindamycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Daptomycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Doxycycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Erythromycin	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)
Gentamicin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Levofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Linezolid	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Minocycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Moxifloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nitrofurantoin	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oxacillin	2 (100)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)
Penicillin	0 (0)	0 (0)	2 (100)	0 (0)	1 (100)	0 (0)

 doi:10.20944/preprints202412.1027.v1

Rifampin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Synercid	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tetracycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*Cell colors represent the prevelance of antibiotic resistance observed. Green < 20%, Yellow 20-39%, and Red ≥ 40%.

4. Discussion

4.1. Comparing the Prevalence of Staphylococci and Fecal Indicator Bacteria in Fitness Center Environments

This study examined 14 pieces of equipment or surfaces in a large fitness center and observed recoverable staphylococci in 92% of all swab samples, indicating the ubiquity of these organisms in the environment. Studies evaluating *S. aureus* transmission potential demonstrated their survival on sports balls for over 72 hours [18], corroborating findings that *S. aureus* can survive for up to 12 days on inanimate surfaces [19]. A Malaysian study that also assessed 42 samples across three gyms found positive results for presumed *S. aureus* on Baird-Parker agar in 100% (14/14), 93% (13/14), and 29% (4/14) of swabs from the three gyms studied [20]. In the context of other studies, the results we obtained further contribute to the evidence that *S. aureus* is very common in fitness environments. Beyond the intuitive knowledge that skin contact with surfaces contributes to their environmental transmission, recent literature also indicates that culturable *S. aureus* can also be obtained from the air inside sports facilities [21].

Regarding fecal indicator bacteria prevalence, there is little scientific literature indicating a significant problem in fitness centers in the U.S. Some literature from a university fitness center in Taiwan documented *E. coli* contamination on the soft handles of bicycles, sit-up benches, and dumbbells [22]. Our study did not examine many handles, which, if sampled more thoroughly, might have yielded more *E. coli* results. The finding of *E. coli* in our study on a bench in the changing area is not entirely unsurprising. While carbapenem-resistant *Enterobacteriaceae* (CRE) remains an urgent public health threat and has the potential to be community-associated, the absence of community-associated CRE is not uncommon in community studies [23], especially since we found little evidence of fecal indicator bacteria in the fitness center we studied. Our results on fecal indicator bacteria may be slightly biased because we used a higher incubation temperature (44°C) for *E. coli*, which, while more selective for thermotolerant coliforms, particularly *E. coli*, may have suppressed the growth of non-thermotolerant coliforms. Some *E. coli* strains may have grown too poorly for detection or not grown at all, which could have led to us underestimating the presence of *E. coli* in the studied environment [24–26]. In contrast, testing at 35°C might have yielded higher *E. coli* counts due to more permissive growth conditions for a broader range of *E. coli* strains, but this could have allowed the growth of other coliforms, potentially resulting in false positives from non-*E. coli* coliforms [13,24–26].

4.2. Comparing the Prevalence of Antibiotic Resistance and MRSA Prevalence in Fitness Environments

Among studies evaluating antibiotic resistance in exercise environments or community settings, particularly regarding MRSA, the results are mixed. In a study regarding MRSA at a large Midwestern (USA) university campus, MRSA prevalence was 6% among 152 samples, with 22% of samples positive for *S. aureus* [27]. Using a similar methodology but focusing on fitness facilities and gym equipment in Northeast Ohio (USA), Dalman et al. [4] found that 38% of samples were positive for *S. aureus*, and 11% were positive for MRSA. Alternatively, Ryan et al. [8] found no MRSA in samples taken from a Florida gym. A noticeable difference between our study and Ryan et al.'s was our use of an enrichment step to optimize recovery [28].

In our study, the prevalence of "presumed" MRSA was as high as 50% in the men's locker room samples, and was 0% in the crevice samples, based on the pink colonies observed on HardyCHROM MRSA agar (Table 1). However, a more detailed assessment of the pink colonies confirmed that most of the "presumed" *S. aureus* were actually *S. aureus*; however, only one (9%) of 11 was "confirmed"

 doi:10.20944/preprints202412.1027.v1

resistant to oxacillin (Table 5) and presumably also methicillin. Prior to plating on the chromogenic agar containing antibiotics, we performed enrichment of the swab samples using TSB with 6.5% saline and 24-hour incubation, similar to the approach described by Lee et al. [28]. During this time, considerable growth of Gram-positive cocci would have occurred in the broth tubes. The inoculum placed onto the chromogenic agar had high bacterial densities, including a diverse community of Gram-positive cocci, many of which may have possessed beta-lactamases, as evident from Table 5. The inoculum density and the potential combined growth of many beta-lactamase-positive bacteria may have allowed breakthrough by borderline or oxacillin-susceptible *S. aureus*. Unlike the method inoculating the chromogenic plates, the MicroScan-based method using the PC-44 panels utilized the Prompt inoculation system, which ensured the wells of the panels were not overloaded with microorganisms, which thus enabled a more precise assessment of the MIC and resistance.

4.3. Antibiotic Resistance among the Coagulase-Negative Staphylococci: A Growing Concern Beyond MRSA

Some of the more interesting results of our study were unintended. Our study aimed to focus on MRSA; however, upon further investigation of our isolates, our study revealed substantial antibiotic resistance among the incidentally-receovered *S. haemolyticus* and *S. saprophyticus*, with over 50% of *S. haemolyticus* isolates resistant to five antibiotics, and more than 38% of *S. saprophyticus* isolates exhibiting resistance to five antibiotics (Table 5). These findings highlight a growing concern regarding antibiotic resistance in coagulase-negative staphylococci (CoNS), particularly *S. haemolyticus* and *S. saprophyticus*, alongside MRSA, for several important reasons. First, these species were likely abundant and the *S. haemolyticus* isolates continue to present high levels of antibiotic resistance, especially when compared to other CoNS, with hospital-acquired strains exhibiting the broadest spectrum of resistance [29]. We are unsure of the origins of our strains. Additionally, *S. haemolyticus* can act as a reservoir for antibiotic resistance genes, which may be transferred to other pathogens, such as *S. aureus*. For example, mupirocin resistance genes have been shown to transfer from *S. haemolyticus* to *S. aureus* via plasmids [30]. Furthermore, many CoNS isolates, including *S. saprophyticus*, exhibit multidrug resistance, with a study reporting 58% of *S. saprophyticus* isolates as multidrug-resistant [31]. Our findings illustrate our environmental isolates of *S. saprophyticus* presenting appreciable levels of multidrug resistance.

Another significant concern is the ability of CoNS, including *S. saprophyticus*, to form biofilms, which significantly enhance their resistance to antibiotics and virulence. Biofilm-associated bacteria are known to be more resistant than non-biofilm formers [31,32]. *S. haemolyticus*, in particular, is emerging as an opportunistic pathogen in hospital environments, where its frequently observed multi-drug resistance presents a serious threat to patient health [29,30]. Meanwhile, *S. saprophyticus* is the second most common cause of community-acquired urinary tract infections (UTIs), and its increasing resistance to antibiotics complicates the treatment of these prevalent infections [31,33]. The initial sources for colonization and/or infection warrant additional study and maybe associated with patient environments and community exposures.

CoNS also possess multiple mechanisms of resistance, including efflux pumps, enzymatic inactivation of antibiotics, and modifications of target sites. For instance, *S. haemolyticus* has been found to carry resistance genes for β-lactams, quinolones, macrolides, and antiseptics [29]. In some strains, certain antibiotics, such as oxacillin, may even enhance biofilm formation, potentially increasing their virulence [32]. The combination of biofilm formation and multidrug resistance makes infections caused by CoNS particularly difficult to treat, often leading to chronic infections and poor patient outcomes [34]. Finally, the rising antibiotic resistance in CoNS adds to the global antibiotic resistance crisis, which places a significant economic burden on patients and healthcare systems worldwide [34,35]. Therefore, while MRSA remains a critical concern, the increasing antibiotic resistance in CoNS, especially *S. haemolyticus* and *S. saprophyticus*, presents a distinct and growing threat that warrants increased attention by clinicians, researchers, and environmental health practitioners responsible for promoting hygiene and sanitary environments.

4.4. Future Studies and Public Health Implications

 doi:10.20944/preprints202412.1027.v1

While our study was limited in having a limited number of samples (n = 42) and having done a limited assessment of isolates (n =48), important inferences for future research and public health interventions are able to be provided. Firstly, our study relied upon a biochemical analysis for species identification. Newer approaches, like MALDI-TOF would have improved our confidence in the species identification, [36] and likely will aid in the identification of MRSA, specifically [37]. A limitation of our study was that we did not include as many handles or surfaces with hand contact (medicine balls, basketballs, equipment handles, dumbbell bars, nautilus bars, etc.) as other studies [7,18,20,22], which may have resulted in our study missing more relevant staphylococci.

For public health relevance, our study adds value to studies examining the considerable diversity of staphylococci, beyond *S. aureus*, which have increased public health relevance given their ability to potentially serve as a reservoir of antibiotic resistance genes. Our study illustrates the ubiquity of presumably health-relevant staphylococci throughout a modern fitness center. From these results, some interventions are recommended that likely have application to most fitness and exercise facilities.

Users of fitness and exercise facilities presumably have an interest in their health and may respond favorably to public health campaigns aimed at informing them of infectious disease risk, antibiotic resistant bacteria being prevalent, and actions they can take to promote self-care and community health. The use of quaternary ammonium wipes in fitness centers, just like their use in food processing, can achieve a 4-5 log reduction in staphylococci on surfaces if given one-minute of contact time [37]. Regular use of wipes or cleaning can prevent the build-up of biofilms which can form in the absence of sanitation in fitness center [7]. Based upon this study, and the finding of *E. coli* on a bench in the women's locker room, it is recommended that more frequent cleaning and mopping (with disinfectant) be done in locker rooms and quaternary ammonium wipes also be provided in the locker rooms for promoting preventive wiping by patrons of contact surfaces.

Lastly, public health campaigns, including large posters, signage [38], or messaging, encouraging exercise/fitness center users to disinfect gym equipment and surfaces can be effective [39]. Creating perceptions of social norms, including disinfecting gym equipment, has been demonstrated to increase the desired behavior of users [39]. If users adopt positive social norms by engaging more regularly in disinfecting gym equipment, even for their own self-care, their actions will result in a healthier environment for all exercise center patrons, including immunocompromised individuals, all while reducing the role the facility may play in the transmission of MRSA, opportunistic pathogens, and antibiotic-resistant bacteria into other settings.

5. Conclusions

Our study highlights the high prevalence of staphylococci, including antibiotic-resistant strains, in an exercise center environment. Like most exercise centers, the need for encouraging personal hygiene and sanitation practices exists. While MRSA remains a concern, our findings also reveal significant antibiotic resistance in coagulase-negative staphylococci, such as *S. haemolyticus* and *S. saprophyticus*, which are among emerging threats to public health. Implementing regular cleaning, particularly in high-contact areas like locker rooms, and promoting hygiene awareness among exercise facility users can help mitigate the spread of resistant pathogens and enhance community health.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Isolate analysis by sample location including the isolate color on HardyCHROM MRSA/*S. aureus* agar along with the isolate identification and antibiotic resistance determination from the MicroScan analysis.

Author Contributions: L.J.K. was responsible for writing—original draft preparation, investigation -field and laboratory data collection, and data curation and preservation; S.T.A. was responsible for co-conceptualization of the study, co-supervision—laboratory management for species identification and antibiotic susceptibility data collection efforts, supporting the development of the methodology, and provision of resources for laboratory methods; J.W.M. supervised all studies, field and laboratory collection, co-conceptualization of the study, and

 doi:10.20944/preprints202412.1027.v1

was responsible for final draft preparation, and managed all studies. All authors have agreed to the published version of the manuscript.

Funding: The research supplies were funded by Eastern Kentucky University's Department of Environmental Health Science & Medical Laboratory Science in 2022.

Data Availability Statement: The data are available in Supplementary Table S1.

Acknowledgments: We are appreciative of the assistance provided by Oluwasefunmi (Sheffie) Fadayomi in helping collect environmental swabs, and the encouragement provided by Dr. D. Gary Brown. We are grateful for the assistance of Sarah Rose and Dr. Ismail El-Amouri with ordering supplies and providing administrative support. Most importantly, we are grateful to the facility staff, notably RF, for providing permission for the sampling effort.

Conflicts of Interest: L.J.K. and T.S.A. declare no conflict of interest. J.W.M. declares being the inventor on an international PCT application and pending U.S. patent application by Eastern Kentucky University for a method for the detection of *E. coli* and antibiotic-resistant bacteria in water. J.W.M. declares being the principal for Eastern Scientific LLC, which is licensed by Eastern Kentucky University to commercialize the method. Eastern Scientific LLC has a U.S. trademark for ColiGlow®. The funders of the research had no role in the design of the study, in the collection, analyses, interpretation of data, writing of the manuscript, or in the decision to publish the results. The content provided here represents the original findings of the authors and does not necessarily represent the views or interpretations of Eastern Kentucky University

References

1. Kourtis, A.P., Staphylococcus aureus bloodstream infections in the United States, 2017: Surveillance trends and associated outcomes. *J. Clin. Infect. Dis.*, 2019, 69, 1719–1724. https://doi.org/10.1093/cid/ciy1163.
2. Champion, E., Goodwin, A., Brolinson, G., Were, R., Prater, M., Inzana, J., Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from healthy university student athletes. *Ann. Clin. Microbiol. Antimicrob.*, 2014, 13, 33. https://doi.org/10.1186/s13643-014-0033-6.
3. Keller, M.B., Turco, R.F., Gray, M.B., Sigler, V., The fate of methicillin-resistant *Staphylococcus aureus* in a synthetic turf system. *Sports Health*, 2020, 12, 263–270. https://doi.org/10.1177/1941738120910795.
4. Dalman, M., Bhatta, S., Nagajothi, N., Thapaliya, D., Olson, H., Naimi, H., Smith, T., Characterizing the molecular epidemiology of *Staphylococcus aureus* across and within fitness facility types. *BMC Infect. Dis.*, 2019, 19, 69. https://doi.org/10.1186/s12879-019-3790-6.
5. Gorwitz, R.J., Kruszon-Moran, D., McAllister, S.K., McQuillan, G., McDougal, L.K., Fosheim, G.E., Jensen, B.J., Killgore, G., Tenover, F.C., Kuehnert, M.J., Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001–2004. *J. Infect. Dis.*, 2008, 197, 1226–1234. https://doi.org/10.1086/533489.
6. Yang, L., Dharmaratne, P., Zhu, C., Sapugahawatte, D.N., Rahman, N., Barua, N., Li, C., Kwok, K.O., Luo, M., Liyanapathirana, V., Ip, M., Global epidemiology of asymptomatic colonization of methicillin-resistant *Staphylococcus aureus* in the upper respiratory tract of young children: A systematic review and meta-analysis. *Arch. Dis. Child.*, 2024, 109, 267–274. https://doi.org/10.1136/archdischild-2023-326592.
7. Dulon, M., Peters, C., Schablon, A., Nienhaus, A., MRSA carriage among healthcare workers in non-outbreak settings in Europe and the United States: A systematic review. *BMC Infect. Dis.*, 2014, 14, 1–14. https://doi.org/10.1186/1471-2334-14-1.
8. Ryan, K.A., Ifantides, C., Bucciarelli, C., Saliba, H., Tuli, S., Black, E., Thompson, L.A., Are gymnasium equipment surfaces a source of staphylococcal infections in the community? *Am. J. Infect. Control*, 2011, 39, 148–150. https://doi.org/10.1016/j.ajic.2010.04.313.
9. Markley, J., Edmond, M., Major, Y., Bearman, G., Stevens, M., Are gym surfaces reservoirs for *Staphylococcus aureus*? A point prevalence survey. *Am. J. Infect. Control*, 2012, 40, 1008–1009. https://doi.org/10.1016/j.ajic.2011.10.014.
10. Schilcher, K., Horswill, A.R., Staphylococcal biofilm development: Structure, regulation, and treatment strategies. *Microbiol. Mol. Biol. Rev.*, 2020, 84, e00001-20. https://doi.org/10.1128/MMBR.00001-20.
11. Smith, J.T., Andam, C.P., Extensive horizontal gene transfer within and between species of coagulase-negative *Staphylococcus*. *Genome Biol. Evol.*, 2021, 13, evab206. https://doi.org/10.1093/gbe/evab206.
12. Zhang, M., Ma, Y., Xu, H., Wang, M., Li, L., Surfaces of gymnastic equipment as reservoirs of microbial pathogens with potential for transmission of bacterial infection and antimicrobial resistance. *Front. Microbiol.*, 2023, 14, 1182594. https://doi.org/10.3389/fmicb.2023.1182594.
13. Boggs, C., Shiferawe, K., Karsten, E., Hamlet, J., Altheide, S.T., Marion, J.W., Evaluation of a tetracycline-resistant *E. coli* enumeration method for correctly classifying *E. coli* in environmental waters in Kentucky, USA. *Pathogens*, 2023, 12, 1090. https://doi.org/10.3390/pathogens12031090.

 doi:10.20944/preprints202412.1027.v1

14. Francy, D.S., Darner, R.A., Comparison of methods for determining *Escherichia coli* concentrations in recreational waters. *Water Res.*, 2000, 34, 2770–2778. https://doi.org/10.1016/S0043-1354(00)00135-9.
15. Beckman Coulter. Conventional Panels. Available online: https://www.beckmancoulter.com/products/microbiology/conventional-panels (accessed on December 6, 2024).
16. Clinical and Laboratory Standards Institute (CLSI). Part B: CLSI vs. FDA Breakpoints. Available online: https://clsi.org/media/wxwfzvpd/part_b_clsi_vs_fda-breakpoints.xlsx (accessed on December 6, 2024).
17. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: March 2023; M100, ED33: Vol. 43, No. 3. Clinical Laboratory Standards Institute: Wayne, PA, USA, 2023.
18. Haghverdian, B.A., Patel, N., Wang, L., Cotter, J.A., The sports ball as a fomite for transmission of *Staphylococcus aureus*. *J. Environ. Health*, 2018, 80, 8–13. https://doi.org/10.1007/s00128-018-2250-x.
19. Boa, T.T., Rahube, T.O., Fremaux, B., Levett, P.N., Yost, C.K., Prevalence of methicillin-resistant staphylococci species isolated from computer keyboards located in secondary and post-secondary schools. *J. Environ. Health*, 2013, 75, 50–58. https://doi.org/10.1016/j.jenvh.2013.04.002.
20. Bilung, L.M., Tahar, A.S., Kira, R., Mohd Rozali, A.A., Apun, K., High occurrence of *Staphylococcus aureus* isolated from fitness equipment from selected gymnasiums. *J. Environ. Public Health*, 2018, 2018, 4592830. https://doi.org/10.1155/2018/4592830.
21. Szulc, J., Cichowicz, R., Gutarowski, M., Okrasa, M., Gutarowska, B., Assessment of dust, chemical, microbiological pollutants, and microclimatic parameters of indoor air in sports facilities. *Int. J. Environ. Res. Public Health*, 2023, 20, 1551. https://doi.org/10.3390/ijerph20021551.
22. Boonrattanakij, N., Yomchinda, S., Lin, F.J., Bellotindos, L.M., Lu, M.C., Investigation and disinfection of bacteria and fungi in sports fitness centers. *Environ. Sci. Pollut. Res.*, 2021, 28, 52576–52586. https://doi.org/10.1007/s11356-021-13052-4.
23. Kelly, A.M., Mathema, B., Larson, E.L., Carbapenem-resistant Enterobacteriaceae in the community: a scoping review. *Int. J. Antimicrob. Agents*, 2017, 50, 127–134. https://doi.org/10.1016/j.ijantimicag.2017.02.024.
24. Hachich, E.M., Di Bari, M., Christ, A.P.G., Lamparelli, C.C., Ramos, S.S., Sato, M.I.Z., Comparison of thermotolerant coliforms and *Escherichia coli* densities in freshwater bodies. *Brazil. J. Microbiol.*, 2012, 43, 675–681. https://doi.org/10.1590/S1517-83822012000300005.
25. Alonzo, L.F., Jain, P., Hinkley, T., Clute-Reinig, N., Garing, S., Spencer, E., Dinh, V.T., Bell, D., Nugen, S., Nichols, K.P., Le Ny, A.L.M., Rapid, sensitive, and low-cost detection of *Escherichia coli* bacteria in contaminated water samples using a phage-based assay. *Sci. Rep.*, 2022, 12, 7741. https://doi.org/10.1038/s41598-022-11747-x.
26. Nowicki, S., Delaurent, Z.R., de Villiers, E.P., Githinji, G., Charles, K.J., The utility of *Escherichia coli* as a contamination indicator for rural drinking water: Evidence from whole genome sequencing. *PLoS One*, 2021, 16, e0245910. https://doi.org/10.1371/journal.pone.0245910.
27. Lee, S., Park, Y.J., Yoo, J.H., Kahng, J., Jeong, I.H., Kwon, Y.M., Han, K., Comparison of culture screening protocols for methicillin-resistant *Staphylococcus aureus* (MRSA) using a chromogenic agar (MRSA-Select). *Ann. Clin. Lab. Sci.*, 2008, 38, 254–257.
28. Thapaliya, D., Taha, M., Dalman, M.R., Kadariya, J., Smith, T.C., Environmental contamination with *Staphylococcus aureus* at a large, Midwestern university campus. *Sci. Total Environ.*, 2017, 599, 1363–1368. https://doi.org/10.1016/j.scitotenv.2017.05.283.
29. Eltwisy, H.O.; Twisy, H.O.; Hafez, M.H.; Sayed, I.M.; El-Mokhtar, M.A. Clinical infections, antibiotic resistance, and pathogenesis of *Staphylococcus haemolyticus*. *Microorganisms* 2022, *10*, 1130. https://doi.org/10.3390/microorganisms10061130.
30. Rossi, C.C.; Ferreira, N.C.; Coelho, M.L.; Schuenck, R.P.; Bastos, M.D.C.D.F.; Giambiagi-deMarval, M. Transfer of mupirocin resistance from *Staphylococcus haemolyticus* clinical strains to *Staphylococcus aureus* through conjugative and mobilizable plasmids. *FEMS Microbiol. Lett.* 2016, *363*, fnw121. https://doi.org/10.1093/femsle/fnw121.
31. Hashemzadeh, M.; Dezfuli, A.A.Z.; Nashibi, R.; Jahangirimehr, F.; Akbarian, Z.A. Study of biofilm formation, structure, and antibiotic resistance in *Staphylococcus saprophyticus* strains causing urinary tract infection in women in Ahvaz, Iran. *New Microbes New Infect.* 2021, *39*, 100831. https://doi.org/10.1016/j.nmni.2021.100831.
32. Qu, Y.; Daley, A.J.; Istivan, T.S.; Garland, S.M.; Deighton, M.A. Antibiotic susceptibility of coagulase-negative staphylococci isolated from very low birth weight babies: comprehensive comparisons of bacteria at different stages of biofilm formation. *Ann. Clin. Microbiol. Antimicrob.* 2010, *9*, 1–12. https://doi.org/10.1186/1476-0711-9-1.
33. Zhang, K.; Potter, R.F.; Marino, J.; Muenks, C.E.; Lammers, M.G.; Dien Bard, J.; Dingle, T.C.; Humphries, R.; Westblade, L.F.; Burnham, C.A.D.; Dantas, G. Comparative genomics reveals the correlations of stress response genes and bacteriophages in developing antibiotic resistance of *Staphylococcus saprophyticus*. *MSystems* 2023, *8*, e00697-23. https://doi.org/10.1128/msystems.00697-23.

34. Onyango, L.A.; Liang, J. Manuka honey as a non-antibiotic alternative against *Staphylococcus* spp. and their small colony variant (SCVs) phenotypes. *Front. Cell. Infect. Microbiol.* 2024, *14*, 1380289. https://doi.org/10.3389/fcimb.2024.1380289.
35. Poudel, A.N.; Zhu, S.; Cooper, N.; Little, P.; Tarrant, C.; Hickman, M.; Yao, G. The economic burden of antibiotic resistance: A systematic review and meta-analysis. *PLoS ONE* 2023, *18*, e0285170. https://doi.org/10.1371/journal.pone.0285170.
36. Martins, K.B., Ferreira, A.M., Mondelli, A.L., Rocchetti, T.T. and de S da Cunha, M.D., 2018. Evaluation of MALDI-TOF VITEK® MS and VITEK® 2 system for the identification of *Staphylococcus saprophyticus*. *Future Microbiology*, 13(14), pp.1603-1609. https://doi.org/10.2217/fmb-2018-0053.
37. Jeon, K., Kim, J.M., Rho, K., Jung, S.H., Park, H.S. and Kim, J.S., 2022. Performance of a machine learning-based methicillin resistance of *Staphylococcus aureus* identification system using MALDI-TOF MS and comparison of the accuracy according to SCC mec types. *Microorganisms*, 10(10), p.1903. https://doi.org/10.3390/microorganisms10101903.
38. Elba, I. and Ivy, J.W., 2018. Increasing the post-use cleaning of gym equipment using prompts and increased access to cleaning materials. *Behavior Analysis in Practice*, 11, pp.390-394. https://doi.org/10.1007/s40617-018-0276-2.
39. Meade, T., 2015. Disinfect the rec: Creating and implementing a disinfection campaign at gyms using the theory of planned behavior. *Recreational Sports Journal*, 39(2), pp.157-169. https://doi.org/10.1123/rsj.2015-0012.